DEVELOPMENT AND VALIDATION OF HPLC ULTRAVIOLET DETECTION METHOD FOR QUETIAPINE FUMARATE ESTIMATION IN PHARMACEUTICAL FORMULATION

Master's Thesis

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Eskişehir
Anadolu University
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The thesis titled "Development and Validation of HPLC Ultraviolet Detection Method for Quetiapine Fumarate Estimation in Pharmaceutical Formulation" has been prepared and submitted by Mohammed ZEDAN in partial fulfillment of the requirements in "Anadolu University Directive on Graduate Education and Examination" for the Master of Science degree in Analytical Chemistry Department has been examined and approved in 28/5/2022.

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ABSTRACT

DEVELOPMENT AND VALIDATION OF HPLC ULTRAVIOLET DETECTION METHOD FOR QUETIAPINE FUMARATE ESTIMATION IN PHARMACEUTICAL FORMULATION

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Anadolu University, Graduate School of Health Sciences, May 2022

For determining quetiapine fumarate, a validated, quick, specific, easy, inexpensive, and accurate high performance liquid chromatography ultraviolet detection HPLC UV approach that may be utilized for regular quality control evaluation and in vitro research has been established. Due to its peak symmetry, resolution, and quick analysis time, the selected column Raptor C_{18} 250 x 4.6 mm provided the best separation. Flow rate was 1 min^{-1} and the injection volume was adjusted to 10 μL, the ambient temperature was 25 °C. The mobile phase was containing a buffer of 60% disodium phosphate Na_2HPO_4 and acetonitrile 40% ACN at pH 6. The analyte was detected using a UV detector at 225 nm. The developed method fulfilled all of the SST's approval requirements. The method has been thoroughly examined, and the results indicate that it may be used for the estimation of quetiapine fumarate in pharmaceutical formulations and in vitro studies.

Keywords: High Performance Liquid Chromatography, Ultraviolet Detection, In Vitro, Quetiapine Fumarate.

ÖZET

KETİAPİN'İN YPSK VE UV SPEKTROFOTOMETRİK YÖNTEMLERLE FARMASÖTİK PREPERATLARDAKİ MİKTAR TAYİNİ

Mohammed ZEDAN

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Ketiapin fumaratın miktar tayini için, düzenli kalite kontrol değerlendirmesi ve in vitro araştırmalar için kullanılabilecek hızlı, spesifik, kolay, ucuz ve hassas yüksek performanslı sıvı kromatografi (HPLC) ve UV spektrometrisi yöntemleri geliştirilmiştir. Pik simetrisi, hızlı analiz süresi ve en iyi ayırım için sabit faz olarak Raptor C_{18} 250 x 4,6 mm kolonu kullanılmıştır. Akış hızı 1 mL/dk, enjeksiyon hacmi 10 uL ve ortam sıcaklığı 25 °C olarak belirlenmiştir. Hareketli faz %60 disodyum hidrojen fosfat Na_2HPO_4 tamponu (pH: 6) ve %40 ACN olacak şekilde izokratik olarak çalışılmıştır. Analitin varlığı 225 nm dalga boyunda tespit edilmiştir. Geliştirilen yöntem, SST'nin tüm onay gereksinimlerini karşılamıştır. Yöntem baştan sona incelenmiş ve sonuçlar, farmasötik formülasyonlarda ve in vitro çalışmalarda ketiapin fumarat miktar tatini için kullanılabileceğini göstermektedir.

Anahtar Sözcükler: Yüksek Performanslı Sıvı Kromatografisi, Ultraviyole dedektör, In Vitro, Ketiapin Fumarat.

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COMPLIANCE DECLARATION, ETHICAL PRINCIPLE, AND REGULATION

This thesis is an original study compiled by myself. Throughout the implementation, information collection, evaluation, and presentation phases of my study, I have committed to scientific ethical standards. This thesis has been checked for plagiarism, and I have cited the sources of all material and data that could be obtained within the context of this project and included them in the references section. If a case is ever discovered in my work that contradicts what I've stated, I accept all legal and ethical repercussions.

Mohammed ZEDAN

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LIST OF ABBREVIATIONS

ACN	: Acetonitrile
AS	: Asymmetry factor
°C	: Degree Celsius
DMSO	: dimethylsulfoxide
EDTA	: Trypsin-ethylenediamine tetra acetic acid
HPLC	: High performance liquid chromatography
ICH	: International Conference on Harmonization
K'	: Capacity factor
LOD	: Limit of Detection
LOQ	: Limit of Quantification
MeOH	: Methanol
N	: Theoretical plate number
RSD	: Relative Standard Deviation
S/N	: Signal to Noise
SD	: Standard Deviation
SST	: System Suitability Test
T	: Tailing Factor
T	: Temperature
USP	: United State Pharmacopeia
UV	: Ultraviolet spectrophotometer
v/v	: Volume by volume

1. INTRODUCTION

In the late nineteenth century, analytical chemistry was first employed to expand medical knowledge. The former workers viewed themselves as chemical pathologists. To increase understanding of the disease's etiology and cause, they were primarily concerned with describing the disease's chemical characteristics. Not until much later were the results of chemical analysis of biological fluids, specifically urine, used to detect and differentiate disease in a single patient.

Words such as pills and medicines are frequently used in regular communication. However, when entering the pharmaceutical industry, appropriate terminology and phrasing become essential. In pharmaceutical preparations, the active pharmaceutical ingredient is a pharmacologically active molecule (or active ingredient). The acronym API stands for active pharmaceutical ingredient. A dosage form is created by combining an active ingredient with excipients (inactive ingredients) in order to provide the patient with an accurate dose.

There is no pharmacological activity associated with the excipients. Possible dosage forms include a pill, an injection for parenteral administration, syrup for oral administration, capsule, or and an ointment for topical administration. In pharmacological compositions, excipients serve a variety of functions, which can be summarized as follows:

- Ensure that the shape and size of the dose form are user-friendly for the patient.
- Verify that the stability of the pharmaceutical preparation is adequate.
- Ensure that the appropriate amount of API is administered to the patient.
- Make it easier to prepare the pharmaceutical preparation.
- Determine if the pharmaceutical preparation has an offensive odor or flavor.

In all areas of the life sciences, such as medical science, veterinary science, agriculture, forensic science, military science, and environmental science, qualitative and quantitative analytic techniques play a crucial role. These techniques are used to detect, identify, and quantify the concentration of numerous biochemically significant components. These analytical methods are divided into several types, including Biological Methodologies (Bioassays). Bioassay methods are used to determine the

activity (stimulus) of any essential biomolecule. A complete multicellular organism, an animal or a plant, separated organ systems or tissue from a multi - celled organism, and unicellular or micro - organisms are examples of biological systems that respond to stimuli. The reaction, which is evaluated like a change in certain characteristics of the complex organism, can be positive or negative. Bioassay has a number of inherent restrictions. These are concerned primarily with specificity, reproducibility, sensitivity, and performance ease. The bioassay evaluates the biological effect of the test material and cannot provide information on its chemical properties [1].

However, physical and chemical methods are more specific in terms of chemistry.-Traditional bio-chemical techniques such as spectrophotometric methods, high performance liquid chromatography HPLC, gas-chromatography GC, mass-spectrometry MS, paper as well as thin layer chromatography PC and TLC and electrophoresis methods paired with ultra - violet UV as well as fluorescence detectors techniques are applied for the detection and quantification of biologically important chemical compounds. These fundamental analytical methods take advantage of a substance's physical and/or chemical properties and are used in both research and diagnostics as validating procedures. The progression of an analysis technique to determine a sample is contingent on the choice of an appropriate procedure, which is relied mostly on chemical and physical properties of the sample. Throughout pharmaceutical analytical techniques, it is adviced to develop unique as well as innovative analytical approaches to enhance specificity, decrease expenses for every analysis, boost productivity, and so on. Moreover, it is essential to acknowledge that the data received from the established technique are accurate and crucial from an analytical standpoint; nevertheless, all this can be accomplished with employing validated techniques or examining the procedures in accordance with international regulations [2].

Quetiapine fumarate is amongst the most last several antipsychotic drugs available. Antipsychotic medication administered orally that functions as an adversary of numerous neurotransmitters, such as serotonin and norepinephrine, which is used to treat Bipolar I disorder manic episodes as well as bipolar II disorder depressive episodes and shizophrenia. Quetiapine fumarate is related to clozapine and olanzapine, two antipsychotics with few extrapyramidal side effects. Seroquel is the brand name for

the medication quetiapine fumarate, and it is the first medication of a new class of antipsychotics [4,7].

There are not many methods developed for the determination of QTP using HPLC UV DAD. Generally, high pressure liquid chromatography (HPLC, LC/MS) are used in the literature.

The purpose of this thesis is to provide comprehensive analytical data on quetiapine fumarate. For the measurement of Quetiapine fumarate in pure and medicinal dosing formilations, a validated, quick, specific, easy, affordable, and accurate high performance liquid-chromatography ultraviolet detection HPLC UV technique was developed..

And for furthermore investigations, an in vitro study was assessed, quetiapine fumarate at various concentrations were applied to human hepatocellur carcinoma cell line HepG2 (ATCC^{R}HB-8065^{TM}) in order to monitor metabolism using HPLC-UV and cytotoxicity using the MTT assay.

2. LITERATURE REVIEW

2.1 Schizophrenia

One of the most serious mental illnesses is schizophrenia, previously known as dementia praecox, which means "divided mind." Delusions, hallucinations, and aberrant cognition and atypical behavior can make it difficult to function on a daily basis in people with schizophrenia [12].

Lifelong treatment is necessary for patients with schizophrenia. It is important to begin treatment as soon as possible to decrease symptoms and enhance the long-term prognosis.

2.1.1 Symptoms

Schizophrenia is defined by problems with cognition, behavior, and mood. Common signs and symptoms include delusions, hallucinations, and muddled speech, all of which indicate a reduced ability to function. Among the symptoms and indicators are:

- Delusions. There is no evidence to back up these claims. For example, a patient may feel that they are being tormented or mistreated; they may have a special ability or

popularity; and perhaps a catastrophic tragedy is approaching. Delusions are common among schizophrenia patients..

- Hallucinations. Things that don't exist can be seen or heard all the time. As a result, a person with schizophrenia sees it as a regular part of everyday life. One of the most frequent hallucinations is hearing things. However, hallucinations can take place in every sense.
- Motor behaviour that is exceedingly unsteady or disorganized. From juvenile foolishness to irrational irritation, this can show up in a number of different ways. It's challenging to finish everyday activities. Resistance to directives, wrong or unusual posture, a complete lack of reactivity, or superfluous and unnecessary movements are all signs of abnormal conduct.
- Disordered thinking (speech). Chaotic speech suggests a lack of focus in one's thinking. A lack of effective communication might lead to replies that are irrelevant or incomplete. Speaking can occasionally include the use of obscure or unintelligible terms.
- Signs and symptoms that are harmful to be under-or non-functional. Some examples of this behavior are a lack of interest in personal cleanliness or a lack of facial emotions or speech patterns that do not vary in pitch or tempo. The individual may also become bored with mundane duties, become socially isolated, or be unable to appreciate the pleasures of life.

Symptoms might evolve over time, with phases of deterioration and recovery. The presence of certain symptoms could be constant.

2.1.2 Causes

Researchers believe that a combination of genes, brain chemicals, and environmental factors play a role in schizophrenia's development.

Schizophrenia may be caused in part by some naturally produced neurochemicals, such as dopamine and glutamate neurotransmitters. Researchers have shown that schizophrenia sufferers have unique brain imaging of the brain as well as the nervous system. Schizophrenia appears to be a condition of the brain, even though the importance of such abnormalities is not yet recognized by experts.

2.1.3 Treatment

Schizophrenia is a long-term condition that requires constant treatment. It is possible to successfully handle the sickness with the use of medication and psychological therapy. In some cases, hospitalization may be necessary.

2.1.4 Medication

The most commonly prescribed antipsychotic drugs, which form the foundation of treatment for schizophrenia, are the antipsychotic pharmaceuticals. Some researchers believe they function by affecting the brain's dopamine neurotransmitter system.

Most indications and symptoms should be controlled with the least quantity of medicine possible by antipsychotic drug treatment. Psychiatrists may try a variety of medications, doses, and combinations to see what works best. It's possible that medications like antidepressants and anxiety reducers may also be helpful. The overall symptoms may not improve for a few weeks after starting treatment.

2.1.4.1 Second-generation antipsychotics

Antipsychotics of the second generation are frequently preferred to those of the first generation due to the lower likelihood of serious adverse effects. The second generation of antipsychotics includes the following medications:

- Abilify (Aripiprazole)
- Saphris (Asenapine)
- Rexulti (Brexpiprazole)
- Fanapt (Iloperidone)
- Vraylar (Cariprazine)
- Clozaril, Versacloz (Clozapine)
- Latuda (Lurasidone)
- Zyprexa (Olanzapine)
- Geodon (Ziprasidone)
- Invega (Paliperidone)
- Risperdal (Risperidone)
- Seroquel (Quetiapine)

2.1.4.2 First-generation antipsychotics

In addition to the potential for movement abnormalities (tardive dyskinesia), these first-generation antipsychotics have such a high incidence of and possibly significant neurological side effects. The first generation of antipsychotics are:

- Fluphenazine
- Chlorpromazine
- Perphenazine
- Haloperidol

Typically, these antipsychotics are less expensive than second generation antipsychotic drugs, especially generic ones, and this can be a significant consideration when long-term treatment is necessary [7 - 14].

2.2 Bipolar disorder

Described as manic depression, bipolar disorder is a brain ailment that causes a person's emotions, energy, and capacity to work to fluctuate. Mood episodes, which can last anywhere from a few days to a few weeks for people with bipolar disorder, are characterized by extremely high or low moods. Depression or manic-hypomaniac (excessively upbeat and annoyed mood) are the two most common types of mood fluctuations (sad mood). Treatment for bipolar disorder can help its sufferers live longer and better lives. Bipolar I, bipolar II, and cyclothymic illness are all subcategories of bipolar disorder. An antipsychotic drug like:

- Risperdal (Risperidone)
- Zyprexa (Olanzapine)
- Abilify (Aripiprazole)
- Seroquel (Quetiapine)
- Vraylar (Cariprazine)
- Saphris (Asenapine)
- Latuda (Lurasidone)
- Geodon (Ziprasidone)

Biopolar disorders can be treated with any of these medications alone or in conjunction with a mood stabilizer.

2.3 Physical and Chemical Properties of Quetiapine Fumarate

Quetiapine Fumarate is the fumarate salt form of quetiapine 2-[2-(4-Dibenzo [b,f]thiazepin-11-yl-1-piperazinyl) ethoxy] ethanol fumarate (2:1) salt (Figure 1), with the molecular weight 883.1 and pKa 7.06 and molecular formula $(C_{21}H_{25}N_3O_2S)_2,C_4H_4O_4$. It is a white or almost white crystalline powder. With 174 -176 °C melting point, and boiling point of 556.5 °C. Quetiapine Fumarate is farirly soluble in water and soluble in methanol [2-7, 15] and in DMSO: >10mg/mL [15].

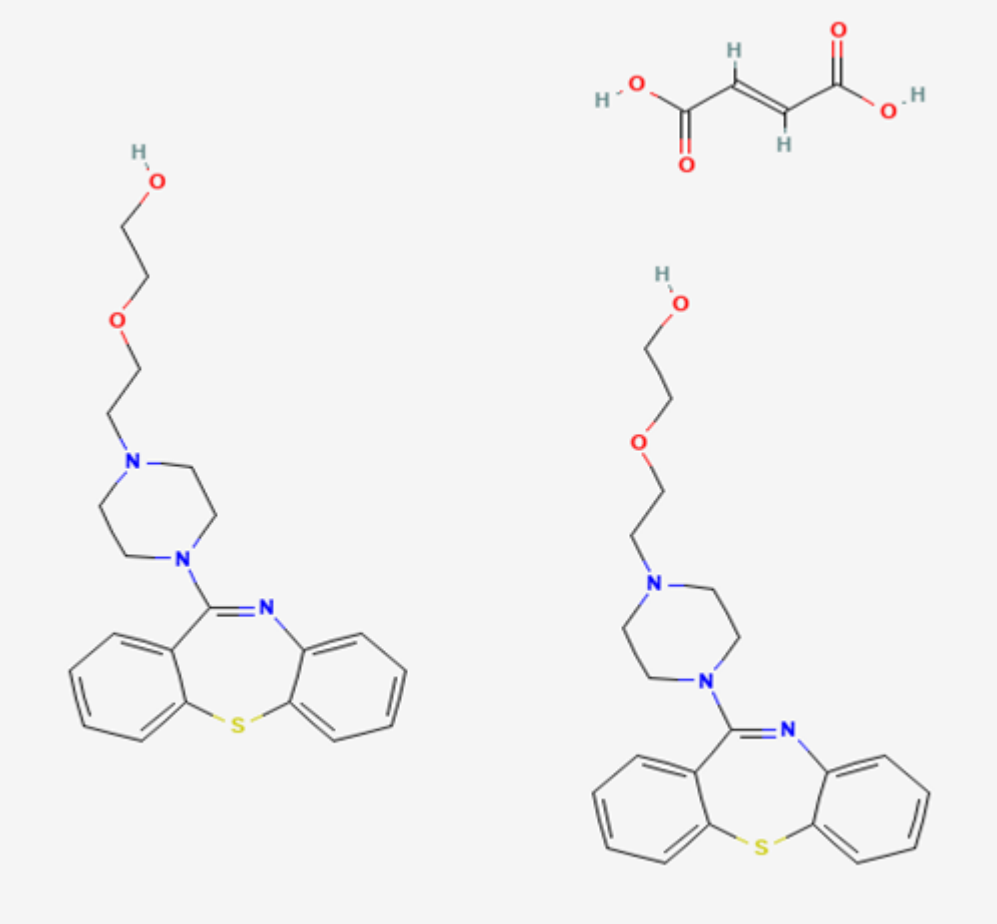

Figure 2.1. *Molecular structure of quetiapine fumarate*

2.4 Pharmacology and Biochemistry of Quetiapine Fumarate

Schizophrenia is treated using the psychoactive medication quetiapine fumarate, which is a benzisoxazole derivative. An antagonist of the dopamine (D2) and serotonin (HT2) receptors, quetiapine fumarate is a sensitive monoaminergic antagonist. In addition to blocking serotonin 5-HT1A and 5-HT2 receptors, quetiapine fumarate also inhibits histamine H1 receptors, dopamine D1 and D2 receptors, and adrenergic receptors alpha 1 and alpha 2. There is no cholinergic, muscarinic, or benzodiazepine receptor affinity for Quetiapine fumarate. The inhibition of histamine H1 and adrenergic alpha 1 receptors by quetiapine may explain drowsiness and orthostatic hypotension associated with its usage. The inhibition of adrenergic a1 receptors by quetiapine fumarate might explain the orthostatic hypotension seen with this medication [2-8, 16].

Quetiapine is rapidly absorbed and extensively biotransformed by cytochromes P450 (CYPs) as well as uridine 5′-diphospho-glucuronosyltransferases (UGTs) in the liver [18-24]. CYP3A4 is known to produce N-desalkylquetiapine, N-desalkylquetiapine sulfoxide, as well as quetiapine sulfoxide, whereas CYP2D6 generates 7-hydroxyquetiapine and 7-hydroxy-N-desalkylquetiapine [18, 21, 22]. In addition, it has been observed that minimal metabolism by CYP3A5 produces O-desalkylquetiapine [21].

2.5 Previous Studies on Analysis of Quetiapine

The phosphate buffer (pH 3.0 modified with orthophosphoric acid) and acetonitrile were used in a 40:60% v/v ratio in the mobile phase in the experiment of D. SUNEETHA and A. LAKSHMANA RAO for the chromatographic separation of quetiapine using C18 Waters column (75x4.6mm 3.5 m). At a rate of 0.8 min^{-1}, the mobile phase was pumped, and detection took place at a wavelength of 291 nm. The detector response was linear across the 20-120 g/mL concentration range. In this study, 0.2 and 0.75 g/mL were shown to be the detection and quantification limits, respectively. There was a 2.929 minute retention time [23].

Pappula Nagaraju, Quetiapine Fumarate Quantification by RP-HPLC Procedure Development and Validation. A Waters Xterra C18 column as well as an isocratic Shimadzu prominence HPLC system were used to extract the medication (250x4.6 mm, 5). With buffer (9.2 0.05) mixed with acetonitrile (51:49 v/v), the technique showed a

linear response between 50 - 150 g/mL when the flow rate was 1.01 min^{-1} and the retention time was 6.588 min [24].

Iury Fernando Rodrigues and his frinds had their chromatographic separation executed on a C18 stationary phase with a mobile phase consisting of phosphate buffer pH 6.6:Acetonitrile : Methanol (45:40:15), a flow rate of 1.0 mL min^{-1}, an injection amount of 20 L, a temperature of 25 °C The relative retention periods of piperazine, lactam, and ethanol compound were around 0.58, 0.69, and 0.88, respectively. Approximately 0.1% of samples included impurities, and the quetiapine hemifumarate assay was more than 98.15 percent [25].

For V G Prasanth and his fellow researchers, their approaches are based on measuring the absorbance of quetiapine fumarate solution in ethanol at 207 nm. For the linear range 1-5 g/mL of QTF, Beer's law is fulfilled by the technique with a molar absorption coefficient value of 1434.41281 L mol^{-1} cm

The intra-day and inter-day relative standard deviation values for accuracy are 100.22 percent and 99.83 percent, respectively. Accuracy is (% RSD 0.39) and recoveries are between 99.34 and 100.11 percent with a standard deviation of 0.39. Range and linearity (r2 =/0.998) [26].

Isocratic elution of quetiapine fumarate in a Grace C18 column was studied using a mobile phase containing 0.1% ortho phosphoric acid, tri ethyl amine like a modifier buffer, and acetonitrile in a 50%:50% (v/v) ratio of the elution buffer. The detection wavelength is 294nm. In the range of 2.01 to 50.2 $x10^{-3}$ g/Lt, the method exhibited good linearity. The method has a 5-minute duration. The proportion of recoveries was close to one hundred percent for provided techniques [27].

In this study the authors offer an auto HPLC method using column exchanges for the determination of quetiapine, olanzapine, perazine, clozapine, and metabolites in blood serum. After washing on silica C8 (20 micron particle size), medicines were separated in 25 minutes on ODS Hypersil C18 (5 micron; column 250 mm x 4.6 i.d.) utilizing UV detection at 254 nm. The quantification limit varied from 10 to 50 ng/ml. At therapeutic levels, the inter assay repeatability of the medications was less than 10%. 139+/-136 ng/ml for quetiapine, 48+/-27 ng/ml for olanzapine, 71+/-52 ng/ml for

perazine, and 328+/-195 ng/ml for clozapine were identified in the serum of 75-295 persons treated with recommended doses of psychotropic medications [28].

Using high-performance liquid chromatography-electrospray mass spectrometry (HPLC-MS/ESI), the authors devised a technique for analyzing quetiapine as well as its sulfoxide-, 7-hydroxy-, and 7-hydroxy-N-dealkyl-metabolites in human plasma. The chemicals were separated using HPLC on a Kromasil C18 (5 micron, 4.6 mm 150 mm) column using water (formic acid: 1.70 mmol/L, ammonium acetate: 5.8 mmol/L)-acetonitrile (65%:35%) as the mobile phase with a flow rate of 0.95 min^{-1}. The chemicals were ionized in the mass spectrometer's electrospray ionization (ESI) ion source and identified in the selective ion recording (SIR) mode. To remove the components, solid-phase extraction columns were employed. 10-2000 microg/L and 1-200 microg/L were linear calibration ranges for quetiapine and its metabolites, respectively. The average extract recovery for all four samples exceeded 85%. Significantly greater than 95% of the time, the strategies were successful. Both intra-day and inter-day RSDs are below 15% [29].

Saracino MA, and colleagues established an HPLC-UV technique for identifying the antipsychotic quetiapine and the geometric isomers of the antidepressant of the second generation, fluvoxamine. The substances were isolated on a reversed-phase C8 column (150 mm x 4.6 mm internal diameter, 5 microns) using a mobile phase of 30 percent acetonitrile and a 10.5mM, pH 3.5 phosphate buffer containing 0.12 percent triethylamine (70 percent). The wavelength utilized for detection was 245 nm, as well as the flow rate was 1.2 mL min^{-1}. In plasma, excellent linearity was seen for each fluvoxamine isomer across the range of concentrations 5.0-160.0 ng $mL^{(-1)}$ and for quetiapine over the concentration range of 2.5-400.0 ng $mL^{(-1)}$. The extraction yield was always more than 93%, while the precision (as measured by relative standard deviation values) was always greater than 4%. Using the methodology, plasma samples from patients with polypharmacy were effectively evaluated. The average rate of recovery was greater than 94%, which was deemed good [30].

SA Bellomarino and colleagues. Atypical antipsychotic drug quetiapine and its principal active and inactive metabolites were detected in human urine and serum using HPLC and tris(2,2-bipyridyl)ruthenium(II) chemiluminescence. To speed up quantification, the method uses a monolithic chromatographic column with a flow rate

of 3 mL/min. To see if quetiapine could be used as a calibration standard, flow injection analysis (FIA) with tris(2,2-bipyridyl) ruthenium(II) chemiluminescence detection and HPLC time of flight mass spectrometry (TOF-MS) was performed. FIA was able to detect 21011 mol L^{-1} in a simple aqueous solution. In urine and serum, the HPLC detection limits were 7108 and 21010 mol L^{-1} correspondingly. FIA had a calibration range of 5109 to 1106 mol L^{-1}. There were HPLC calibration ranges of 1 mol L^{-1} for urine and 1 molL^{-1} for serum. The levels of quetiapine found in clinical samples were 3 x 106 mol L^{-1} in urine and 7 x 107 mol L^{-1} in serum. HPLC-TOF-MS was utilized to identify metabolites without the need for preconcentration, compared to previously described approaches [31].

Using high-pressure liquid chromatography–tandem mass spectrometry (LC–MS/MS), researchers were able to determine the concentration of quetiapine in human Na2EDTA plasma. An internal control drug, clozapine (CLO), was employed. Extraction of the samples was carried out using solid phase extraction (SPE). Oasis HLB cartridges were tested for quetiapine concentration using isocratic HPLC–MS/MS. The SRM option was used to detect MS/MS. Atlantis dC18 and Pelliguard LC-18 (20 mm 4 mm) guard columns were used for the separation of QUE and CLO using a Waters 2695 liquid chromatograph Tested were the 3.0-mm 10-mm Waters Waters MS C8 as well as C18 columns (both 3.0-mm 10-mm). Acetonitrile–methanol–0.01 M ammonium acetate (v/v/v) was used as the mobile phase, and acetic acid was used to alter the pH. (pH 3.5). Vacuum filtering with a 0.45 m filter degassed the mobile phase prior to use. In this experiment, the flow rate was set at 0.4 min^{-1}. A range of values, from 1.0 to 382.2 ng/mL, were used to verify the method. Inter-day precision and intra-day high accuracy were assessed using R.S.D. and percentage of variation (which are both below 8%), respectively, using the recommended approach [32].

Two UV spectrophotometric approaches are provided for measuring quetiapine in bulk medicines and pharmaceutical dosage forms. The absorbance of quetiapine solution in 0.1 N HCl at 209 nm (procedure A) or methanol at 208 nm (method B) is measured (procedure B). Across the linear range of 1.25–12.50 I1/4g mL^{-1} quetiapine, Beer's law is obeyed by both approaches, with molar absorptivity levels of 6.21 A—104 and 5.9 A—104 L mol^{-1} cm^{-1}, respectively. Also included are the Sandell sensitivity, limits of quantification (LOQ), and limits of detection (LOD). The methodologies were

validated according to ICH regulations. The accuracy of the results is good (2.50 percent RSD) when expressed as intra-day and inter-day relative standard deviation values. Likewise, the precision is adequate (2.50 percent). Excipients used as ingredients in pharmaceutical formulations did not interact with the methods proposed, as shown by the recovery studies employing the standard addn. methodology, which produced average percent recoveries ranging from 101.50 to 108.25 percent with a standard deviation of 1.25 percent [33].

In this study, the authors created a high-performance liquid chromatographic method for the determination of classical neuroleptics, atypical antipsychotics (clozapine, quetiapine, as well as risperidone), and their metabolic products in human plasma. The extraction was accomplished using a C8 reversed-phase column and then a mobile phase composed of 70 percent water phosphate solution containing triethylamine at pH 3.0 and 30 percent acetonitrile. The UV monitor was calibrated to 238 nm using amitriptyline as the internal reference. A meticulous pre-treatment procedure for plasma samples was established using solid-phase separation with cyanopropyl cartridges and attaining high extraction yields (> or =93 percent). For all sample matrices, the (LOQ) and (LOD) were always below 2.6 ng mL^{-1} and 0.9 ng mL^{-1}, respectively. Plasma specimens from schizophrenia patients undergoing polypharmacy using two or even more antipsychotics were effectively analyzed using the approach. Data on precision and accuracy were satisfactory, and no impact from other central nervous system (CNS) medications was found [34].

The authors in this study and for the analysis of the novel antipsychotic medicine quetiapine in plasma, they devised an HPLC approach using high-performance liquid chromatography (HPLC). The analysis was done on a C8 (150x4.6 mm 5 mm) reverse phase column with mobile phase acetonitrile, methane, and pH1.9 phosphate buffer with triprolidine used as an internal standard. Solid-phase extraction was used to properly pre-treat the biological materials (SPE). At values between 4 and 400 ng $ml^{(-1)}$, quetiapine plasma concentrations showed excellent linearity. People using Seroquel (R) tablets showed favorable results when plasma samples were tested. This study has high precision and accuracy (mean RSD=3.3 percent) for quetiapine. For quetiapine-treated individuals, the technique appears to be appropriate [35].

For the chromatographic study of the antipsychotic drug quetiapine, the scientists devised a reversed-phase high-performance liquid technique. The presence of quetiapine Noxide as well as quetiapine lactam, two of its metabolic byproducts, was employed to determine the amount of quetiapine. Analysis was performed on a Zorbax SBPhenyl column with a 250 mm 4.6 mm 5m Zorbax SB Phenyl diameter. In order to measure the concentration of acetonitrile in the mobile phase, we used a flow rate of one microliter per minute and a 254 nm UV detector to measure the concentration of acetonitrile in the mobile phase. The linearity in the 0.08–20 g/mL range was proven by the method, with a detection limit of 0.03 g/mL. (3.3108 M). Quetiapine in bulk, pills, and human plasma were all successfully analyzed using the new method, with recovery rates of 99.961.25 percent, 101.370.481 percent, and 100.821.53 percent, on average. Other drugs, such as clomipramine, carbamazepine, as well as fluconazole, were also tested using the suggested method [36].

A liquid-chromatography tandem mass spectrometry (LC-MS/MS) experiment designed and validated for this objective was used to investigate the pharmacokinetics of the psychoactive drug quetiapine and four related metabolites in human plasma. Quetiapine and its metabolites are extracted from human blood plasma samples and separated using Luna C(18) columns (50mm) for dual-column separation. The method provides a linear response from 0.70ng/ml to at least 500ng/ml for each component when using 40 microl of plasma. By diluting with a blank matrix, the useable range was increased by a factor of 100. The accuracies and precisions of quetiapine were less than 6% and 6%, respectively. Only 9.4 percent (5.9 percent) of norquetiapine's accuracy and precision were found, whereas 6.4 percent (6.2 percent) of M2 and 10.0 percent (6.4 percent) of M3 were found to have similar results. The pharmacokinetics of quetiapine and its metabolites in human plasma samples were studied using this approach, and an example of its application is provided [37].

The esearchers here created a liquid chromatographic technique for quantifying quetiapine hemifumarate. For the separation procedure, a simple isocratic mobile phase combination was utilized in conjunction with a C18 stationary phase. The findings were quantified using UV detection at 225nm and a flow rate of 1.0 mL per minute. It was shown that the LC technique could differentiate between quetiapine hemifumarate and its three possible contaminants with a resolution better than 2.0. As shown by regression

analysis, the correlation coefficient between quetiapine hemifumarate and its three impurities was greater than 0.99. All three quetiapine hemifumarate impurities were detected at a level of 0.003 percent, as opposed to a concentration value of 1.0mgmL1 for a 3L injection volume using this technology. The bulk active pharmaceutical ingredient was subjected to hydrolysis, oxidation, photolysis, and thermal degradation stress conditions. Significant degeneration was seen under oxidative stress. Using a recognized norm as a benchmark, measurements revealed that the mass balance was close to 99.5% [38].

The authors created a reverse-phase HPLC method for measuring the antipsychotic drug quetiapine fumarate in tablet and in its forms. In this procedure, a C18, 25 cm x 5 m x 4.6 mm ID column was employed in an isocratic mode with a mobile phase consisting of methanol: water: triethylamine in the ratio of 73%:27%:0.4% v/v pH 3.0 adjusted with phosphoric acid. The detecting wavelength is 289 nm, and the flow rate is one minute per minute. In the range of 100 to 300 g/ml, the linearity of quetiapine has a regression value of 0.9996. The suggested approach is sufficiently selective to distinguish between the parent drug and its hydrolysis, photolysis, or chemical oxidation degradation products, as well as excipients [39].

3. MATERIALS

3.1 Chemicals

The Table 3.1. shows the brands and analytical purity of the chemical components used in the analyses. All materials used were of analytical purity, which was carefully chosen.

Table 3.1. *List of chemical materials used in studies*

Name of Chemical Material Used	**Company**	**Purity**
Metanol	Sigma – Aldrich (Germany) and Carlo Erba (France)	99,9%
Acetonitrile	Sigma – Aldrich	99,9%

	(Germany)	
	and Carlo Erba (France)	
Disodium phosphate HNa_2PO_4	Sigma – Aldrich (Germany)	99,9%
Water CHROMASOLV®	Sigma – Aldrich (Germany)	99,9%
Orthophosphoric acid	Sigma – Aldrich (Germany)	85-90%
Quetiapine Fumarate	Sigma – Aldrich (Germany)	100%
3-(4,5-dimethylthaizol-2-yl)-2,5-diphenyltetrazolium	Sants Cruz Biotechnilogy	
Tetrazolium blue (MTT)	Sigma	
Dimethyl sulfoxide (DMSO)	Carlon Erba	
(DMEM)		
Fetal Bovine Serum	Capricorn Scientific	
Phosphate buffer solution	Biomatik	
Penisilin streotomycin	Wisent Bio Products	
Tripsin-EDTA	Wisent Bio Products	

3.2 Devices and Instruments

The brands and models of the devices used in this study are shown in the table 3.3.

Table 3.2. *Brands and models of the devices*

Devices name	**Company/ Model**
High Performance Liquid Chromatography (HPLC)	Shimadzu (Japan) Model: Nexera – i (LC–2040C 3D) (Japan) Liquid Chromatograph
UV Detector for HPLC	Shimadzu (Japan) Model: Nexera – i(

	LC–2040C 3D) (Japan)
Sensitive scale	Model: Ohaus PA214C and Ohaus Pioneer PA64
PH Metre	Model: Ohaus Starter 3100
Safety control magnetic stirrer	IKA RCT STANDARD
Water bath	Model: Lab companion BW – 20 H
Mobile phase filter device	BFC Model: BF Mobile phase filtration system integrated with – S2500 vacuum device
Syringe and tips	Part No: SN02522 - P
Biosafty cabinet	Heal Force HF Safe 1800
Refrigerator	Arçelik 2470 NEX
Deep freezer (-20C and -80C)	Arçelik 2470 NEX
ELISA reader	Biotek Eon
Cell culture dish 75 cm^2	Nest
Cell culture 96 well	Nest
Cell counting device	Bio Rad TC20
Incubator CO2 alternately	Cell 240 Thermo Fisher Hera
Micro-centrifuge Tube with Safe-lock Cap (2ml)	Nest
Cryo Tube	Nest
Autoclave	Hirayama
Automatic pipette(10, 20, 100, 200, and 100um)	Eppendorf
Pipette tip(10, 20, 100, 200, 300 and 1000um)	Nest
Polypropylene tube(15 and 50 mL)	Nest
Refrigerated centrifuge	Eppendorf Centrifuge 5810 R
Sterile polypropylene centrifuge tube (15 and 50 mL)	Isolab
Sterile serological pipette	LP
Thermostat water bath	Wish Clean Wisd WUC-D06H
Inverted microscope	Leica DMi 1

4. METHODS

4.1 HPLC UV/DAD

4.1.1 Preparation of mobile phase

The mobile phase was made up of disodium phosphate 60% Na_2HPO_4 pH 6 ±0.01 buffer and acetonitrile 40% ACN. Buffer was prepared by diluting 709.8 mg of HNa_2PO_4 in 500 ml ultrapure water in a volumetric flask (V=1000 mL) and then left for 5 minutes in the ultrasonic bath to make sure that it diluted very well. The pH was adjusted to pH 6 ±0.01 by adding controlled drops from Orthophosphoric acid for HPLC purity 85-90% using Ohaus Starter 3100 pH meter and Ika rct Standard safety control magnetic stirrer. Under vacuum, the buffer mixture was filtered using a 0.22 m pore size filter to get rid of any insoluble particles. To make sure that the solution doesn't contain any air bubbles, the solution was placed in the ultrasonic bath cleaner for 15 minutes. The mixing unit of the HPLC device was used to mix the specified mobile phase in the desired ratios.

4.1.2 Preparation of calibration standard samples

Analyte standard stock solution was preoared, 10mg of Quetiapine fumarate %100 was accurately weighed and dissolved in 10ml methanol and kept in the refrigerator at temperature 2C. A serial dilution sample vials were washed with methanol for 5 min and left for 5 min to dry and used to prepare the final standard concentrations from our stock sample 1000 ppm by diluting it with methanol to(200, 150, 100, 80, 60, 40, 20, 10, 5, 2 , 1) ppm.

4.1.3 Analysis parameters

For preliminary research, the sample standard stock solution from the preceding section was utilized, and the composition of the mobile phase was altered by varying the amounts of Na_2HPO_4 and ACN. The system suitability parameters, including resolution, theoretical plate and asymmetry factor, were evaluated, and the conditions that led to High Performance Liquid Chromatography UV detection were selected.

Four HPLC columns of varying brands and models were evaluated throughout the method development trials. The Raptor C18 250 x 4.6 mm Column had the highest

performance based on an analysis of the system suitability data and the chromatograms obtained; so, it was chosen for further study. Flow rate of 1 min^{-1}, The injection volume was adjusted to 10 uL, the ambient temperature was 25 °C, and the pressure was 32.5.

4.1.4 Evaluation of results

The analytical response was determined throughout the investigation by the area under the curve for each chromatographic peak. The calibration curve was generated by matching the peak area against the equivalent concentration; for quantification, the linearity equation was utilized.

4.1.5 Method validation

The proposed technique was validated in compliance with the International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use ICH Q2(R1) and U.S. Pharmacopeia USP requirements, which include accuracy, precision, LOD, LOQ, and other specifications. Also, the system's suitability was checked to make sure that the analytical performance of the instrument and column was correct.

4.1.5.1 System suitability test (SST)

Verifying the performance of the analytical instrument is a vital step in the method development process. Number of Theoretical Plates (N), Resolution (RS), Tailing (T) Symmetry Factor (As) and Capacity Factor (K') are examples of parameters. The equations used in the calculations are listed in Table 4.1.

Table 4.1. *System suitability test equations*

Parameter	**Equation**	
Tailing factor	$T = \frac{W_{0.05}}{2 * \alpha_{0.05}}$	T: Tailing factor. W0.05: The peak width at 5% height. α0.05: The Front half width at 5% height.
Theoretical plate	$N = 16(t_R / W)^2$	tR: Retention time.

		W: Width of the peak.
Asymmetry factor	$A_s = B / A$	A_s = back-to-front ratio asymmetry of a bisected peak measured at 10% of height.
Reproducibility of the obtained peak area	$\%RSD=SD/X_{average}$ x 100	RSD: Relative standard deviation. SD: standard deviation X average=average of the analyses performed

4.1.5.2 Linearity

The linear connection between concentration of the analyte and instrumental response characterizes the linearity of an analytical method [41]. The linearity of the established technique for Quetiapine in the concentration range (200, 150, 100, 80, 60, 40, 20, 10, 5, 2.1) ppm was examined.

4.1.5.3 Accuracy

Accuracy is a crucial parameter for the validation of an analytical technique; it is the evaluation of the level of consistency between the calculated value and the actual value [41]. This was accomplished using the recovery percentage and the standard addition approach. High, medium, and low concentration levels (5 ppm, 20 ppm, and 80 ppm) were evaluated three times over a period of three days.

4.1.5.4 Precision

Precision is the degree of similarity between repeated measurements throughout a series of tests; it is necessary to produce accurate findings. Precision was evaluated for repetition intra-day precision, intermediate precision inter-day precision, and reproducibility between laboratories precision. Standard deviation (SD) and relative standard deviation (RSD) are utilized to express accuracy.

4.1.5.5 Limit of detection and quantification (LOD) and (LOQ)

The smallest quantity of an analyte that can be identified but not measured is known as the limit of detection (LOD). The LOQ is the smallest amount that can be accurately and precisely calculated. In the HPLC section of the experiment, signal-to-

noise ratios were utilized to determine LOQ and LOD. It was determined by utilizing a S/N ratio of 3 for the LOD and 10 for the LOQ in the chromatograms..

4.1.5.6 Specificity and selectivity

When determining the concentration of a single chemical in a combination, composition, or in the presence of other compounds in the environment, the ability to precisely assess its specificity and selectivity is an essential analytical parameter to consider. Biological substances, djuvants, biological metabolites (unknown and known), contaminants and byproducts present in the environment shouldn't alter the detection of a single component [40]. In this study, validation techniques for permeability testing and in vitro testing were examined for selectivity.

4.1.5.7 Robustness and ruggedness

The capacity of the designed analytical approach to withstand minor changes in operating parameters is called robustness..

4.1.5.8 Stability

Standard solutions were also tested for their stability under a variety of situations during technique validation. As with real-world sample analysis and testing, stability testing was carried out under realistically-simulated conditions. The following stability conditions are being evaluated during stability testing..

- ***Short-Term Temperature Stability***

  After 24 hours at room temperature, the standard solutions were evaluated for their stability. It was referred to as "benchtop stability" in the past.

- ***Stability in Freeze***

  The analytes stability was tested after leaving it in the freezer (-8°C) cycles.

- ***Stability in the Long Run***

  The frozen stock solutions were tested for sevral monthes stability.

4.1.6 Sample preparation for pharmaceutical dosage forms

The described approach was used for tablets of quetiapine (Brand1 100 mg, and Brand2 50 mg). Each pill was then weighed, and the mean weight of 10 tablets was

determined. To achieve a fine powder, the pills were completely ground in a mortar. One tablet's powder was dissolved in 10 mL of methanol and sonicated for 15 min to trigger dissolution; the resultant solution was filtered using 0.22-micron nylon syringe filters and then injected into the system.

4.2 In Vitro Applications

Due to their identical genotypic and phenotypic qualities to the functional enzymes required for phase I and phase II metabolic in normal hepatocytes, liver cells are the optimal candidate for detecting harmful substances and evaluating hepatotoxicity. Numerous in vitro liver toxicity models are used. HepG2, Hep3B, HBG, and HepaRG are immortal liver-derived cell lines often used for in vitro assessment of liver damage [42]. Tuschl and Schwab compared the toxicity of the HepG2 human hepatocellular carcinoma cells to that of other cells in their investigation, as well as the identification of certain chemical compounds. According to their findings, it is the best in vitro model for comparing cell cycle impacts [43].

HepG2 (ATCC®HB-8065TM) human hepatocellular carcinoma cells were utilized to evaluate quetiapine-induced hepatotoxicity. HepG2 cells were cultured in (DMEM) Dulbecco's modified Eagle's medium supplemented with fetal calf serum, 100 IU/mL penicillin and 100 mg/mL streptomycin, and at 37 °C 7.5% $NaHCO_3$ in a humidified environment containing 5% CO_2 95% air and. Once or twice each week, trypsinization was used to subculture and proliferate cells cultivated in 25 cm^2 and 75 cm^2 flasks [44, 45].

4.2.1 Cell proliferation

In order to reproduce and prepare the HepG2 cells used in the experiments, they were routinely passaged every 2-3 days. The cell culture flask was gently shaken to allow dead cells to pass into the medium and the medium was removed. To clean the cells, phosphate buffer (5 mL) was added to each flask, followed by the removal of the washing solution. Trypsin-ethylenediamine tetraacetic acid (EDTA) solution (1X) was added to the flask and left in the incubator for about 5 minutes (5% CO_2, 95% humidity and 37°C). The cells were suspended by adding the medium on the flask taken from the incubator and transferred to new flasks by dividing 1:2, 1:3. Flasks were placed in the incubator and left for incubation.

4.2.2 Application of MTT cytotoxictiy test to cells and calculation of IC values

After the incubation period, the medium was removed. Trypsin-EDTA solution (1X) (3-5 mL for 75 cm^2 flasks, 1-3 mL for 25 cm² flasks) was added to the flasks and kept in the incubator for 3-5 minutes. At least twice the volume of trypsin-EDTA solution was added to the cells, which were separated from the surface by gently tapping when necessary, and a cell suspension was prepared by pipetting. The cell suspension was pipetted into the centrifuge tube and centrifuged at +4°C at 1200 rpm for 5 minutes, after which the supernatant was discarded. Using a pipette, a gentle cell suspension was created by adding a sufficient amount of media to the cell pellet. 10 L of cell suspension and 10 L of trypan blue mixture were combined, and a device was used to count the cells.

Each well of a 96-well plate was seeded with 1x104 HepG2 cells in 100 L of medium and incubated for 24 hours. At the conclusion of the incubation time, the plates were inverted to remove the top portion, the cells were washed with phosphate buffer, and the washing solution was withdrawn from the medium. Then, 8 different concentrations of quetiapine fumarate prepared in the range of 1-0.000316 mM were added to each well to incubate for 24 hours or 48 hours. At the completion of each incubation phase, the medium was discarded. The phosphate buffer solution (PBS)-prepared MTT solution at a concentration of 5 mg/mL was diluted 1:10 with the medium and applied to each well in a volume of 100 L (0.5 mg/mL). At the completion of the 3-hour incubation period, the MTT solution was withdrawn and the plate was inverted. Each well was then supplied with 100 L of dimethylsulfoxide (DMSO). The absorbance readings of the plates were measured at 540 nm in a microplate reader after being gently shaken. For each quetiapine fumarate concentration, inhibition percentage values were computed. The IC 50 values of quetiapine fumarate were calculated using nonlinear regression analysis, and their cytotoxic features were interpreted [46-50]. The MTT cytotoxicity test was performed twice on thesis items.

4.2.3 Metabolism evaluation

Each well of a 96-well plate was seeded with 1x104 HepG2 cells in 100 L of medium and incubated for 24 hours. At the conclusion of the incubation time, the plates were inverted to remove the top portion, the cells were washed with phosphate buffer, and the

washing solution was withdrawn from the medium. Then, eight different doses of quetiapine fumarate ranging from 1 to 0.000316 mM were applied to each well and allowed to incubate for 24 or 48 hours. At the completion of the incubation periods, pipettes were used to collect the medium solutions in the plates. The solutions were then put into sample tubes. The samples were centrifuged at 1000 g for 5 minutes at 4 °C to obtain the supernatant. For the HPLC analysis, the supernatants were filtered over a 0.2 m syringe membrane.

5. RESULTS AND DISCUSSION

5.1 Development and Optimization of the HPLC Method

According to the literature review, sevral HPLC ultravilot detection methods have been developed. To develop an analytical method utilizing an HPLC instrument, a number of influencing factors and parameters must be considered, such as the chemical and physical properties of the substances to be assessed, the mobile phase substance selected, the column where components are separated, as well as other instrumental and analytical criteria. The purpose of this research was to develop a simple and rapid HPLC technique for determining quetiapine fumarate in the aforementioned pharmaceutical formulations and pure materials, as well as for use in in vitro experiments.Selection of mobile phase.

Utilizing the Raptor C18 250 x 4.6 mm Column, the mobile phase components and ratio were determined. The selection of the mobile phase is influenced by the chemical characteristics of the substances, such as partition coefficient, pKa, polarity and solubility. If the chemicals can be ionized, the buffer is employed to regulate retention and provide consistent findings. The pH effect was investigated using a mobile phase containing a buffer of 60 % disodium phosphate Na_2HPO_4 and acetonitrile 40 % ACN. Quetiapine fumarate solubility (pKa 7.06). Using a buffer with a proper pH causes this basic compound to ionize and become more hydrophilic, resulting in a shorter retention time. pH 6 was chosen after trying different of pH values and decided for further investigation.

Due to a number of characteristics, including the production of a low UV cut-off point of 200 and minimal back-pressure, ACN is the solvent of choice in HPLC. Different percentages of ACN were tested, with 40% by volume generating the best results in this method. SST was utilized to assess the target compound's outcome, with

the quickest analysis duration selected. With a 60 percent disodium phosphate Na_2HPO_4 and 40 percent acetonitrile ACN buffer, the maximum separation with high resolution was accomplished.

5.1.1 Selection of the column

The column is the most important component of the HPLC system because it is where the separation takes place. The column selected is determined by the chemical properties of the target compounds. The column packing material's physical and chemical properties are critical. In terms of providing effective separation octyl bonded silica OBS known as C8 and Octadecyl-bonded silica ODS also known as C18 , are two commonly used stationary phases.

In the technique development research, four HPLC columns of different brands and models were evaluated. figure 5.1. The Raptor C18 250 x 4.6 mm Column demonstrated the best performance and was used for further research after an examination of the system suitability results and chromatograms recorded.

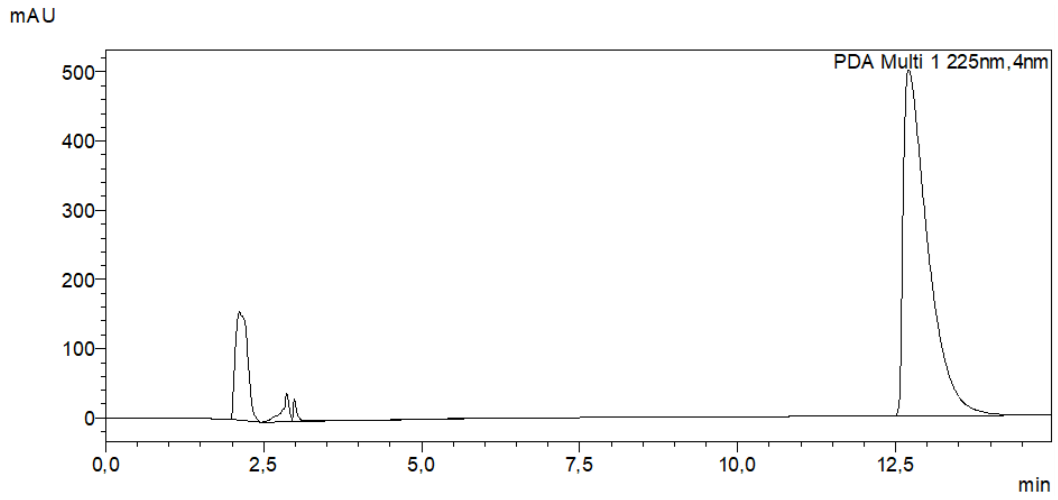

Figure 5.1. *Chromatogram of one of the examined columns*

5.1.2 SST

Many analytical procedures include system suitability testing as part of the process. The suitability parameters of the system were examined and used to determine

the best conditions. The asymmetry factor, tailing factor (T), theoretical plate number (N), selectivity (α) and capacity factor (k), were all assessed for this purpose. Table 5.1 shows the results of the system suitability test.

Table 5.1. *System suitability testing data of quetiapin*

SST parameters	Observed value Quetiapine	Recommended value
Tailing factor T	1.963	T < 2.00
Capacity factor k’	3.091	k’= 2 - 10
Asymmetry factor As	1	As = 0.95-1.20
Theoretical plate number N	9259	N > 2000

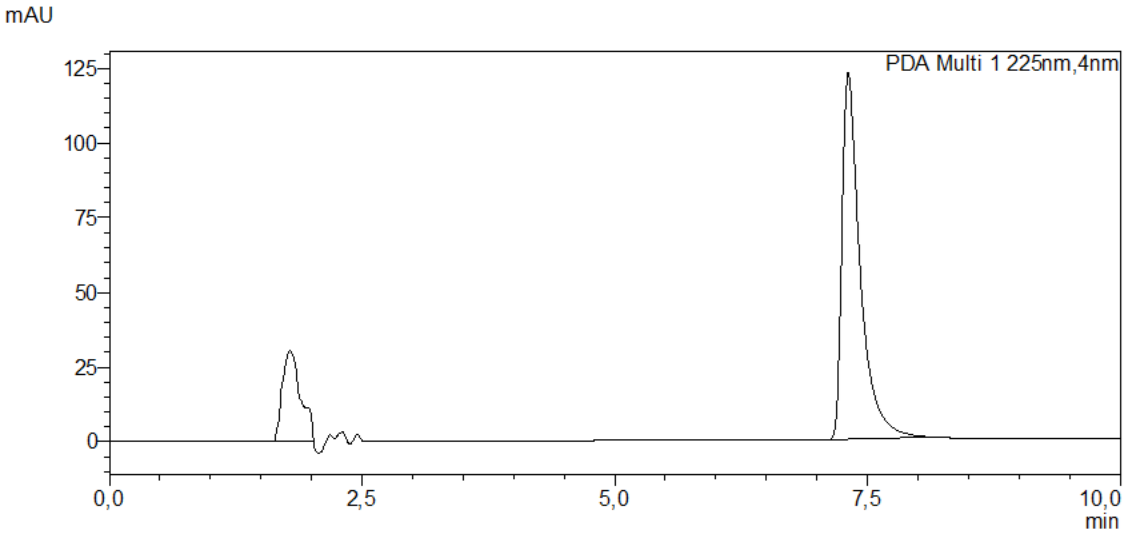

Figure 5.2. *60 ppm standard chromatogram of quetiapine*

5.1.3 Validation of HPLC method

The validation of the analyte's established analysis technique adheres to the ICH guideline on analytical procedure validation.

5.1.3.1 Linearity

The developed method's linearity was tested using the chosen column, Quetiapine in the range of (200, 150, 100, 80, 60, 40, 20, 10, 5, 2, 1) ppm.

Validation parameters were calculated to investigate linearity within intra-day and inter-day. It was discovered that the assayed compounds produced linear results over the specified concentration range. LOD and LOQ values were calculated using the signal intensity in the chromatograms and the noise intensity ratios that were the closest. The chromatogram (S/N) value is multiplied by 3,3 and 10 for the LOD and LOQ, respectively. Quetiapine statistics are provided in Table 5.2 for linearity range, LOQ, LOD, regression equation, and correlation coefficient.

Table 5.2. *LOQ , LOD ,Linearity range, regression equation and correlation coefficient data for quetiapine*

Compound	Linear range ppm	Regression equation	R^2	LOD	LOQ
Quetiapine	1−200	y = 26275x - 13652	0,9999	0.058	0.175

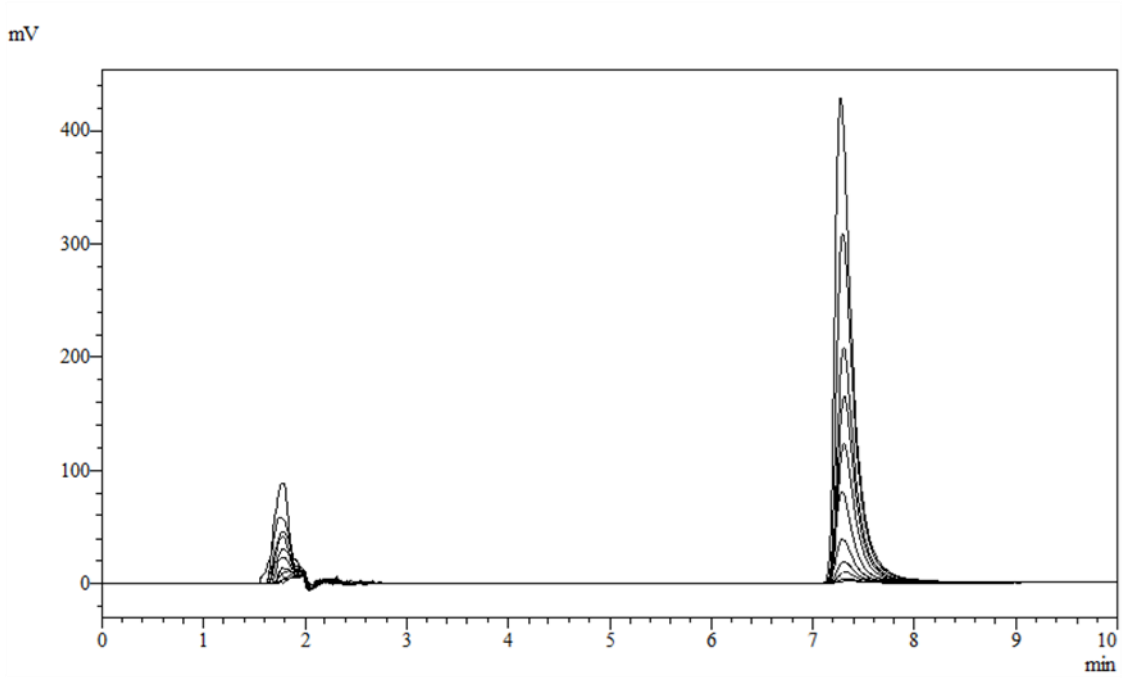

Figure 5.3. *1-200 ppm standard overload chromatograms of quetiapine*

5.1.3.2 Accuracy

The method's accuracy was assessed using the standard addition method, which involved adding a known amount of quetiapine to pre-analyzed tablet samples. The percentage of recovery was calculated using three different concentrations: low level 5 ppm, medium level 20 ppm, and high level 80 ppm. Each level's tests were repeated three times over the course of three days. Table 5.3 contains the validation data for accuracy studies.

Table 5.3. *Validation data for accuracy studies*

Compound	Nominal value PPM	1st	2nd	3rd	Mean Recovery (%)
	5	5,36	5,46	5,4	108
	20	20.13	19.71	19.72	100.7
	80	78,9	80.1	79.6	99.4

5.1.3.3 Precision

To assess the new method's accuracy, intermediate precision (inter-day precision) and repeatability (intra-day precision) were determined. On three days, quetiapine solutions with concentrations of 5 ppm, 20 ppm, and 80 ppm were analyzed three times. RSD % values of less than 2% indicated that the method's precision was acceptable. Table 5.4 shows the precision results.

Table 5.4. *Evaluation of precision studies quetiapine standard solutions intra day (n = 3) inter day (n = 9) (%RSD)*

Intra day (n=3)	1st	2st	3st	Whole day
5	0.59	0,23	0.35	0.39
20	0.99	0.87	0.81	0.89
80	0.64	0.01	0.07	0.67

5.1.3.4 Specificity and selectivity

Specificity and selectivity, which show the capacity to correctly identify the concentration of a single chemical in a combination, formulation, or in the presence of other compounds in the environment, are among the most essential analytical criteria. Other environmental components should not impede the measurement of a single component. The selectivity of the validation methods for the permeability test and the validation method for the in vitro test was tested in this study and according to the collected data from the pharmaceutical formulation analysis, in vitro analysis and the chromatogram found that there was no interfere peak in the chromotogram, the results shows that the specificity and selectivity is valid in this method.

5.1.3.5 Robustness and ruggedness

The influence of a minor, subtle adjustment to the operating parameters on the ultimate result was investigated. The RSD value was below 2%, suggesting the robustness of the proposed approach.

5.1.3.6 Stability study

The results of short-term (1 day), freeze-thaw, and long-term (many months) stability investigations on QTP standard solutions revealed that the compound is stable under all circumstances.

5.1.4 Analysis of quetiapine in the pharmaceutical formulations.

Two commercial tablet brands were selected to evaluate the applicability and validity of the suggested approach for measuring quetiapine in pharmaceutical formulations. The quantity of quetiapine included in the tablet formulation was assessed by examining the peak area of the standard against the quantity of quetiapine contained in the pharmaceutical formulations. Table 5.5 shows the outcomes.

Table 5.5. *Quetiapine in pharmaceutical formulations*

Formulation	Label claim, mg	Amount found, mg	% Amount found
Brand-1	115.13	114.14	99.14
Brand-2	57.57	57.45	99.79

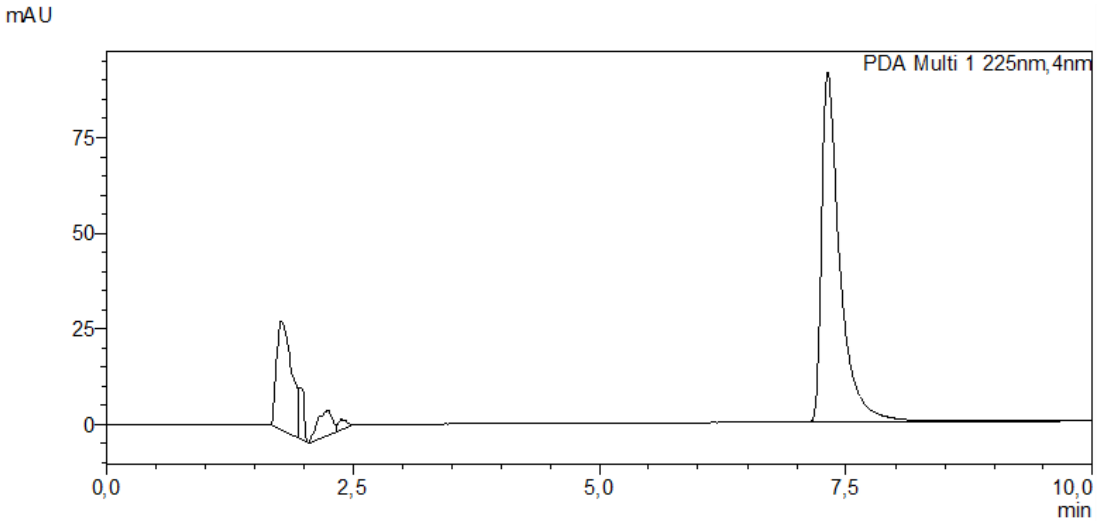

Figure 5.4. *Tablet brand-1*

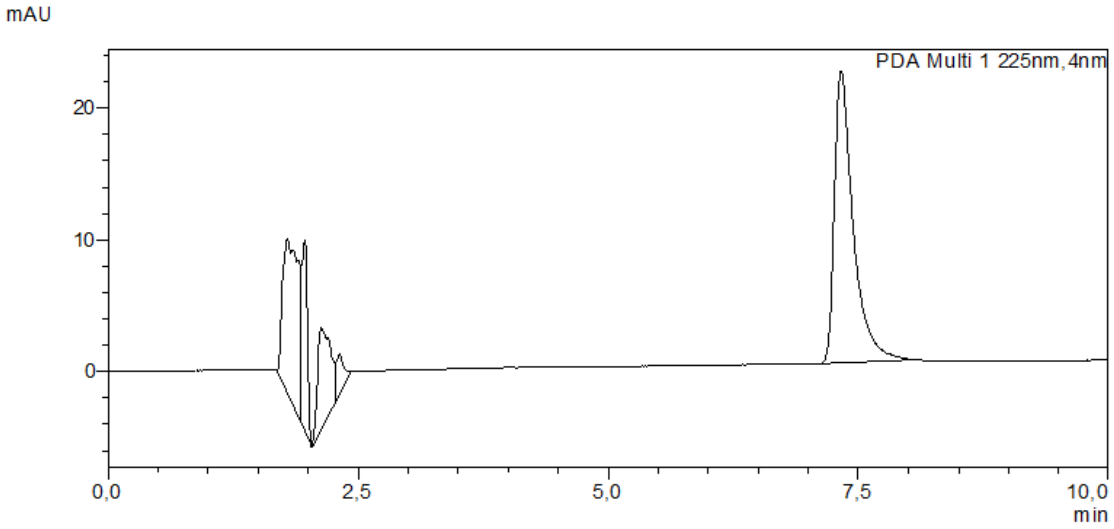

Figure 5.5. *Tablet brand-2*

5.1.5 Over all assessment of HPLC method

This thesis devised a simple isocratic HPLC technique with a 10-minute run time for the quantitative measurement of quetiapine. Additionally, it has been used well to identify quetiapine in pharmaceutical dose forms. With strong peak symmetry, resolution, and a quick analysis time, the selected column provided the best separation. The developed approach meets all SST approval requirements. The present approach has been carefully evaluated, and the findings demonstrate that it is robust, accurate, and has a low LOD.

5.2 In Vitro Results

5.2.1 MTT cytotoxictiy test to cells and calculation of IC values

To investigate the cytotoxic effect of qutiapine fumarate on HepG2, cell concentration response curve was assessed and compared. Cell viability was decreased with increasing concentrations of qutiapine fumarate. After incubation period for 24 hours, the cell viabilities were decreased by about 1.29%, 1.90%, 3.49%, 7.99%, 15.46%, 18.82%, 61.65%, and 86.90% at 0.000316, 0.001, 0.00316, 0.001, 0.0316, 0.01, 0.316, and 1 mM of qutiapine fumarate, respectively. After incubation period for 48 hours, the cell viabilities were decreased by about 3.28%, 4.77%, 7.71%, 7.65%, 10.41%, 21.72%, 83.58%, and 93.60% at 0.000316, 0.001, 0.00316, 0.001, 0.0316, 0.01, 0.316, and 1 mM of qutiapine fumarate, respectively. The half-maximal inhibitory concentrations (IC50) of qutiapine fumarate were 120.87 ± 4.47 and 130.64 ± 4.70 following the corresponding incubation periods; 24 and 48, respectively (Table 5.6).

Table 5.6. *IC50 of qutiapine fumarate*

Incubation period	IC50
24 hours	130.64 ±4.47
48 hours	120.87 ±4.70

5.2.2 Metabolism evaluation

According to Table 5.7, we were able to apply our analytical method to identify and quantify the extracellular quetiapine fumarate in HepG2 cells applied with 8 different concentrations of quetiapine fumarate in the cell culture medium.

Table 5.7. *After incubation period, concentrations of quetiapine fumarate (ppm)*

In vitro concentrations of quetiapine fumarate (ppm)	Found concentration (24h)	Found concentration (48h)
883.1	24.95901	27.43524
279.06	8.900285	8.171874
88.31	2.977621	2.978344
27.906	1.158021	1.193111
8.831	0.701161	0.676841
2.7906	No peak	No peak
0.8831	No peak	No peak
0.27906	No peak	No peak

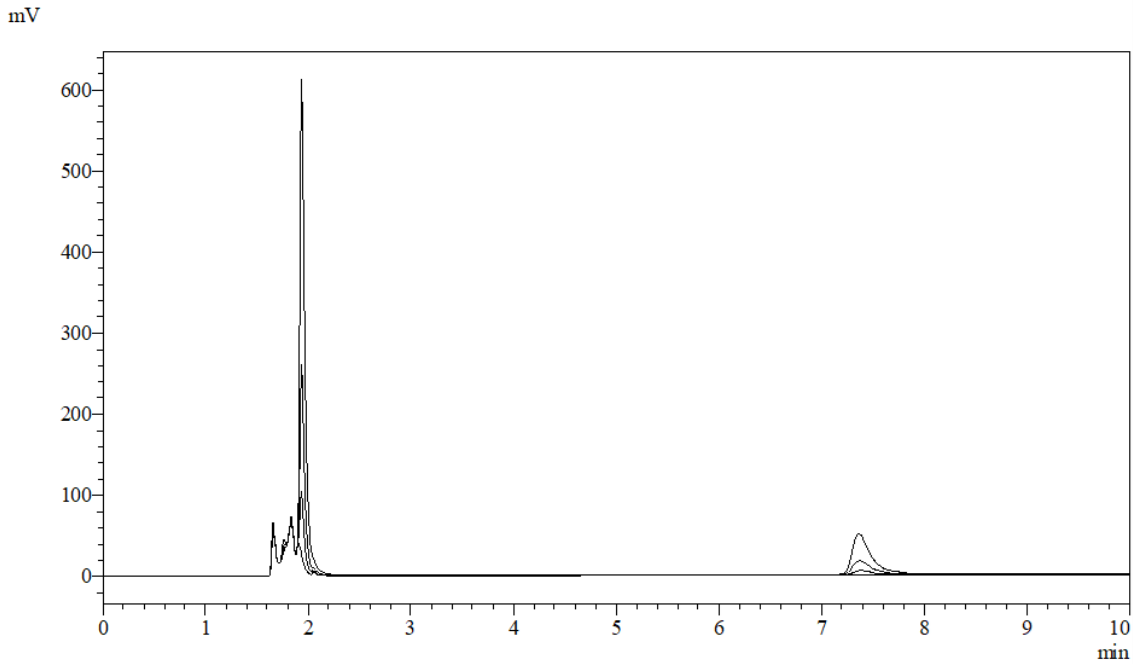

Figure 5.6. *24 Hours chromatogram of Quetiapine metabolism assay*

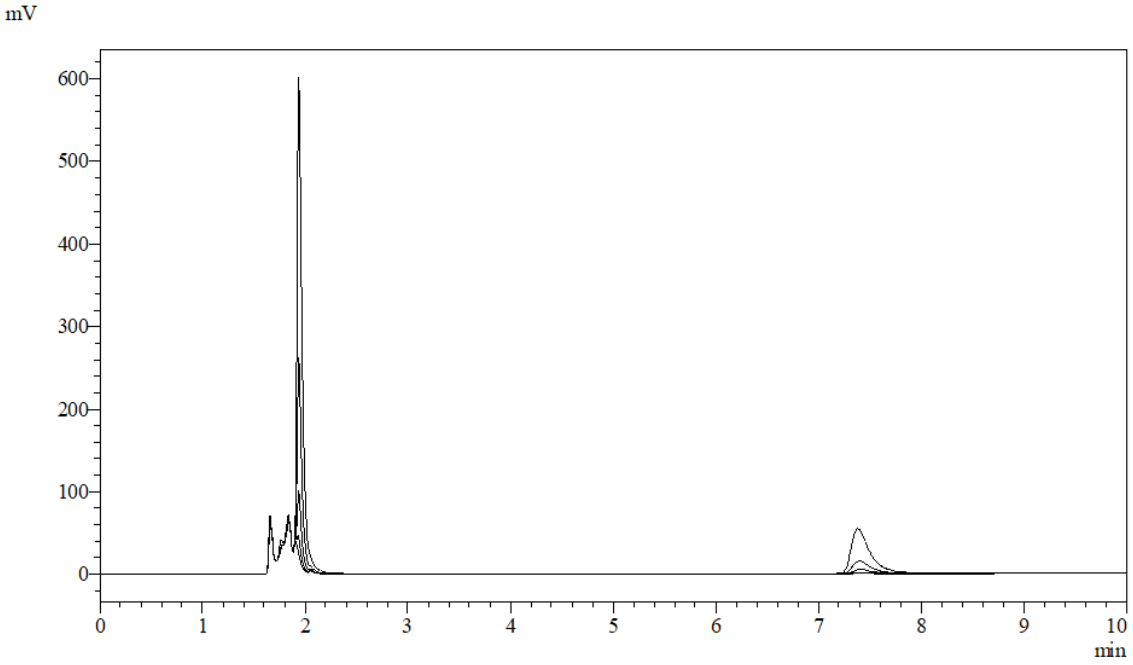

Figure 5.7. *48 Hours chromatogram of Quetiapine metabolism assey*

CONCLUSION

The suggested method for determining Quetiapine fumarate is a validated, quick, specific, easy, inexpensive, and accurate high performance liquid chromatography ultraviolet detection HPLC UV approach that may be utilized for regular quality control evaluation and in vitro research. Due to its peak symmetry, resolution, and quick analysis time, the selected column provided the best separation. The created approach fulfilled all of the SST's approval requirements. The approach has been thoroughly examined, and the data indicate that it may be used for the estimation of quetiapine fumarate in pharmaceutical formulations and in vitro study.

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