T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

OPTIMIZATION AND STATISTICAL ANALYSIS OF DISSOLUTION TEST FOR DRUG DEVELOPMENT

HÜLYA ÖZER
A THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF SCIENCE
DEPARTMENT OF CHEMICAL ENGINEERING

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ASST. PROF. MUHAMMED ENES ORUC

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T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ

İLAÇ GELİŞTİRMEDE ÇÖZÜNME TESTİNİN OPTİMİZASYONU VE İSTATİKSEL ANALİZİ

HÜLYA ÖZER
YÜKSEK LİSANS TEZİ
KİMYA MÜHENDİSLİĞİ ANABİLİM DALI

DANIŞMANI
DR. ÖĞR. ÜYESİ MUHAMMED ENES ORUÇ

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JÜRİ

ÜYE
(TEZ DANIŞMANI) : Dr. Öğr. Üyesi Muhammed Enes ORUÇ

ÜYE : Dr. Öğr. Üyesi Hasan ŞILDIR

ÜYE : Prof. Dr. Hasan SADIKOĞLU

ONAY

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SUMMARY

In this study, development, understanding, control strategy and specifications of dissolution method of Axitinib film coated tablets were determined by using Quality by Design approach. Design of Experiment (DOE) was formed for analytical development with Minitab software. Results of the experiments were firstly analyzed by Minitab software capable of linear regression modelling. Quality and design (QbD) is a systematic scientific approach to controlling and understanding analyze and method parameters in method design and development. Critical Quality Attributes (CQAs), method variables that affect critical quality attributes and Critical Process Parameters (CPPs) were determined and experimental studies were carried out. c) were selected as Critical Quality Attributes (CQAs). Dissolution rate (50 rpm and 75 rpm) and dissolution volume (500 ml and 900 ml) parameters at three different dissolution media (0.01 N HCl, pH 4.5 Acetate + 0.1% SLS and pH 6.8 Phosphate +%) 0.1 SLS) has been studied for Axitinib film-coated tablet dissolution method optimization. With the dissolution studies and the Minitab program, it was decided which of the most critical factors were and the optimum analysis method was selected.

Key Words: Axitinib, Quality by Design (QbD), Dissolution, Minitab.

ÖZET

Bu çalışmada, Axitinib içeren film kaplı tabletlerin dissolüsyon metodunun geliştirilmesi, anlaşılması, kontrol stratejisi ve spesifikasyonlarının oluşturulması, Tasarımla Kalite (Quality by Design, QbD) yaklaşımı kullanılarak belirlenmiştir. Analitik geliştirme çalışmalarında Minitab programında Deney Tasarımı (Design of Experiment, DOE) oluşturulmuş olup, elde edilen veriler önce doğrusal regresyon modellemesi yapabilen Minitab programında değerlendirilmiştir. Tasarım ile kalite (QbD), ürün ile metot dizaynı ve geliştirilmesinde, metot değişkenlerini kontrol etmeyi ve anlamayı sağlayan sistematik bilimsel bir yaklaşımdır. Kritik Kalite Özellikleri (Critical Quality Attributes - CQAs), kritik kalite özelliklerini etkileyen metot değişkenleri ve Kritik Test Parametreleri (Critical Test Parameters - CTPs) belirlenmiş ve deneysel çalışmalar yapılmıştır. Kritik Kalite Özellikleri (Critical Quality Attributes - CQAs) olarak orijinal ürün ve jenerik ürün arasındaki benzerlik faktörü (f2) ve dissolüsyon etkinliği (%DE) seçilmiştir. Axitinib film kaplı tablet dissolüsyon metot optimizasyonu için dönme hızı(50 rpm ve 75 rpm) ve çözünme hızı hacmi (500 ml ve 900 ml) parametreleri 3 farklı çözünme hızı ortamında (pH 1.2, pH 4. 5 ve pH 6.8) çalışılmıştır. Yapılan çözünme hızı deney çalışmaları ve Minitab programı ile en kritik faktörlerin hangileri olduğuna karar verilmiş ve optimum analiz metodu seçilmiştir.

Anahtar Kelimeler: Axitinib, Tasarımla Kalite (QbD), Dissolüsyon, Minitab.

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LIST of ABBREVIATIONS and ACRONYMS

Abbreviations and Acronyms		Explanations
(v / v)	:	Volume/volume
API	:	Active Pharmaceutical Ingredient
BCS	:	Biopharmaceutical Classification System
CTP	:	Critical Test Parameters
CQA	:	Critical Quality Attributes
QTTP	:	quality target test profile
ATP	:	analytical target profile
DoE	:	Design of Experiment
QbD	:	Quality by Design
AQbD		Analytical Quality by Design
DE	:	Dissolution Efficiency
f2	:	Similarity factor
USP	:	United States Pharmacopeia
EP	:	European Pharmacopoeia
FDA	:	Food and Drug Administration
ICH	:	International Council for Harmonisation
GI	:	Gastrointestinal
HPLC	:	High Pressure Liquid Chromatography
AUC	:	Area Under The Curve
IVIVC	:	In Vitro In-Vivo Correlation
PAT	:	Process Analytical Technology
BE	:	Bioequivalence

MODR	:	Method Operable Design Region
UV	:	Ultraviolet Visible
A_N	:	Average of the Axitinib peak area that obtained from test solution
A_S	:	Average of the Axitinibpeak area that obtained from standard-1 solution
W_S	:	Weighing of Axitinib Working Standard as a mg
P	:	Potency of Axitinib Working Standard as % (as is)
LC	:	Label claim

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1. INTRODUCTION

1.1. Motivation

Drugs are an important part of the health system from past to present. Drugs improve our health and quality of life by helping us recover from illness. With the development of the pharmaceutical industry, many diseases and epidemics are treated. For countries, the pharmaceutical industry is strategically important as it is directly linked to the health and well-being of the constructions. The development of society is ensured by healthy individuals, and the more developed the pharmaceutical industry, the easier it is to have healthy societies. When we look at the world and our country, the pharmaceutical industry has a large share and is growing and developing at an increasing rate [1].

Generally, the pharmaceutical industry discovers, develops, produces, and sells drugs that will be used as (or self-administered) drugs to patients for the aim of remedial, vaccinating or decreasing symptoms. Companies of pharmaceuticals may manufacture generic or original drugs and medical devices. They are dependent on several laws and regulations which drug patents controlling, drug testing and safety, efficacy, marketing of drugs. The important thing in the manufacture of pharmaceutical drugs is to deliver a quality, effective and at the same time safe drug to patients [1]. While developing and producing a new drug, it goes through many pharmaceutical processes and one of them is analytical tests. Analytical tests guide us on many issues about how to proceed in product development, whether the developed/produced product will be put on the market. Analytical tests need to provide reproducible and reliable results. Therefore, it is important to develop analytical test methods in the most appropriate way. As a result, the quality of the process during drug development and production also affects the quality of the final product [1], [2].

Depending on the method and route of administration, dosage forms are divided into different types; liquid, solid and semi-solid dosage forms. Solid dosage forms are more preferred in clinical applications because they are practical, stable and safe [2]. Besides their advantages, they can cause problems with bioavailability, absorption. While the absorption of the API from a solid dosage form takes place, the release of this active substance from the drug, its solubility and its permeability from

the gastrointestinal tract play an important role. The first two of these steps, the release and dissolution of the active ingredient, are critical factors and therefore determination of the in vitro dissolution rate can be a suitable indicator for predicting in vivo performance. At the same time in vitro dissolution rate tests are the most important criteria that indicate the performance and quality of the drug used in defining the bioavailability effect of formulation factors in the drug development process [3].

An appropriate and optimum dissolution analytical method should be used to determine the amount of solute. There are many parameters in dissolution tests and many samples are obtained as a result of each analysis. Therefore, the chosen method should be sensitive, selective, robust and fast [4]. Experimental Design (DoE) is defined as a strategy for planning experiments to effectively obtain information from trial data. DoE is a practical method used to describe the relationship between different parameters (independent variable) and response variables (dependent variable) affected by parameters [5].

1.2. The objective of the Thesis

Axitinib is used in combination with avelumab (Bavencio) or pembrolizumab (Keytruda) to treat advanced renal cell carcinoma. Axitinib is in a class of medications called kinase inhibitors and orally administered, particular tyrosine kinase inhibitor of the vascular endothelial growth factor receptor-1 (VEGFR-1), VEGFR-2, and VEGFR-3. As a second-generation VEGFR inhibitor, Axitinib offers increased potency and specificity for the VEGFR when compared to before VEGFR inhibitors that showed multikinase activity with off-target effects. It works by blocking the action of an abnormal protein that signals cancer cells to multiply [6], [7].

For the drug active ingredient, pharmacopoeias such as, American, European, British, Japanese, may include methods for dissolution testing. However, there is no method in any pharmacopoeia for the active ingredient Axitinib [4].

The purpose of thesis is to evaluate the effect of critical dissolution test parameters on Axitinib dosage form through experimental and statistical experimental design methodologies to optimize the dissolution test method.

1.3. Scope of the Thesis

The research conducted performs an optimization design and determines the test parameters to determine or find a combination of factors corresponding to the optimum method according to the desired release of the drug. The analysis made according to the determined test parameters can be repeated and reliably done. While doing this study, the following steps will be followed;

- DOE (Design of Experiment)
- Perform experiments
- ANOVA analysis (statistical analysis)

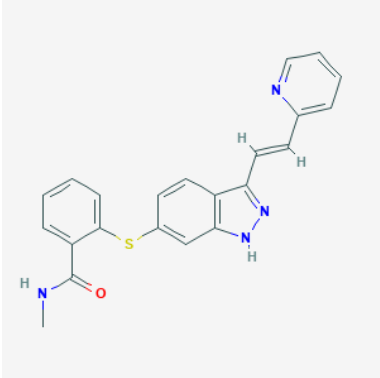
Solid form of drug including Axitinib requires a delicate optimization for the dissolution parameters. In the literature, we were not able find a study about a correlation between dissolution process and parameters. The aim of the current work is to perform an optimization and statistical analysis of dissolution test for Axitinib. During this study, an experimental design will be created using the QbD approach. QbD is a scientific, systematic and risk-based approach that aims to develop a defined process or method. This approach uses statistical experimental design (DOE) to develop an analytical methodology. In this study, experiments designed with mixed level factorial design($2^2 \times 3$) will be carried out. The effects of stirring speed (50 rpm and 75 rpm), the dissolution volume (500 ml and 900 ml) and dissolution mediums (pH 1.2 , pH 4.5 and pH 6.8) on dissolution at different levels will be examined. These parameters have a significant impact on the dissolution of the drug, so the most appropriate parameters should be used while developing the method. In conclusion, we will estimate the importance of each parameter that has an impact on the dissolution method of Axitinib and determine the optimal conditions for incorporating the method into routine laboratory testing.

2. GENERAL INFORMATION

2.1. Axitinib

Renal cell cancer (RCC) is the sixth leading cause of cancer-related death. Tyrosine kinase inhibitors are widely used in the treatment of advanced RCC [6]. Kinase is an enzyme that promotes cell growth. There are many types of kinases that control different phases of cell growth. By blocking a particular enzyme from working, this drug therapy can slow the growth of cancer cells. Axitinib is a type of targeted therapy called a tyrosine kinase inhibitor that specifically blocks vascular endothelial growth factor (VEGF). This means it works by targeting receptors found on cancer cells. The tyrosine kinase inhibitor Axitinib is available in numerous countries worldwide, including the US and the EU, for use in previously-treated advanced [6], [7]. The physicochemical properties of Axitinib are given in Table 2.1.

Table 2.1: Physicochemical characterization of Axitinib

Structural formula	
Established names	Axitinib
Molecular Weight	386.5 g/mol
Molecular Formula	$C_{22}H_{18}N_4OS_5$
Chemical Name	N-Methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1H-indazol-6-ylsulfanyl]- benzamide
Solubility	As pH increases, the solubility of Axitinib in aqueous media decreases.
Melting Point	225°C

2.2. The Development of Drugs

The drug is used in the treatment, prevention or treatment of diseases. It is a basic health-related product used in diagnosis thorough research and rigorous subjected to controls and approved by authorized parties it is put into use after obtaining license.

According to the Human Medicinal Products Licensing Regulation ready-to-use drugs, content to be an active ingredient and excipients in terms of it consists of two parts. Active ingredient in medicines it constitutes the pharmacological active substances used. Excipient constitutes the substances in the composition of the pharmaceutical form of the drug [8].

Drugs based on a patented molecule and unlike any before are considered original drugs. It has the same properties as original drugs and proven by scientific studies that it provides the same treatment but after the original drugs' patent expires products available for market is expressed as “generic (equivalent) drug”. A generic drug cannot be "superior" or "better" than the original drug. In order for a drug containing the same active substance as the original drug in the same doses but produced by different organizations (companies) to be prescribed instead of the original drug, it is necessary to prove its bioequivalence [8]. As with all human studies, bioequivalence studies are conducted according to the rules of “Good Clinical Practice”. Bioequivalence studies control is carried out by the FDA in the United States, the European Drug Product Evaluation administration in the European Union, EMEA, and the Ministry of Health in Turkey. Whether drugs show biological equivalence (bioequivalence) is measured by in vitro (dissolution tests) and in vivo (clinical) trials [8], [9].

For in-vitro dissolution tests, in vitro dissolution tests are used to foresee the bioavailability of the drug in humans measured in vivo. The most usually used method in in vitro experiments is the dissolution test. The dissolution of the drug is determined under certain experimental conditions in an environment such as artificial gastric or intestinal juice. Three different buffer solutions are used with a pH between 1-8, the preferred pH values are 1.2, 4.5, 6.8, representing the pH values of gastric juice, small intestine and large intestine, respectively. As a result of the test, the amount of solute percentage of the drug in the pharmaceutical form is evaluated within a certain period of time [9].

For in-vivo dissolution tests, in vivo (clinical) bioequivalence studies, area is calculated that under the plasma drug concentration-time curve. For this, blood samples are taken from healthy individuals at appropriate intervals during at least 3 half-lives of the drug. Blood samples are taken frequently enough to fully characterize the descending and ascending parts of the concentration-time curve. This is about 10-15 blood samples. In bioequivalence studies, the area under the curve (AUC) of the test (generic) and reference (original) drug products, the maximum drug concentration (cmax) and the time taken to reach the cmax (tmax) are measured and a comparison is made over them. “AUC is a measure of the amount of drug released into the systemic circulation. Cmax is related to the degree of absorption and tmax is related to the absorption rate”. The lower and upper limits of the 90% confidence interval of the mean of the test product / original product ratio for the two primary variables AUC and cmax, on which the comparison is based, are within the 0.8-1.25 range for AUCs and in the same range (wider range in certain cases) for cmax must [9].

2.3. Dissolution Test for Drugs

IUPAC has defined the term "dissolution" as: Formation of a new homogeneous phase by mixing two different phases. Figure 2.1 for a schematic representation of this situation: The green colored stars represent the molecules of the first phase and the blue colored stars represent the molecules of the second phase. Initially, the separation between the two phases can be clearly observed. Dissolution can be defined as the formation of a new homogeneous phase by mixing two different phases (right side in Figure 2.1). (right hand side in Figure 2.1) [10].

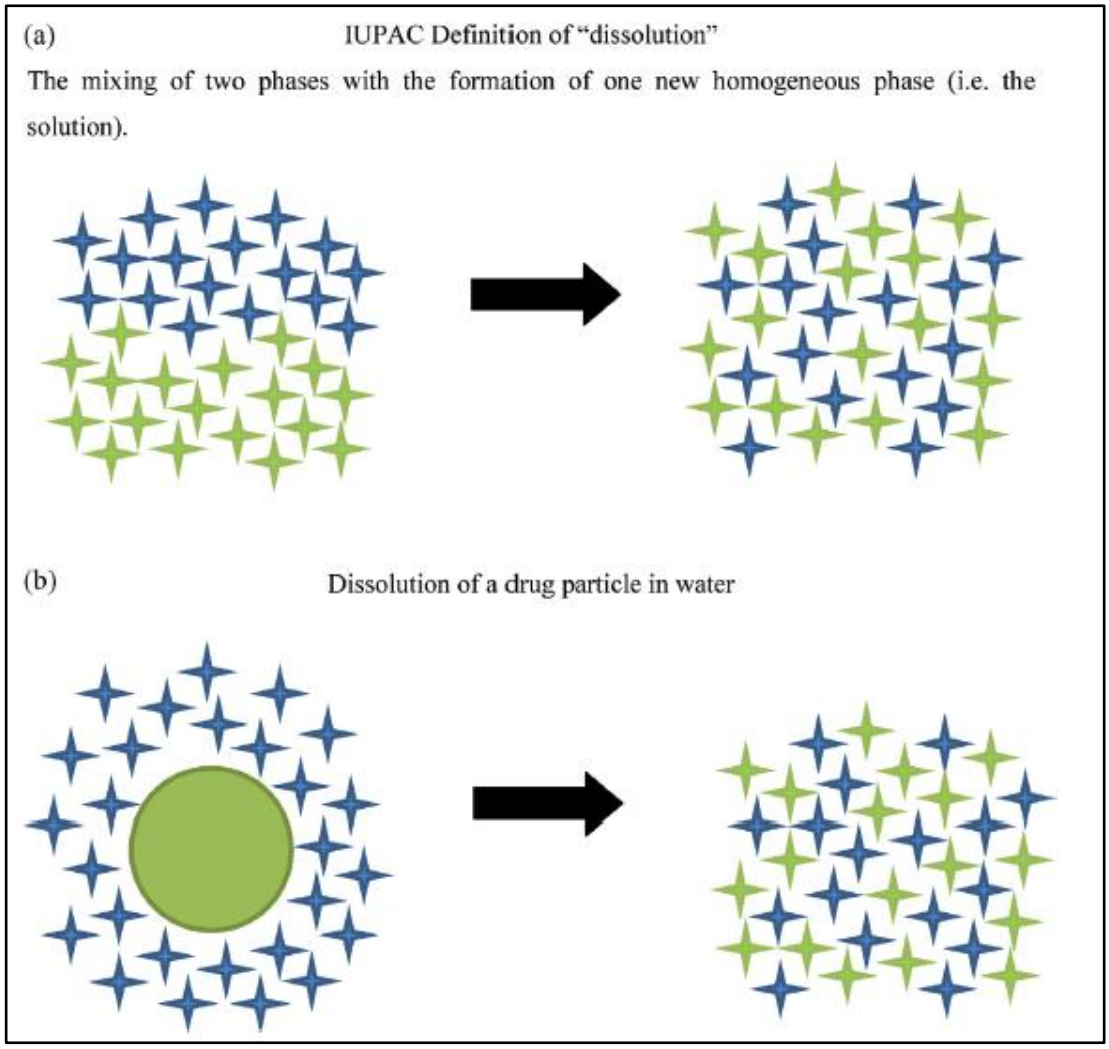


Figure 2.1: Dissolution for different two molecules

The physical phenomena that occur sequentially during the dissolution of the drug are shown in Figure 2.2. We can summarize the 5 main steps as follows;

- The drug particle surface comes into contact with water and gets wet.
- Drug particle bonds are broken.
- Water molecules surround the drug molecules(solvation).
- Drug molecules begin to dissolve from the boundary layer and spread through the liquid.
- If the underlying bulk liquid is well mixed, drug molecules are transferred by convection in the liquid that is not part of the unmixed boundary layer [10], [11].

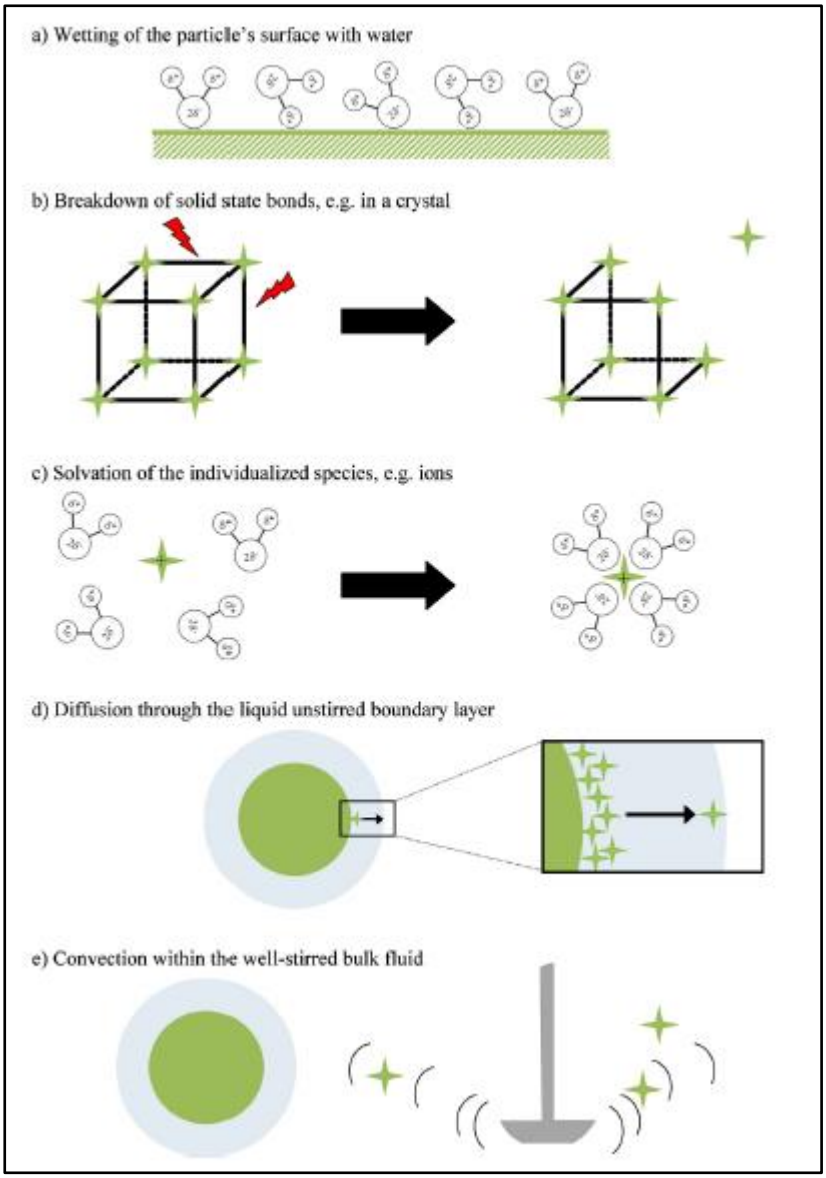


Figure 2.2: Schematic representation of the 5 main steps

Many factors have an effect on the dissolution rate. We can collect these factors in 5 basic groups [12]:

- Factors related to the physicochemical properties of the drug; The physicochemical properties of the drug have an important effect in controlling dissolution. The physicochemical properties added below are some of the properties that may affect the dissolution of the drug [12] :

- Drug solubility; To control drug dissolution, drug solubility is very important and drug solubility is the main factor determining dissolution rate.

- Salt formation; Salt formation provide many advantages to pharmaceutical products. It can improve the solubility, dissolution rate, permeability and efficacy of the drug.
- Particle size; The surface area of the drug directly affects the dissolution rate. When we reduce the particle size, we increase the surface area of the drug. As the surface area of the drug increases, our possibility of obtaining higher dissolution increases.
- Solid state characteristics; Properties of solid phase are one of the parameters that have an effect on the solubility of the drug. Solid phase properties such as the polymorphic nature of the drug, amorphousness or crystallinity. For example, the hydrated forms dissolve more slowly than the anhydrous form due to their thermodynamic properties; the anhydrous forms are more active than the hydrates. Another feature is that the material has an amorphous or crystallinity form. When crystal forms and amorphous forms are compared, it is observed that the dissolution rates of amorphous forms are faster than crystal forms.

- Factors related to the drug product formulation; Drug dissolution depends on the active substance in binders, diluents, etc. It can change when mixed with excipients [12].

- Diluents; The diluent used in the capsule and tablet has an effect on the dissolution rate of the drug.
- Disintegrants; Disintegrants added before and after granulation affect the dissolution rate.
- Binders and granulating agents; The addition of the hydrophilic binder increases the dissolution rate of the poorly wetting drug. A large amount of binder increases hardness and reduces tablet disintegration / dissolution. On the other hand, non-aqueous binders delay the dissolution of the drug.
- Lubricants; Lubricants are hydrophobic materials that, when added, form a hydrophobic layer around the tablets, prolonging their dissolution time. This is one of the parameters affecting the dissolution rate of the drug.
- Surfactants; When surfactants are used, the interfacial tension decreases, so the effective surface area increases. It provides a rapid dissolution when used for drugs with low solubility.
- Factors related to the processing;

- Method of granulation; Granulation process is an effective method to increase the dissolution rate of poorly soluble drugs. Wet granulation is considered superior to dry granulation.
- Compression force; The compression process affects many tablet-related outcomes, for example the porosity, density, hardness, disintegration time and dissolution of the tablet. The greater the compression of the tablet, the greater its density and hardness. With their increase, the bonds between the drug particles become tighter and their porosity decreases. Therefore, the solvent affects the tablet more difficult and the dissolution rate decreases. On the other hand, the increase in the compression force may cause the drug particles to deform, crush or break into smaller particles.

- Factors related to the dissolution testing apparatus [11], [12];

- Agitation; Depending on the type of mixing used, the laminar and turbulent flow that occurs in the system due to mixing, the shape of the mixer, and the physical and chemical properties of the solid, mixing affects the dissolution rate. The flow that occurs due to the mixing speed changes the liquid-solid interface formed between the drug and the solvent. The important thing at this stage is to prevent turbulence, to obtain reliable results and to maintain laminar flow. Therefore, it is preferable to keep the mixing speed at a low speed.
- Sampling probe position-Filter; The USP/NF defines where the sampling probe will be located in the system. The sampling location is important because of the fluid dynamics of the system and can influence changes in dissolution. In addition, the sampling probe should be used with a filter to avoid inaccurate measurements.

- Factors related to the test parameters of dissolution;

- Temperature; The solubility of the drug depends on temperature, so it is very important to carefully control the temperature during the dissolution process. Generally, during the determination of the dissolution of oral dosage forms and suppositories, the temperature should be maintained at $37^{o}\pm0.5$.

- Dissolution medium; It is one of the most important factors affecting the dissolution of the drug. The dissolution medium also consists of many parameters as follows;
    i. pH; With the increase in pH, the rate of dissolution of weak acids increases, while the rate of dissolution of weak bases decreases.
    ii. Volume of dissolution medium; If the drug is difficult to dissolve, if it is to be completely dissolved, a relatively large amount of liquid should be used.
    iii. Deaeration of dissolution medium; Dissolved gases can have many effects on dissolution testing. In general, dissolved gases can lead to higher and more variable results. Changes in the behavior of the dosage form may be due to: causing particles to float, generating abrasive forces on the tablet/capsule, causing the dosage form to rotate or dance, and clustering the product together, etc.

While developing the method for the dissolution rate, the developed method should be applicable, systematic, scientific and reproducible. A robust method should give low sensitivity and create a good profile. The developed method of analysis should be selective between different formulations or batches. At the same time, it must meet the criteria of precision and robustness [8].

The dissolution test is used at many stages during drug development and production, such as formulation selection, in vitro in-vivo correlation (IVIVC), quality control tests for release and stability purposes. The dissolution medium to be used in the dissolution test during method development is one of the most important parameters. The dissolution medium chosen must be both selective and such that we can obtain robust results. It should also deliver our targeted results. When determining the dissolution medium, we must consider the analytical target profile (ATP) and the characterization of active substance solubility. These are guidelines for media selection [13], [14].

Analyze the ATP should specify the dosage form type for which it will develop tests. This can have a major effect on the choice of media. For example, if a delayed-release product is targeted, it is appropriate to use two dissolution rate media, starting with a low pH medium first and then continuing with a high pH medium.

The pH of the dissolution medium used for immediate release products is usually 1.2 to 6.8 whereas the pH of the dissolution medium used for sustained release products is usually 1.2 to 7.5.

Dissolution Testing Apparatus:

7 different apparatus have been defined by the USP to be used in the dissolution rate test. However, apparatus 1 (basket) and apparatus 2 (paddle) are generally used for tablet and capsule dosage forms in dissolution tests. The table 2.5 provides a summary for comparison of USP apparatuses [11].

The basket method is designed to be used up to 150 rpm, although it is usually used at a stirring speed of 50 rpm or 75 rpm. Tablet and capsule forms are one of the most preferred apparatus for dissolution analysis. The paddle method is another most preferred apparatus. Stirring speed is working as 50 rpm or 75 rpm. In addition, this apparatus can be used for testing oral suspensions at a speed of 25-50 rpm. It is known that the use of the Reciprocating cylinder (USP Apparatus 3) is suitable for bead type modified release dosage forms. Some active substances are soluble to a limited extent. The flow-through cell (USP Apparatus 4) is used for this dosage form. They may also be advantageous for modified-release dosage forms. Paddle over disc (USP Apparatus 5) and cylinder (USP Apparatus 6) are used for the analysis of transdermal dosage forms. Finally, the reciprocating disk (USP Apparatus 7) is applied to non-disintegrating oral modified-release dosage forms as well as transdermal dosage forms [11].

Table 2.2: Comparison of USP apparatuses

Apparatus	Name	Drug Formulation Tested
Apparatus 1	Basket	Conventional tablets, chewable tablets, controlled-release formulations
Apparatus 2	Paddle	Tablets, orally disintegrating tablets, chewable tablets, capsules, controlled release products, suspensions
Apparatus 3	Reciprocating Cylinder	Controlled release formulations, chewable tablets
Apparatus 4	Flow-Through Cell	Formulations containing poorly soluble drugs, powders and granules, microparticles, implants
Apparatus 5	Paddle Over Disc	Transdermal formulation
Apparatus 6	Cylinder	Transdermal formulation
Apparatus 7	Reciprocating Disk	Controlled-release formulations (non-disintegrating oral formulations and transdermal formulations)

2.4. Evolution of Drug Dissolution Testing

A drug that enters the body follows different pathways in the stomach and intestines until it enters the systemic circulation. Accordingly, a drug must be dissolved / released and absorbed from the dosage form during the transit time through the gastrointestinal tract. The Biopharmaceutical Classification System (BCS) is a system that separates drugs by considering their water solubility, permeability in the small intestine, and the amount of active substance dissolved from the dosage form (in vitro dissolution test) [15]. The aim of the system is to foresee the drug's in vivo study based on solubility, permeability measurements and in vitro dissolution rate evaluations, to minimize in vivo studies, to provide in vitro - in vivo correlation and bioavailability/bioequivalence. BCS is a system recognized by the authorities and applied in terms of bio exemption, especially in regulatory studies, and is very important for the pharmaceutical industry [15], [16].

Solubility, permeability and dissolution rate are three important factors that affect both the absorption rate and the amount of immediate release drug forms.

Considering solubility and permeability, BCS has categorized drugs in 4 parts as shown in Table 2.3 [15].

Table 2.3: BCS Calssification

		Solubility	
		High	Low
Permeability	High	Class 1	Class 2
	Low	Class 3	Class 4

2.5. General Information on Quality by Design

Quality by design (QbD) has recently become an important paradigm in the pharmaceutical industry. Aim of QbD is to create meaningful requirements by more effectively using recent pharmaceutical information during a product's lifecycle. The relationship between active ingredient properties, drug formulation, and parameters of process can be established with QbD. The ICH Q8, Q9, and Q10 guidelines are all part of the QbD strategy [17], [18].

Analytical approaches may be added to the QbD definition. Pharmaceutical development processes such as raw material analysis, in-process verification, release testing, and stability studies all include analytical testing [17]. To ensure that the consistency of the substance is maintained, the analytical approach should be in accordance with the QbD and PAT. As a result, regulatory guidelines for AQbD should be discussed, explaining the development of methods in accordance with DoE, including risk management processes and specifics of quality systems needed [19], [24].

Because of the high degree of variability involved at each level of method development, the traditional approach is very unfavorable. As a result, efforts have

been made to apply the QbD approach to the application of analytical methods, which has become known as "Analytical QbD (AQbD)." Traditional and scientific approaches to developing analytical methods differ significantly. The differences between traditional analytical method development and analytical method development using the AQbD approach are shown in Figure 2.3 [17], [20].

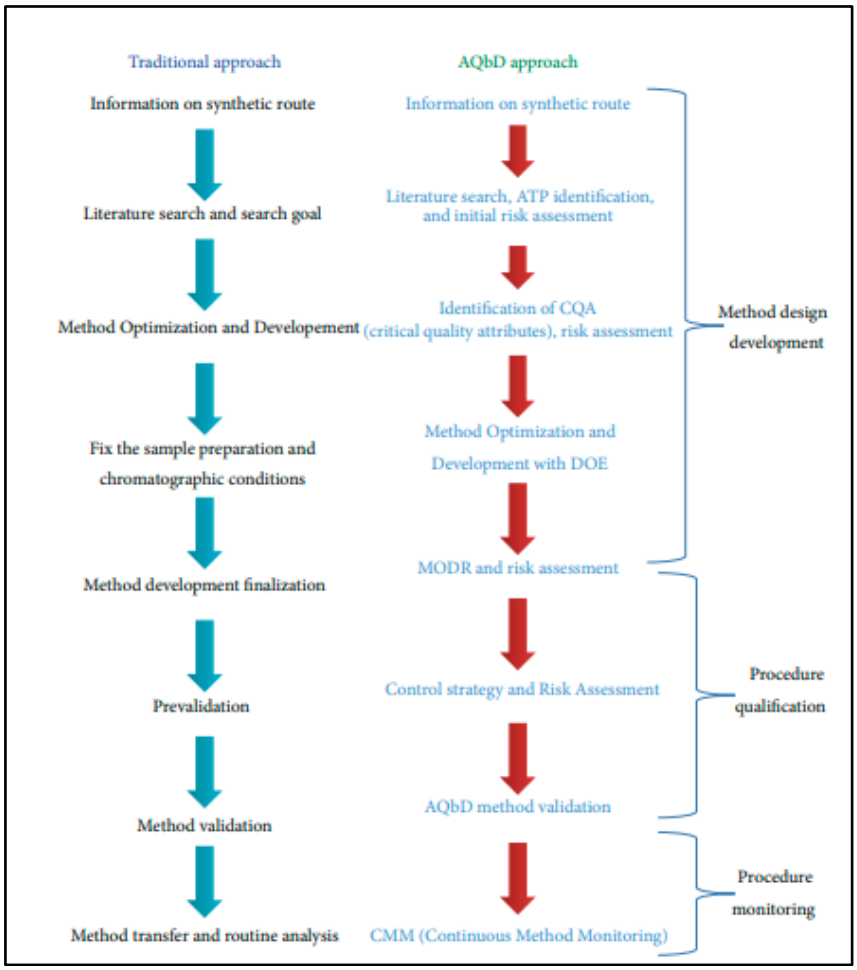


Figure 2.3: Traditional and AQbD approach for analytical method development

ATP (Analytical Target Profile), CQA, Risk Assessment, Process Optimization and Development with DoE, MODR (method operable design region), Control Strategy and Risk Assessment, AQbD Method Validation, and Continuous Method Monitoring are some of the tools used in the AQbD life cycle. The AQbD life cycle is presented in Figure 2.4. with each steps [17].

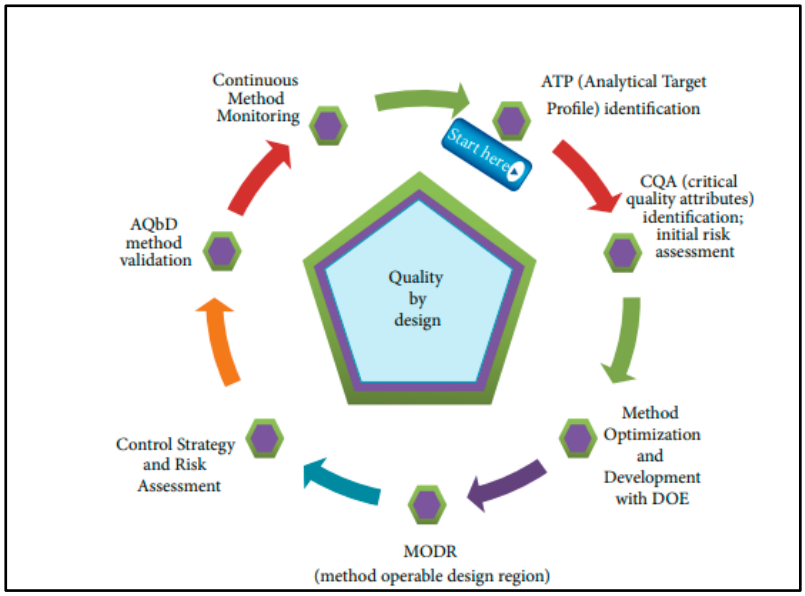


Figure 2.4: AQbD tools and life cycle.

The first step in Analytical Quality by Design (AQbD) is the definition of the Analytical Target Profile. ATP specifies analytical method's objective, which will guide method specification, design, and development. In particular, ATP is the description of the material to be analyzed, the parameter to be analyzed in this material, and the stage at which the measurement will be made [17].

Defining Critical Quality Attributes (CQA) is the next step. A developed or manufactured pharmaceutical product must meet specifications in order to be used. These specified specifications are defined as CQA. Appearance, content uniformity, impurities, assay, residual solvents, drug release or dissolution, can all be part of the CQA procedure [17].

DoE can be used to validate and optimize critical system variables based on statistical significance after analytical method variables have been identified by the initial risk assessment [17], [21].

Method operable design region (MODR) is used to create a multidimensional space dependent on method variables and settings.

Control strategy is a pre-determined system of controls based on analyte design and MODR knowledge. The statistical data obtained from the DoE and MODR steps can be used to develop a method control strategy. Correlations between system and analyte attributes for the ability to satisfy ATP requirements can be drawn using this statistical experimental data. By comparing the AQbD approach to the traditional approach, the method control strategy does not seem to be significantly different. To

provide a better correlation between the aim of method and results of method, method can be developed based on CQA, DoE, and MODR experimental data [17], [21].

In general, analytical method development with the QbD approach has good business and regulatory implications. On the business side, the method developed with QbD is more robust, which reduces the problems associated with the method.. QbD enhances regulatory evaluation, compliance, and inspection coordination, resulting in more valuable details in regulatory submissions. As a result, there is more consistency, flexibility, and improved review quality [25].

2.6. Design of Experiment (DoE)

Experiment Design (DoE) is a strategy for planning the experiments that need to be done to obtain the maximum amount of information. It is an organized method used to determine the relationship between different parameters (independent variable) and the response variables affected by them (dependent variable). The basic principle of DoE is to create a mathematical model for a designed set of experiments. Results can be explicated and predicted, and the design space can be determined using mathematical models [22].

Application of DOE in QbD enables the following to happen;

- Getting more information from a small number of experiments,
- To examine the effects one by one by changing all process parameters simultaneously,
- To consider experiment, operator, raw material or process variability,
- Defining interactions between process parameters instead of single factor experiments,
- To characterize the acceptable ranges of CPPs that help define the design area that provides quality assurance.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals

The chemicals used in the analysis during the thesis study are given below, along with their sources.

Chemicals	**Manufacturer**
Axitinib	ALEMBIC
Inlyta 5 Mg Tablets	Pfizer
Axitinib 5 Mg Tablets	Onko Koçsel Pharmaceuticals
Potasium Dihydrogen Phospate	Merck
1-Octane Sulphonic Acid Sodium Salt	Sigma Aldrich
Ortho Phosphoric Acid	Merck
Acetonitrile (HPLC Grade)	Merck
Ultra Pure Water	N/A
Hydrochloric Acid (37.%)	Merck
Glacial Acetic Acid	Merck
Sodium Acetate	Merck
Sodium Lauryl Sulphate	Merck
Disodium Hydrogen Phospate	Merck
Citric Acid Monohydrate	Isolab

3.1.2. Equipment

The equipments used in the laboratory scale trial series analysis are given below with their brands and models.

Equipments	**Brand, Model**

Micro Balance	Mettler Toledo Xp 26
High Pressure Liquid Chromatography(HPLC)	Waters,Alliance 2695
Ultrasonic Bath	Bandelin Sonorex Rk510
pH Meter	Mettler Toledo, S470 PH/COND
Dissolution	Distek, 3200
Magnetic Stirrer	Heidolph Mr Hei-Standard
Vortex	Heidolph Reax Control

3.1.3. Computer Software and Programs

Software / Programs	**Company**
Word	Microsoft
Excel	Microsoft
Empower	Waters
Minitab Statistical Software	Minitab

3.2. Methods

Dissolution is a comprehensive test and demonstrates the properties of the formulation and API. The dissolution test has been developed to evaluate the in vitro dissolution of solid pharmaceutical dosage forms and also provides information on the release of the drug over time. It is desirable in cases where absorption of the drug is necessary for the therapeutic effect [23].

The aim of the study is to determine the relationship between independent variables (parameters) and dependent variables (results) that affect method development. By doing a DoE study, we can see optimal conditions, analytical target profile (ATP), quality target test profile (QTTP), critical test parameters (CTP) and how these can affect critical quality attributes (CQA). For this study these are determined in below;

- CTP: Volume of dissolution, stirring speed(rpm) and dissolution medium

- CQA: Similarity factor (f2) of between original product and generic product and dissolution efficiency (%)
- QTTP: Similarity factor (f2) value of between original product and generic product is more than 50 and dissolution efficiency (%) is more than %80 at 45 minutes for generic drug

3.2.1. Experimental Design

To develop an appropriate dissolution method for Axitinib, the following parameters were evaluated for original and generic products according to USP, FDA and ICH criteria;

- **dissolution medium** at pH 1.2, pH 4.5 and pH 6.8 buffer solution,
- **dissolution medium volume** of 500 ml and 900 ml
- **stirring speed** of 50 and 75 rpm.

Experimental studies were carried out to evaluate the effect of each determined parameter on the solubility of Axitinib and accordingly to develop the optimum dissolution method to be used in routine laboratory tests. In the study, 12 analyzes were carried out within the scope of 3×2^2 mixed level factorial design, with three levels (-1, 0, +1) for the dissolution medium and two levels (-1 and +1) for the dissolution volume and stirring speed. Each study was done with 6 repetitions.The factors, and levels are listed in Table 3.1.

The Minitab software was used to evaluate the influence of each factor on the responses in order to optimise the best conditions of the dissolution test.

Table 3.1: Mixed Factorial Design of Experiment

Experiment	Dissolution medium volume, x_1	Stirring speed (rpm), x_2	Dissolution medium, x_3
1	500 ml	50 rpm	pH 1.2
2	500 ml	50 rpm	pH 4.5
3	500 ml	50 rpm	pH 6.8
4	500 ml	75 rpm	pH 1.2
5	500 ml	75 rpm	pH 4.5
6	500 ml	75 rpm	pH 6.8
7	900 ml	50 rpm	pH 1.2
8	900 ml	50 rpm	pH 4.5
9	900 ml	50 rpm	pH 6.8
10	900 ml	75 rpm	pH 1.2
11	900 ml	75 rpm	pH 4.5
12	900 ml	75 rpm	pH 6.8

3.2.2 In Vitro Dissolution Experiments

The experimental design for Axitinib tablet was studied within the dissolution parameters shown in the tables below Table 3.2.

Table 3.2: Dissolution Equipment Parameters

Dissolution Medium	pH 1.2 /pH4.5/pH6.8
Medium Volume	500 mL/900 mL
Dissolution Apparatus	USP Paddle (Apparatus 2)
Stirring Rate	50 rpm/75 rpm
Time	5, 10, 15, 20, 30, 45 and 60. minute
Temperature	37.0°C± 0.5°C

All dissolution media used (pH 1.2, pH 4.5 and pH 6.8) were degassed in ultrasonic bath for 30 min prior to use.

pH 1.2 buffer solution: 0,85 mL of hydrochloric acid (37.%) is diluted with 1000 ml of water and mixed properly. pH measurement is performed.

pH 4.5 buffer solution: Dissolve 3.0 g sodium acetate trihidrate in 1000 ml volumetric flask and add 14.0 ml 2.0 N acetic acid solution, the add water to volume and mix. pH measurement is performed and it is confirmed that the pH value is 4.5. Finally add 1.0 g Sodium Lauryl Sulphate and mixed properly.

pH 6.8 buffer solution: Mix 773 ml of a 71.5 g/L disodium hydrogen phosphate with 227 ml of a 21 g/L solution pf citric acid. pH measurement is performed and it is confirmed that the pH value is 6.8. Finally add 1.0 g Sodium Lauryl Sulphate and mixed properly.

The pallets were placed 2.5 cm above the bottom of the containers. In vitro dissolution media were filled in vessels. The tablets were thrown into the vessels and experiments were carried out under the conditions mentioned above. After the 1.5 mL sample taken from all cells at the specified time intervals was filtered through a 0.45µm Nylon filter, the amount was determined by HPLC (high performance liquid chromatography). The peak areas obtained were converted to concentrations and the percentage of soluble axitinib was calculated. Details, mobile phase and standard preparations are presented in Table 3.3.

Table 3.3: HPLC Equipment Parameters

Standards to be used	Axitinib Working Standards
Reactive to be used	Potasium dihydrogen phospate 1-octane sulphonic acid sodium salt Ortho phosphoric acid Acetonitrile (HPLC Grade) Ultra-pure water
Method	HPLC
Detector	UV
Column	InertSustain C18, 250×4.6 mm, 5µm
Wavelength	220 nm
Flow Rate	1.0 ml/minute
Injection Volume	10 µl
Column Temperature	45°C
Sample Temperature	25°C
Mobile Phase	Mobile phase: Buffer: Acetonitrile (1300:700) (V:V)
Run Time	16 minute

Preparation of Dissolution Medium: Dissolution medium preparations are given in page 22.

Preparation of diluent: 0.1% of orthophosphoric acid solution : acetonitrile in the ratio of 50:50 (v/v).

Buffer solution preparation: Dissolve about 6.8 g potassium dihydrogen phospate in 1000 ml volumetric flask. And add 2.5 g 1-octane sülfonicacid sodium salt into this solution. Make up the volume with water. Adjust the pH 2.50 ± 0.05 with dilute orthophosphoric acid solution. Mix well. Filter through 0.22 µ nylon membrane filter and degas.

Preparation of Mobile Phase Solution: Buffer: Acetonitrile (1300:700)(v:v)

Preparation of Standard Solution: Weigh and transfer about 27.0 mg of Axitinib reference standard into a 100 mL volumetric flask. Add about 60 mL of diluent and sonicate to dissolve. Make up to the volume with diluent and mix well. Pipette out 2

mL of this solution into a 100 mL volumetric flask and make up the volume with dissolution medium and mix well.

Preparation of Test Solution: Dissolution rate equipment parameters are provided. 6 Axitinib tablets are weighted, transfered to dissolution rate vessels separately and analysis is initiated. 70 µm filter is used. 1.5 mL samples are taken automatically to vial from each vessel.

Calculation;

$$\textbf{\%Axitinib} = \frac{A_n}{A_s} \times \frac{W_s}{100} \times \frac{2}{100} \times \frac{900}{LC} \times 100 \times P \quad (3.1)$$

A_N: Average of the Axitinib peak area that obtained from test solution
A_S: Average of the Axitinibpeak area that obtained from standard-1 solution
W_S: Weighing of Axitinib Working Standard as a mg
P: Potency of Axitinib Working Standard as % (as is)
LC: Label claim

Comparison of in vitro dissolution results;

One of the QTTP requirements is the similarity factor between the original product and the generic product. Any inference from dissolution profile tests and results (eg justification for a biowaiver) can only be considered valid if the dissolution profile has been adequately characterized using a sufficient number of time points. The similarity of in vitro dissolution rate profiles is made according to the f2 similarity factor given in Equation 3.2.

$$f_2 = 50.\log\left[\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{t=n}[R_{(t)}-T_{(t)}]^2}{n}}}\right] \quad (3.2)$$

In this equation;
f_2: similarity factor,
n: number of time points,
R (t): original product dissolved at time t after the start of the study average percentage,

T (t): is the average percentage of the test product dissolved at the time t after the start of the study.

After the dissolution percentages are determined for both the generic product and the original product, the similarity factor (f2) evaluation should be made. The following conditions should be considered while making the evaluation;

- At least three time points other than the zero point should be considered.
- Must have time point for both generic and original product.
- Six analysis results should be evaluated for each time point.

An f2 value between 50 and 100 indicates that the two dissolution profiles are similar.

4. RESULTS

Dissolution rate recommendation of the European Pharmacopoeia for conventional immediate-release tablets of drugs for which there is no monograph in the Pharmacopoeia; It is in the form of “at least 75.0% of the value stated on the label of the drug should dissolve in 45 minutes or less”.

We determined acceptance criteria in this study as follows;

- Similarity factor (f2) value of between original product and generic product is more than 50,
- Dissolution efficiency (%) is more than %80 at 45 minutes for generic drugs

Results consist of two parts. One of them experimental results and the other part is statistics results.

4.1. Experimental Results

The twelve experiments listed in Table 3.1 were carried out and concluded. The DE values obtained from each experiment for the generic product were tabulated at between Table 4.1 and Table 4.12 as 6 samples depending on time. The values of DE% obtained in the comparison of the dissolution test methods are shown in Figure 4.1, Figure 4.2. and Figure 4.3.

Table 4.1: Results of the dissolution tests for the Experiment 1(500ml/50rpm/pH1.2)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	35,5	34,1	34,4	34,7	33,8	31,5	34,0
10	61,0	58,1	59,7	60,2	59,5	54,3	58,8
15	72,5	69,7	69,2	69,9	71,5	68,1	70,1
20	79,3	76,9	77,1	77,7	77,5	73,1	76,9
30	87,0	86,7	87,1	87,0	85,3	83,6	86,1
45	**94,3**	**94,7**	**95,0**	**94,1**	**93,1**	**90,3**	**93,6**
60	99,5	98,7	96,5	95,5	96,1	96,0	97,0

Table 4.2: Results of the dissolution tests for the Experiment 2(500ml/50rpm/pH 4.5)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	28,6	32,9	31,3	31,6	31,0	35,0	31,7
10	36,7	41,3	38,7	39,0	40,5	45,3	40,2
15	42,9	47,0	46,2	45,6	46,5	48,7	46,1
20	47,6	49,0	51,0	50,9	54,9	56,6	51,7
30	53,5	57,6	57,7	58,6	55,1	63,9	57,7
45	**57,4**	**62,5**	**63,5**	**62,4**	**67,7**	**71,0**	**64,1**
60	76,3	70,9	67,8	66,4	68,2	63,3	68,8

Table 4.3: Results of the dissolution tests for the Experiment 3(500ml/50rpm/pH 6.8)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	15,2	13,0	13,1	12,2	8,5	7,9	11,7
10	20,7	16,7	17,0	16,1	19,9	13,5	17,3
15	24,2	21,1	21,7	20,6	17,3	21,0	21,0
20	27,7	24,5	23,8	23,3	19,7	19,8	23,1
30	32,8	30,1	29,0	28,1	30,1	24,4	29,1
45	**39,3**	**35,3**	**36,3**	**36,2**	**35,0**	**34,9**	**36,2**
60	31,5	34,0	33,7	39,8	40,7	41,2	36,8

Table 4.4: Results of the dissolution tests for the Experiment 4(500ml/75rpm/pH 1.2)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	47,0	46,5	46,6	46,9	45,1	45,7	46,3
10	64,0	63,2	63,1	63,5	63,4	63,1	63,4
15	73,4	73,8	73,4	74,1	73,4	73,9	73,7
20	79,9	80,5	80,6	80,9	81,1	81,1	80,7
30	87,7	87,5	87,8	89,3	89,3	88,0	88,3
45	**90,2**	**94,2**	**94,4**	**96,0**	**97,3**	**96,3**	**94,7**
60	97,0	97,2	97,2	98,0	100,8	100,9	98,5

Table 4.5: Results of the dissolution tests for the Experiment 5(500ml/75rpm/pH 4.5)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	41,3	40,9	39,1	39,8	38,3	34,7	39,0
10	49,3	49,5	52,8	53,6	54,5	55,5	52,5
15	65,0	64,5	63,4	63,6	61,7	59,4	62,9
20	71,4	72,1	72,2	71,3	65,1	65,8	69,7
30	81,9	81,7	81,0	82,2	75,2	75,8	79,6
45	**90,2**	**89,7**	**88,7**	**90,8**	**87,2**	**84,9**	**88,6**
60	96,2	96,9	94,8	95,5	93,8	91,9	94,9

Table 4.6: Results of the dissolution tests for the Experiment 6(500ml/75rpm/pH 6.8)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	32,8	33,1	33,2	33,1	33,3	33,0	33,1
10	33,7	33,9	33,8	33,9	34,2	33,8	33,9
15	37,8	37,7	37,6	37,8	37,8	37,8	37,7
20	41,0	41,1	40,8	41,0	41,2	41,5	41,1
30	46,3	46,4	46,3	46,6	46,8	46,7	46,5
45	**51,8**	**52,2**	**52,6**	**54,4**	**52,7**	**53,1**	**52,8**
60	59,7	56,5	56,9	56,9	56,5	56,8	57,2

Table 4.7: Results of the dissolution tests for the Experiment 7(900ml/50rpm/pH 1.2)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	34,6	35,1	36,5	39,8	38,9	37,8	37,1
10	50,0	51,5	51,2	54,5	56,8	53,8	53,0
15	60,6	60,3	60,1	62,4	65,6	58,4	61,2
20	67,3	67,1	66,7	68,7	72,5	68,5	68,5
30	76,3	75,5	75,3	76,6	81,4	77,7	77,1
45	**84,1**	**83,0**	**83,0**	**84,5**	**88,1**	**84,9**	**84,6**
60	90,2	88,4	89,1	90,0	88,4	88,8	89,1

Table 4.8: Results of the dissolution tests for the Experiment 8(900ml/50rpm/pH4.5)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	26,4	36,3	23,4	34,7	32,6	25,9	29,9
10	45,3	51,1	41,7	47,2	43,8	42,0	45,2
15	56,8	58,9	53,2	55,7	50,7	51,1	54,4
20	63,3	64,4	60,5	61,8	56,5	56,9	60,6
30	72,5	70,9	69,2	70,2	62,9	64,5	68,4
45	**80,4**	**77,5**	**75,9**	**76,5**	**68,8**	**72,9**	**75,3**
60	85,5	80,8	80,2	85,1	80,1	79,8	81,9

Table 4.9: Results of the dissolution tests for the Experiment 9(900ml/50rpm/pH6.8)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	1,0	3,5	1,7	2,9	8,3	2,9	3,4
10	11,5	12,3	11,4	10,1	19,3	11,9	12,7
15	16,1	15,7	16,4	14,6	24,1	16,3	17,2
20	19,3	18,1	20,1	18,3	28,4	18,5	20,4
30	24,3	23,5	25,1	24,2	33,2	22,1	25,4
45	**30,3**	**28,7**	**32,4**	**30,8**	**38,6**	**27,1**	**31,3**
60	34,6	32,6	38,4	35,7	45,8	32,0	36,5

Table 4.10: Results of the dissolution tests for the Experiment 10 (900ml/75rpm/pH1.2)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	40,6	41,2	46,8	42,8	42,6	42,7	42,8
10	62,0	59,3	61,9	60,7	59,7	58,3	60,3
15	73,5	66,8	71,2	72,4	70,0	68,4	70,4
20	82,0	76,3	76,8	75,9	75,6	76,8	77,2
30	90,8	84,4	84,6	88,6	84,2	85,8	86,4
45	**97,9**	**89,3**	**87,6**	**94,1**	**88,5**	**93,3**	**91,8**
60	93,2	92,8	91,4	98,9	91,9	96,2	94,1

Table 4.11: Results of the dissolution tests for the Experiment 11 (900ml/75rpm/pH4.5)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	39,5	42,3	43,4	38,5	37,1	40,4	40,2
10	55,6	58,4	56,2	56,0	54,8	53,8	55,8
15	66,2	70,6	66,1	67,0	64,0	63,5	66,2
20	74,5	73,4	75,5	75,2	72,0	70,6	73,5
30	85,3	82,6	82,3	86,9	80,7	77,3	82,5
45	**93,4**	**90,7**	**92,6**	**95,5**	**89,3**	**85,1**	**91,1**
60	98,7	97,6	96,3	100,0	94,1	90,4	96,2

Table 4.12: Results of the dissolution tests for the Experiment 12 (900ml/75rpm/pH6.8)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	0,4	0,3	0,6	0,5	0,3	0,6	0,4
10	15,3	11,7	18,4	14,5	10,8	5,4	12,7
15	29,3	24,3	34,5	26,4	17,9	14,9	24,6
20	34,3	33,4	38,2	33,4	32,6	22,4	32,4
30	41,8	41,3	44,8	36,8	41,2	31,4	39,6
45	48,7	48,7	52,2	48,7	52,4	39,4	48,4
60	53,8	53,6	57,4	59,2	58,6	47,1	54,9

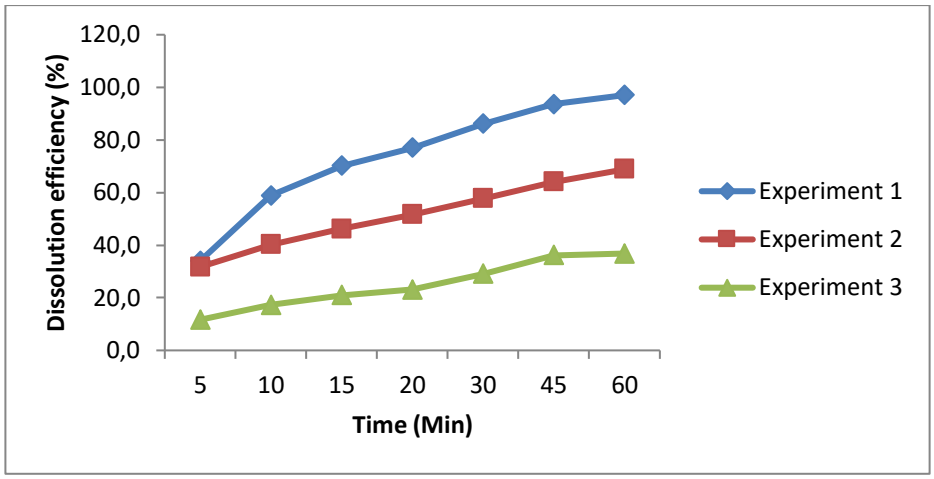


Figure 4.1: Dissolution profiles of Axitinib tablets obtained in pH1.2, pH4.5 and pH6.8 at 50 rpm and 500 mL of dissolution medium

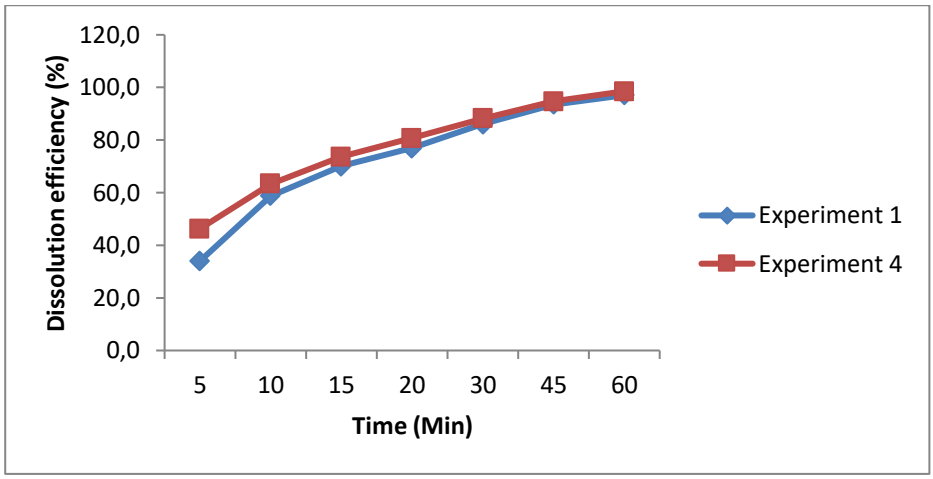


Figure 4.2: Dissolution profiles of Axitinib tablets obtained in pH1.2, 500 mL of dissolution medium and at 50 rpm / 75 rpm

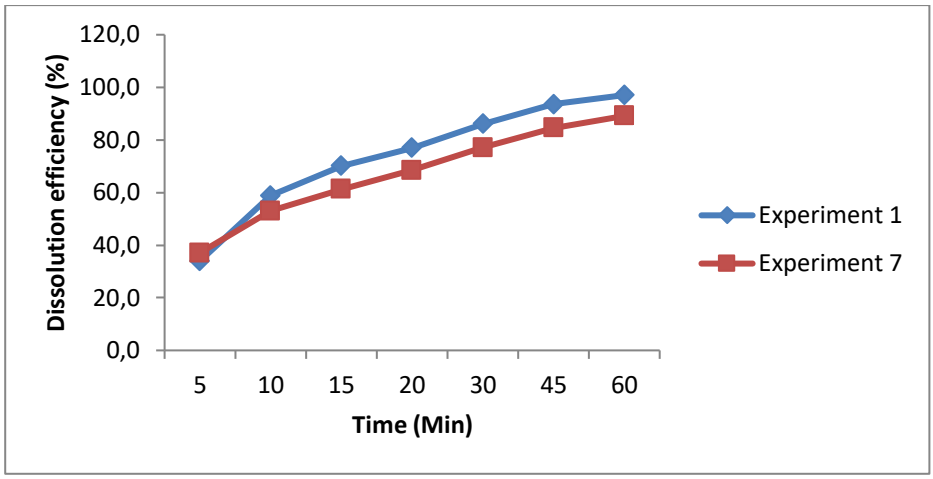


Figure 4.3: Dissolution profiles of Axitinib tablets obtained in pH1.2, 500 mL/ 900 mL of dissolution medium and at 50 rpm

The DE values obtained from each experiment for the original product were tabulated at between Table 4.13 and Table 4.24 as 6 samples depending on time. The values of DE% obtained in the comparison of the dissolution test methods are shown in Figure 4.4, Figure 4.5. and Figure 4.6.

Table 4.13: Results of the dissolution tests for the Experiment 1 (500ml/50rpm/pH1.2)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	33,5	33,9	34,1	34,2	34,0	33,9	33,9
10	48,4	49,4	49,6	48,1	47,7	48,4	48,6
15	59,0	50,6	59,5	58,7	59,9	59,5	57,9
20	65,3	66,7	66,9	66,8	66,9	66,7	66,6
30	76,4	76,5	76,8	76,9	75,5	76,8	76,5
45	**85,8**	**84,9**	**85,9**	**86,3**	**85,6**	**79,4**	**84,6**
60	91,4	91,7	91,4	85,3	85,7	91,6	89,5

Table 4.14: Results of the dissolution tests for the Experiment 2 (500ml/50rpm/pH4.5)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	30,2	31,3	30,6	30,1	34,1	30,2	31,1
10	38,4	38,4	38,4	38,0	38,3	37,8	38,2
15	43,8	43,9	43,6	43,8	43,6	43,8	43,7
20	48,4	48,1	48,3	48,2	47,7	48,2	48,2
30	53,6	54,7	54,8	55,1	54,6	55,1	54,7
45	**63,2**	**63,3**	**63,2**	**62,9**	**63,3**	**62,4**	**63,0**
60	70,0	69,4	70,1	69,8	70,3	70,1	69,9

Table 4.15: Results of the dissolution tests for the Experiment 3 (500ml/50rpm/pH6.8)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	16,5	16,5	16,3	16,5	16,5	16,4	16,5
10	19,4	19,5	20,2	19,6	19,5	19,6	19,6
15	21,5	21,8	21,6	21,9	22,2	21,7	21,8
20	23,5	23,5	23,3	23,1	23,8	24,2	23,6
30	27,3	27,8	27,2	27,4	27,3	27,8	27,5
45	**31,7**	**31,7**	**31,6**	**31,6**	**31,8**	**31,8**	**31,7**
60	34,5	34,4	34,6	34,3	34,4	34,4	34,4

Table 4.16: Results of the dissolution tests for the Experiment 4 (500ml/75rpm/pH1.2)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	41,5	42,0	42,0	41,2	41,6	40,8	41,5
10	57,5	58,5	58,3	58,3	57,9	58,5	58,2
15	67,8	68,3	68,7	68,7	69,1	69,2	68,6
20	76,1	76,0	76,3	76,8	77,0	76,3	76,4
30	85,7	86,4	86,7	86,1	86,4	86,5	86,3
45	**89,3**	**95,3**	**95,6**	**95,5**	**95,4**	**93,2**	**94,1**
60	94,6	101,1	102,1	101,3	100,8	89,0	98,1

Table 4.17: Results of the dissolution tests for the Experiment 5 (500ml/75rpm/pH4.5)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	47,7	47,8	47,5	48,0	47,9	48,1	47,8
10	57,9	58,0	57,6	58,1	57,7	57,3	57,8
15	64,7	65,1	65,3	65,2	65,5	65,0	65,1
20	71,7	71,1	71,4	71,2	71,6	71,8	71,5
30	80,1	79,6	80,6	81,0	80,7	79,8	80,3
45	**89,4**	**89,0**	**89,6**	**89,9**	**89,8**	**89,5**	**89,5**
60	94,8	94,9	94,9	95,1	94,8	95,2	95,0

Table 4.18: Results of the dissolution tests for the Experiment 6 (500ml/75rpm/pH6.8)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	19,2	24,1	25,3	26,1	26,2	26,8	24,6
10	31,2	31,4	30,9	31,4	31,6	31,3	31,3
15	33,8	33,7	33,8	33,8	33,7	33,6	33,8
20	36,4	35,5	35,6	35,5	35,5	35,6	35,7
30	37,5	37,8	37,8	37,8	38,0	37,9	37,8
45	**40,3**	**40,3**	**40,4**	**40,5**	**40,1**	**39,9**	**40,2**
60	42,6	42,7	42,5	42,7	42,0	42,7	42,5

Table 4.19: Results of the dissolution tests for the Experiment 7 (900ml/50rpm/pH1.2)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	36,7	34,3	31,2	36,9	35,9	35,5	35,1
10	56,0	51,6	46,4	55,4	53,0	53,4	52,6
15	67,8	61,7	58,6	66,8	65,0	64,7	64,1
20	74,0	69,0	65,7	74,8	72,4	73,7	71,6
30	85,1	78,5	75,2	84,9	81,9	84,4	81,7
45	**92,8**	**85,9**	**82,3**	**92,2**	**88,8**	**91,8**	**89,0**
60	96,4	88,8	86,6	97,4	93,2	94,9	92,9

Table 4.20: Results of the dissolution tests for the Experiment 8 (900ml/50rpm/pH4.5)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	29,5	37,8	38,5	33,4	35,8	30,5	34,3
10	38,1	46,3	46,2	41,4	44,6	39,5	42,7
15	43,9	51,6	51,9	47,3	50,1	44,8	48,3
20	47,6	55,7	55,9	50,8	54,5	49,5	52,3
30	63,9	61,0	61,8	57,4	59,4	54,1	59,6
45	**58,0**	**66,1**	**67,8**	**62,5**	**64,3**	**60,4**	**63,2**
60	66,5	69,8	66,2	66,0	67,7	65,0	66,9

Table 4.21: Results of the dissolution tests for the Experiment 9 (900ml/50rpm/pH6.8)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	20,0	15,1	19,3	22,6	16,3	17,8	18,5
10	26,0	20,4	24,8	28,8	19,6	22,0	23,6
15	31,3	26,4	29,9	31,3	23,5	29,1	28,6
20	35,1	29,0	34,3	35,3	25,7	32,0	31,9
30	38,0	32,7	37,4	38,0	29,2	36,0	35,2
45	**41,6**	**36,2**	**43,6**	**41,7**	**33,2**	**41,2**	**39,6**
60	44,5	39,1	46,3	44,2	38,6	44,8	42,9

Table 4.22: Results of the dissolution tests for the Experiment 10 (900ml/75rpm/pH1.2)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	64,5	53,4	49,4	64,2	66,6	54,6	58,8
10	67,0	66,8	70,4	73,0	67,6	69,3	69,0
15	68,8	75,0	74,8	73,2	72,2	72,2	72,7
20	78,9	81,6	81,7	79,6	79,4	79,2	80,1
30	88,2	90,9	91,4	89,2	88,6	88,8	89,5
45	**95,6**	**98,3**	**99,2**	**97,1**	**96,5**	**96,4**	**97,2**
60	100,3	102,2	103,2	101,7	100,9	101,2	101,6

Table 4.23: Results of the dissolution tests for the Experiment 11 (900ml/75rpm/pH4.5)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	55,2	52,3	55,4	56,7	54,8	56,5	55,2
10	63,7	62,8	64,2	66,1	63,9	65,7	64,4
15	70,5	69,7	71,1	72,2	71,2	72,7	71,3
20	77,0	74,8	74,9	77,6	75,0	77,3	76,1
30	89,9	89,3	91,0	91,7	93,5	93,3	91,4
45	**95,4**	**94,7**	**94,6**	**96,3**	**95,9**	**96,5**	**95,6**
60	98,5	97,4	98,5	106,9	99,6	100,6	100,3

Table 4.24: Results of the dissolution tests for the Experiment 12 (900ml/75rpm/pH6.8)

Time (Min)	Sample-1 (DE%)	Sample-2 (DE%)	Sample-3 (DE%)	Sample-4 (DE%)	Sample-5 (DE%)	Sample-6 (DE%)	Average (DE%)
5	29,6	31,1	30,6	30,8	31,6	30,6	30,7
10	32,8	33,7	33,6	33,8	33,5	33,1	33,4
15	36,0	37,0	36,7	36,7	36,9	36,5	36,6
20	37,1	39,5	39,1	39,5	39,5	38,6	38,9
30	42,7	43,6	43,2	43,0	43,4	42,5	43,1
45	**46,8**	**47,9**	**47,6**	**47,3**	**46,9**	**47,2**	**47,3**
60	51,0	52,1	51,2	51,3	50,7	50,6	51,1

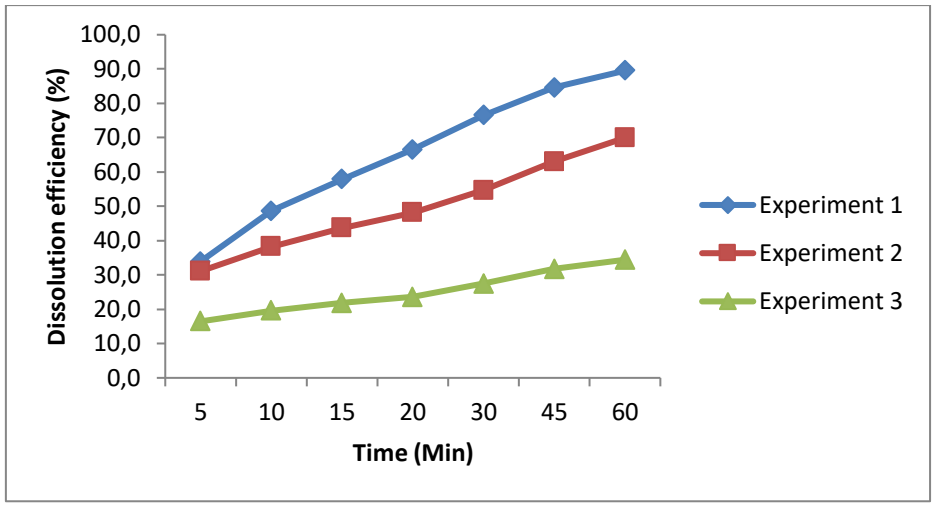


Figure 4.4: Dissolution profiles of Axitinib tablets obtained in pH1.2, pH4.5 and pH6.8 at 50 rpm and 500 mL of dissolution medium

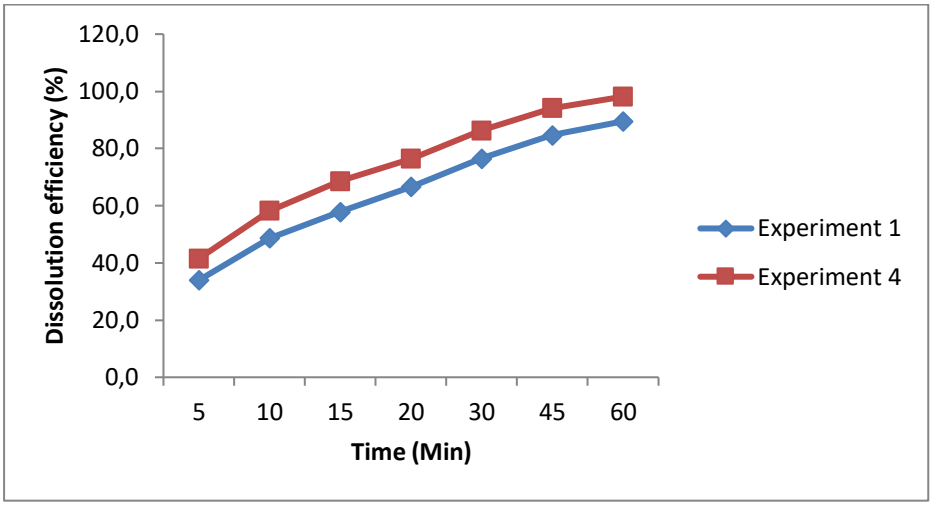


Figure 4.5: Dissolution profiles of Axitinib tablets obtained in pH1.2, 500 mL of dissolution medium and at 50 rpm / 75 rpm

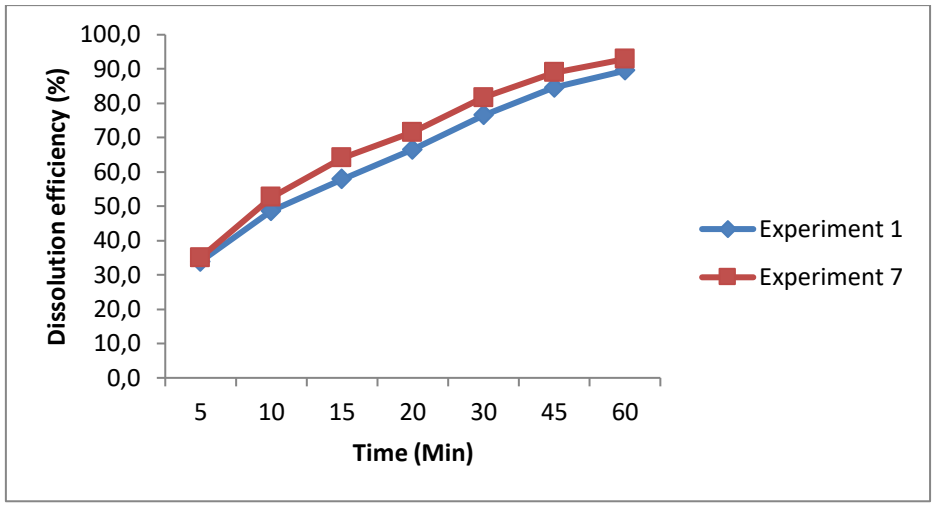


Figure 4.6: Dissolution profiles of Axitinib tablets obtained in pH1.2, 500 mL/ 900 mL of dissolution medium and at 50 rpm

The similarity factors (f2) between the generic product and the original product are given in Table 4.25. to Table 4.37. And the results for each experimental setup are shown in Figure 4.7. to Figure 4.18.

Table 4.25: Results of the Similarity factor (f2) for Experiment 1

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	51,7
5	33,9	34,0	
10	48,6	58,8	
15	57,9	70,1	
20	66,6	76,9	
30	76,5	86,1	
45	84,6	93,6	
60	89,5	97,0	

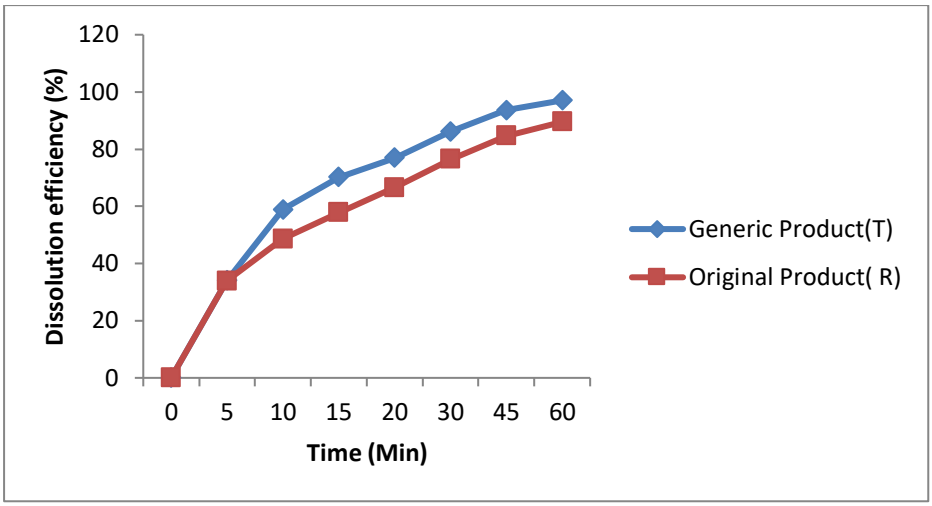


Figure 4.7: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 1

Table 4.26: Results of the Similarity factor (f2) for Experiment 2

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	80,7
5	31,1	31,7	
10	38,2	40,2	
15	43,7	46,1	
20	48,2	51,7	
30	54,7	57,7	
45	63,0	64,1	
60	69,9	68,8	

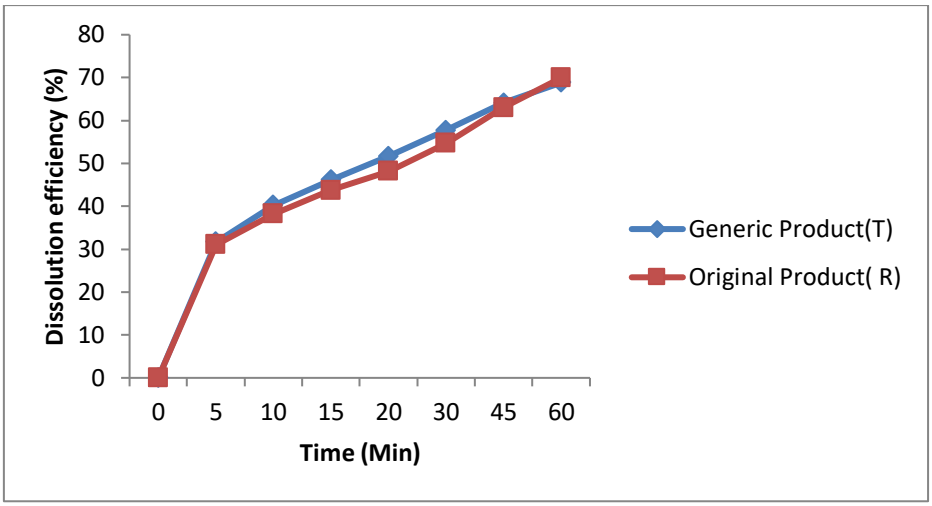


Figure 4.8. Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 2

Table 4.27: Results of the Similarity factor (f2) for Experiment 3

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	75,9
5	16,5	11,7	
10	19,6	17,3	
15	21,8	21,0	
20	23,6	23,1	
30	27,5	29,1	
45	31,7	36,2	
60	34,4	36,8	

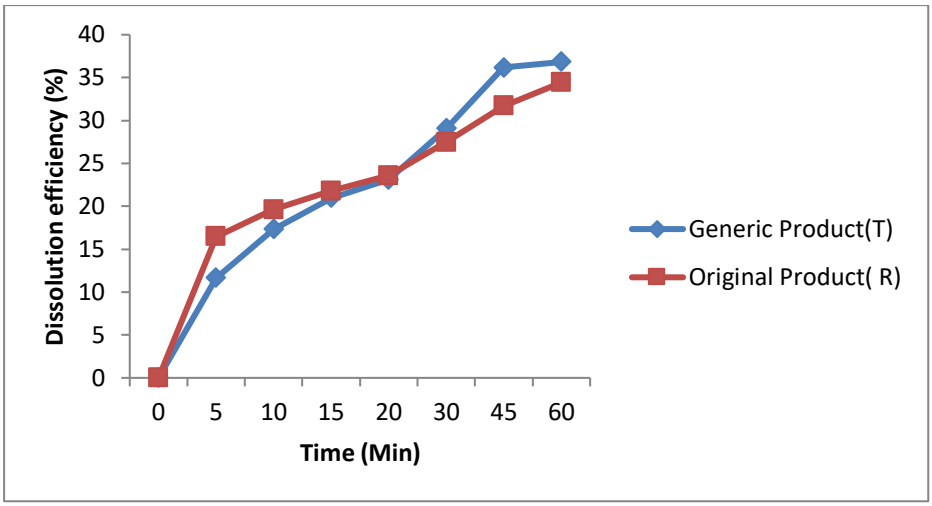


Figure 4.9: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 3

Table 4.28: Results of the Similarity factor (f2) for Experiment 4

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	70,5
5	41,5	46,3	
10	58,2	63,4	
15	68,6	73,7	
20	76,4	80,7	
30	86,3	88,3	
45	94,1	94,7	
60	98,1	98,5	

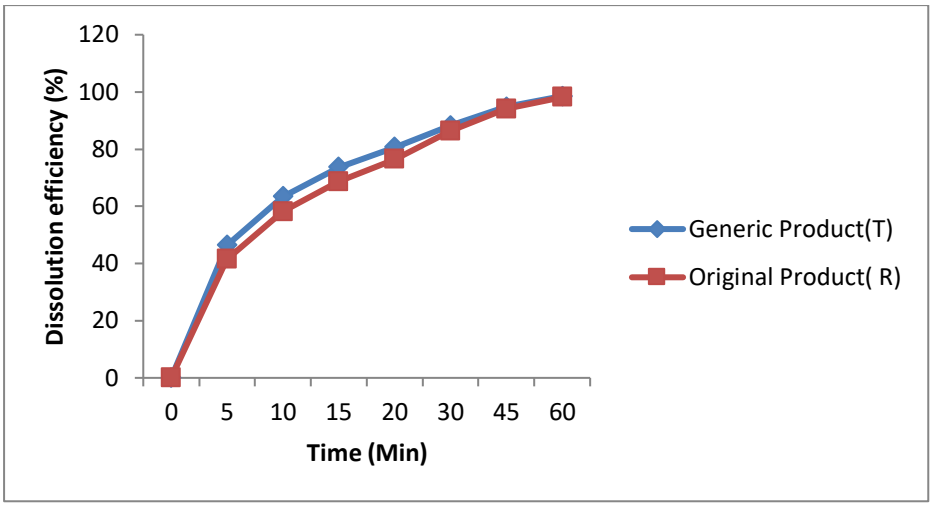


Figure 4.10: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 4

Table 4.29: Results of the Similarity factor (f2) for Experiment 5

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	69,0
5	47,8	39,0	
10	57,8	52,5	
15	65,1	62,9	
20	71,5	69,7	
30	80,3	79,6	
45	89,5	88,6	
60	95,0	94,9	

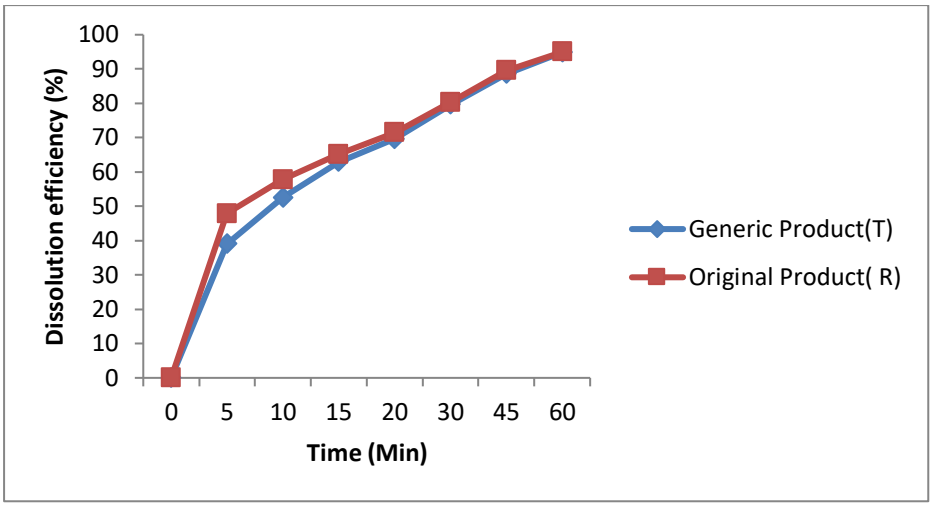


Figure 4.11: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 5

Table 4.30: Results of the Similarity factor (f2) for Experiment 6

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	52,0
5	24,6	33,1	
10	31,3	33,9	
15	33,8	37,7	
20	35,7	41,1	
30	37,8	46,5	
45	40,2	52,8	
60	42,5	57,2	

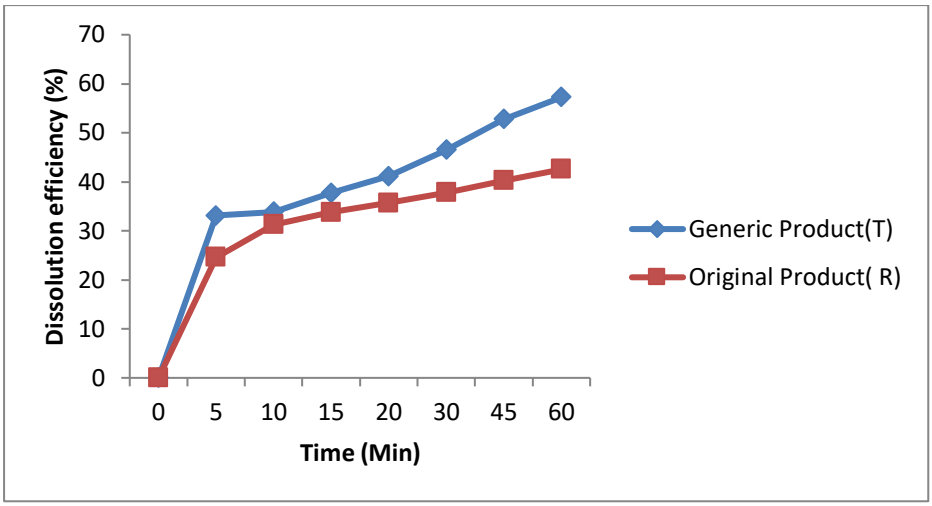


Figure 4.12: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 6

Table 4.31: Results of the Similarity factor (f2) for Experiment 7

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	73,1
5	35,1	37,1	
10	52,6	53,0	
15	64,1	61,2	
20	71,6	68,5	
30	81,7	77,1	
45	89,0	84,6	
60	92,9	89,1	

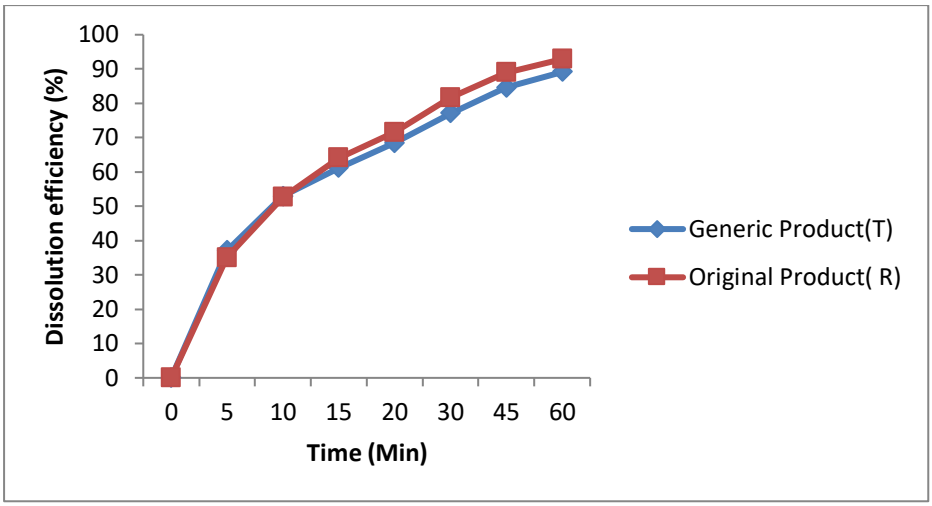


Figure 4.13: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 7

Table 4.32: Results of the Similarity factor (f2) for Experiment 8

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	51,9
5	34,3	29,9	
10	42,7	45,2	
15	48,3	54,4	
20	52,3	60,6	
30	59,6	68,4	
45	63,2	75,3	
60	66,9	81,9	

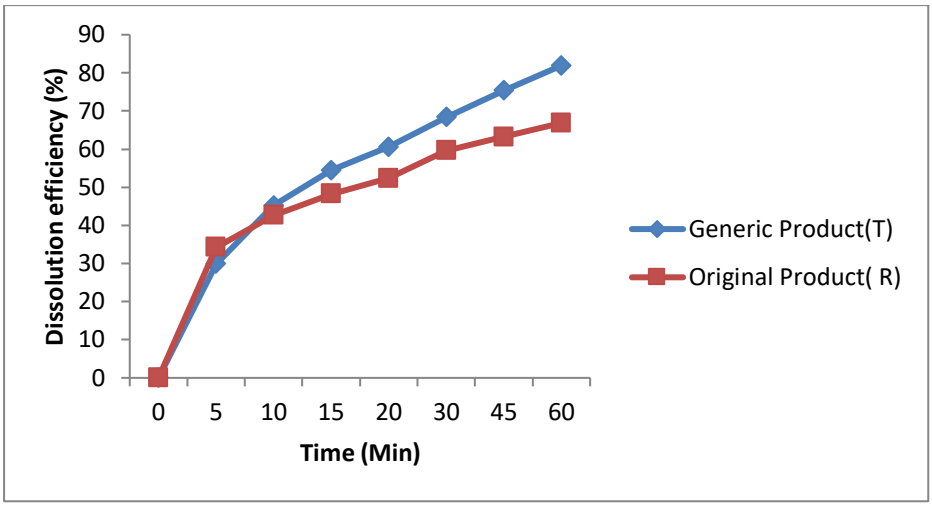


Figure 4.14: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 8

Table 4.33: Results of the Similarity factor (f2) for Experiment 9

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	48,3
5	18,5	3,4	
10	23,6	12,7	
15	28,6	17,2	
20	31,9	20,4	
30	35,2	25,4	
45	39,6	31,3	
60	42,9	36,5	

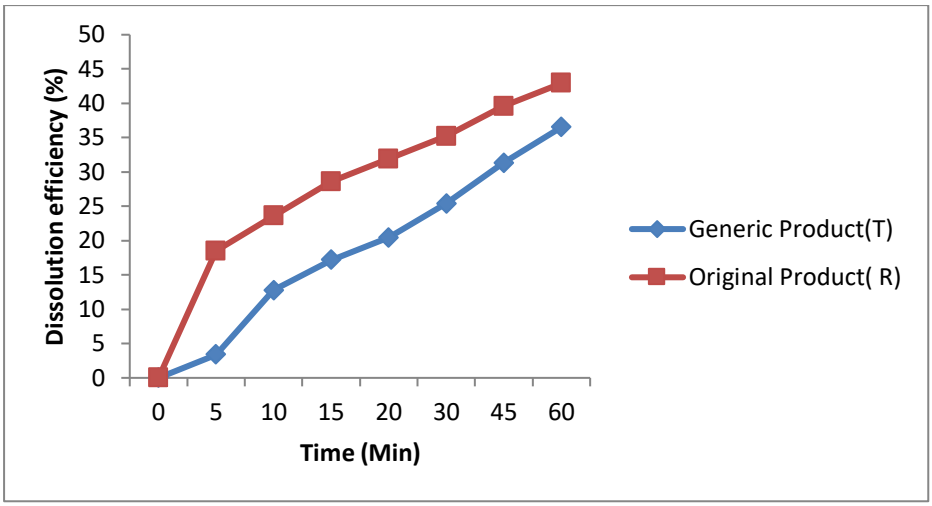


Figure 4.15: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 9

Table 4.34: Results of the Similarity factor (f2) for Experiment 10

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	54,9
5	58,8	42,8	
10	69,0	60,3	
15	72,7	70,4	
20	80,1	77,2	
30	89,5	86,4	
45	97,2	91,8	
60	101,6	94,1	

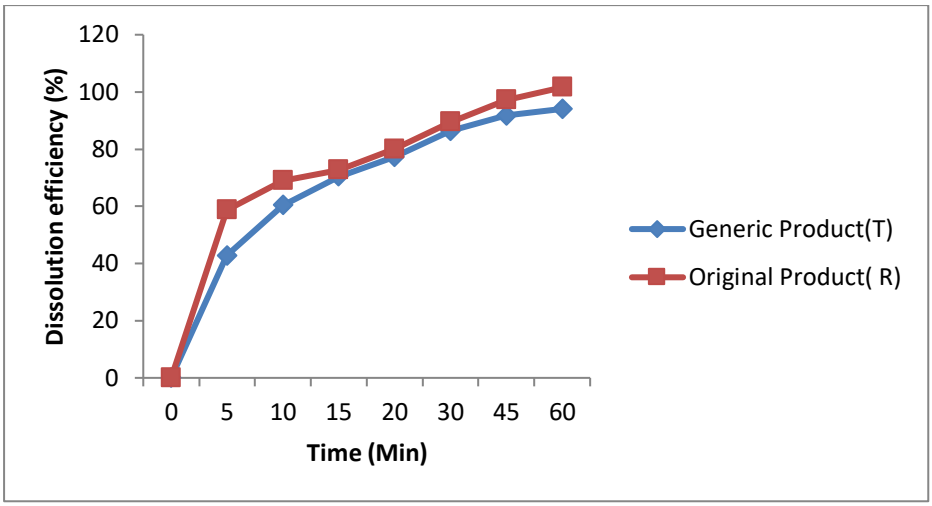


Figure 4.16: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 10

Table 4.35: Results of the Similarity factor (f2) for Experiment 11

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	54,7
5	55,2	40,2	
10	64,4	55,8	
15	71,3	66,2	
20	76,1	73,5	
30	91,4	82,5	
45	95,6	91,1	
60	100,3	96,2	

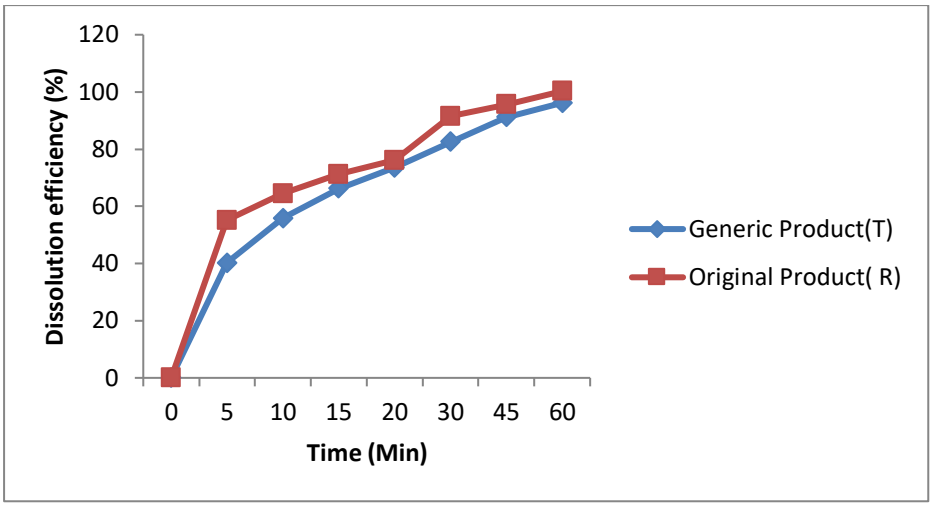


Figure 4.17: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 11

Table 4.36: Results of the Similarity factor (f2) for Experiment 12

Time (Min)	**Original Product(R) (DE%)**	**Generic Product(T) (DE%)**	**Similarity factor (f2)**
0	0	0	41,2
5	30,7	0,4	
10	33,4	12,7	
15	36,6	24,6	
20	38,9	32,4	
30	43,1	39,6	
45	47,3	48,4	
60	51,1	54,9	

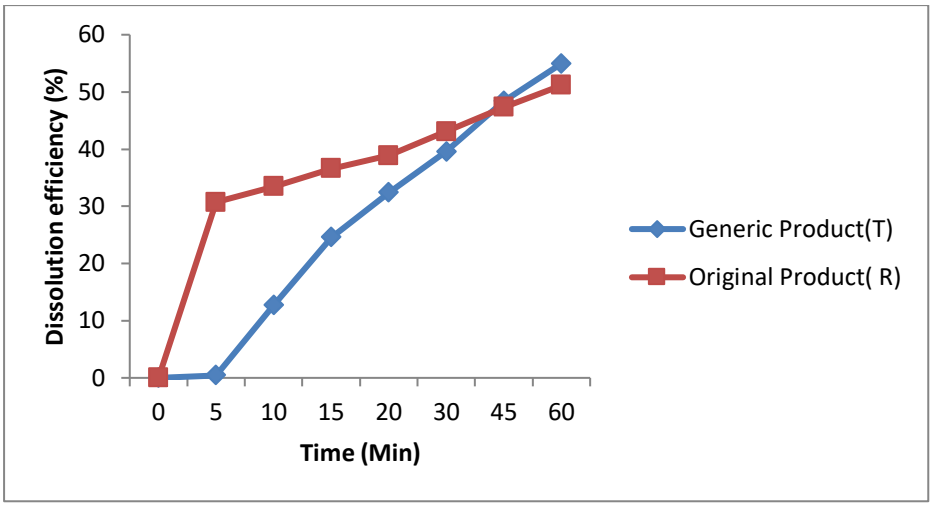


Figure 4.18: Comparison between the dissolved amount of the active pharmaceutical ingredient in terms of percentage for Experiment 12

Summary results of experiment presented in Table 4.37.

Table 4.37. Results of experiment for DE% and f2

Experiment Number	Generic Product(T) (DE%) at 45th min.	f2 (Similarity factor)
Experiment 1	93,6	51,7
Experiment 2	64,1	80,7
Experiment 3	36,2	75,9
Experiment 4	94,7	70,5
Experiment 5	88,6	69,0
Experiment 6	52,8	52,0
Experiment 7	84,6	73,1
Experiment 8	75,3	51,9
Experiment 9	31,3	48,3
Experiment 10	91,8	54,9
Experiment 11	91,1	54,7
Experiment 12	48,4	41,2

4.2. Statistical Results

Statistical analyzes were made with Minitab 19 program. Table 4.38 presents the statistical analysis data made with ANOVA. Drug efficacy results were compared. The results obtained with ANOVA show that there are significant effects for the main factors as well as some interactions. Finally, the results were modeled in Excel according to the parameters affecting the dissolution efficiency. The regression models created for both the original and the generic product are seen in Equations 4.1 and 4.2.

Table 4.38: ANOVA one way for the exploratory study of the dissolution parameter for generic product

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	6071.11	674.57	48.32	0.020
Linear	4	5828.75	1457.19	104.38	0.010
Dissolution medium volume	1	4.69	4.69	0.34	0.621
Stirring speed	1	564.44	564.44	40.43	0.024
Dissolution medium	2	5259.63	2629.81	188.38	0.005
2-Way Interactions	5	242.35	48.47	3.47	0.239
Dissolution medium volume*Stirring speed	1	0.37	0.37	0.03	0.886
Dissolution medium volume*Dissolution medium	2	99.26	49.63	3.56	0.220
Stirring speed*Dissolution medium	2	142.73	71.36	5.11	0.164
Error	2	27.92	13.96		
Total	11	6099.03			

Table 4.39: ANOVA one way for the exploratory study of the dissolution parameter for original product

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	6601.34	733.48	1421.94	0.001
Linear	4	6345.35	1586.34	3075.29	0.000
Dissolution medium volume	1	49.21	49.21	95.39	0.010
Stirring speed	1	649.74	649.74	1259.59	0.001
Dissolution medium	2	5646.40	2823.20	5473.09	0.000
2-Way Interactions	5	255.99	51.20	99.25	0.010
Dissolution medium volume*Stirring speed	1	0.04	0.04	0.08	0.805
Dissolution medium volume*Dissolution medium	2	21.91	10.96	21.24	0.045
Stirring speed*Dissolution medium	2	234.03	117.02	226.85	0.004
Error	2	1.03	0.52		
Total	11	6602.37			

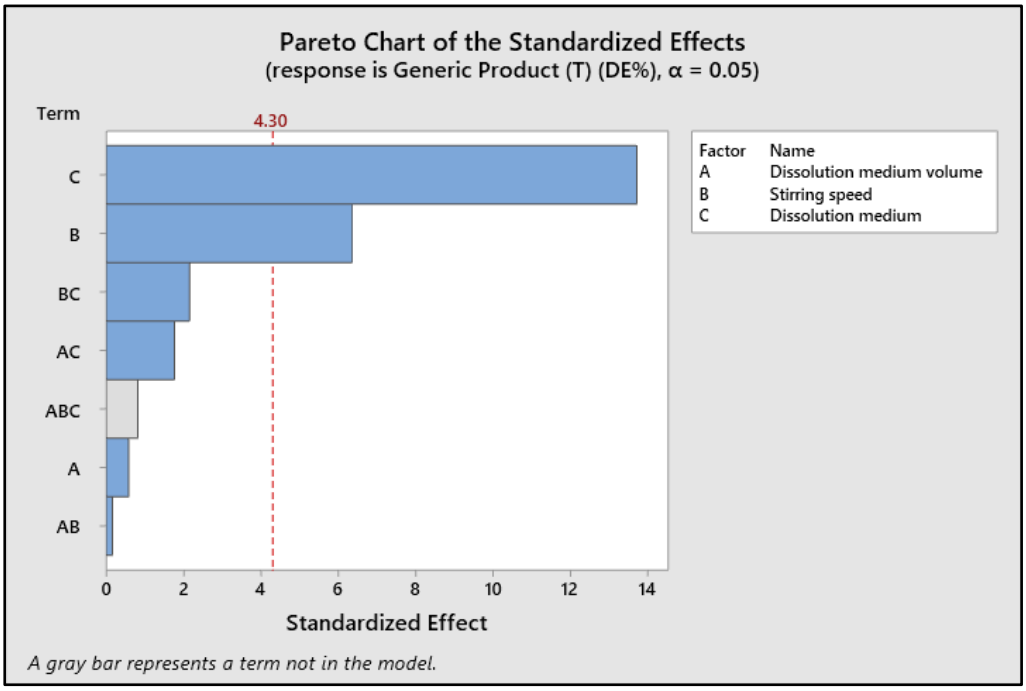


Figure 4.19: Pareto diagram for the analysis of standardized effects for generic product

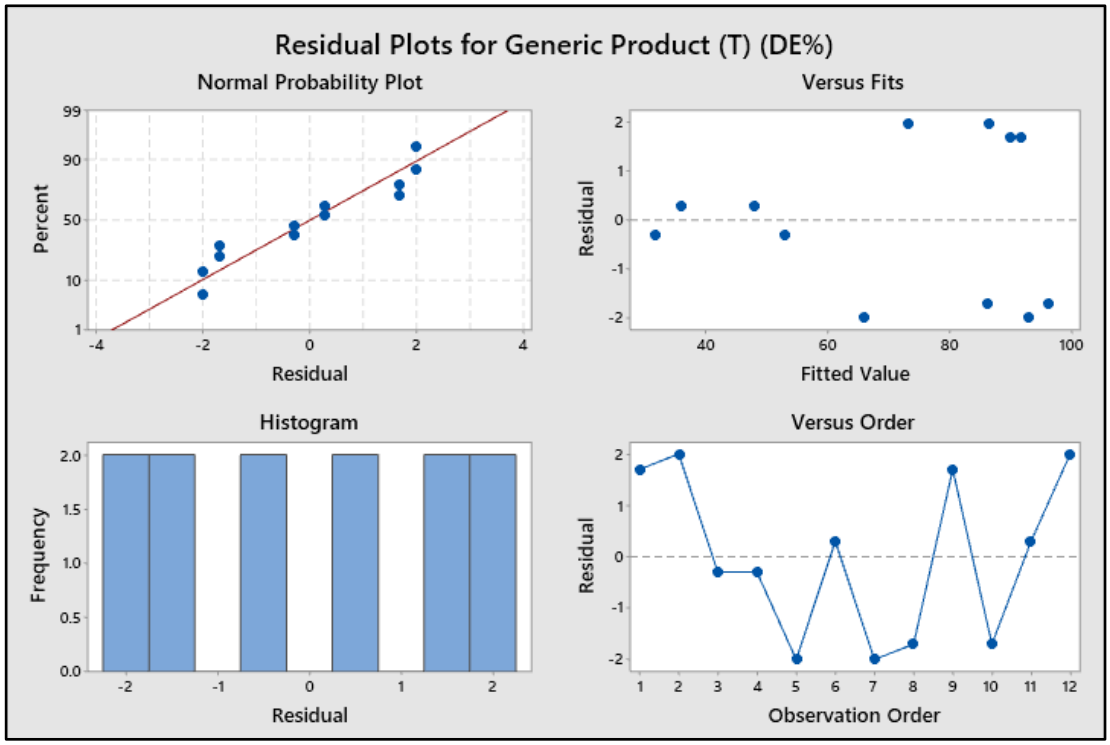


Figure 4.20**:** Residual plots for the DoE for generic product

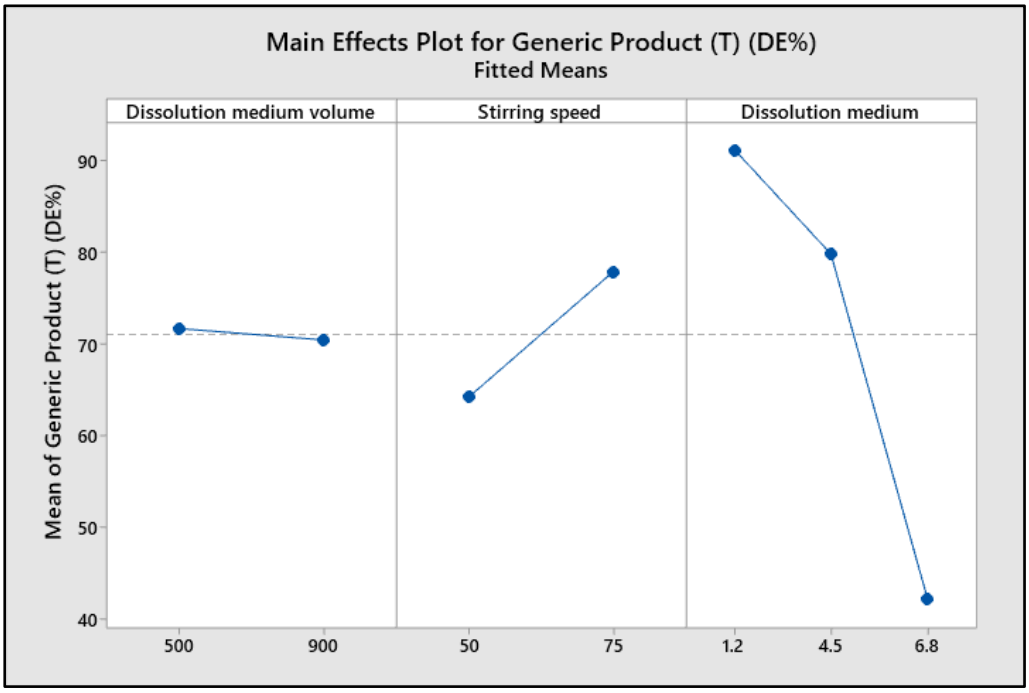


Figure 4.21: Main effects diagram for generic product

Table 4.40: Response Optimization: Generic Product (T) (DE%)

Solution

Solution	Dissolution medium volume	Stirring speed	Dissolution medium	Generic Product (T) (DE%) Fit	Composite Desirability
1	500	75	1.2	96.4	1

Multiple Response Prediction

Variable	Setting
Dissolution medium volume	500
Stirring speed	75
Dissolution medium	1.2

Response	Fit	SE Fit	95% CI	95% PI
Generic Product (T) (DE%)	96.40	3.41	(81.72, 111.08)	(74.63, 118.17)

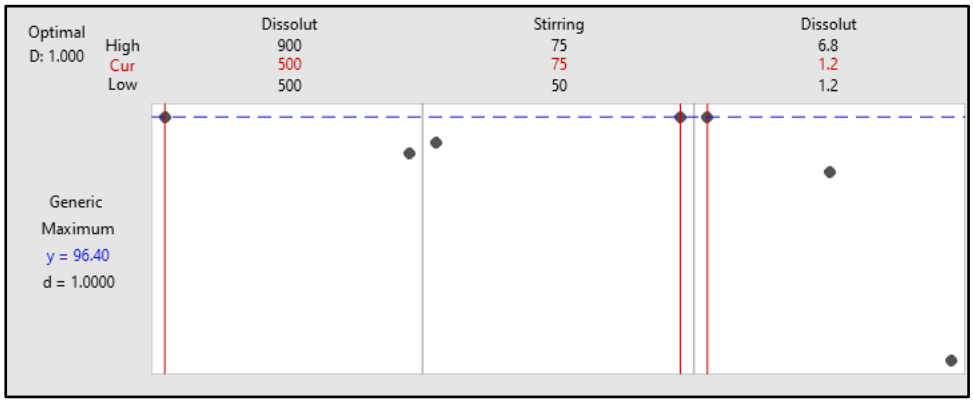


Figure 4.22: Response Optimization: Generic Product (T) (DE%)

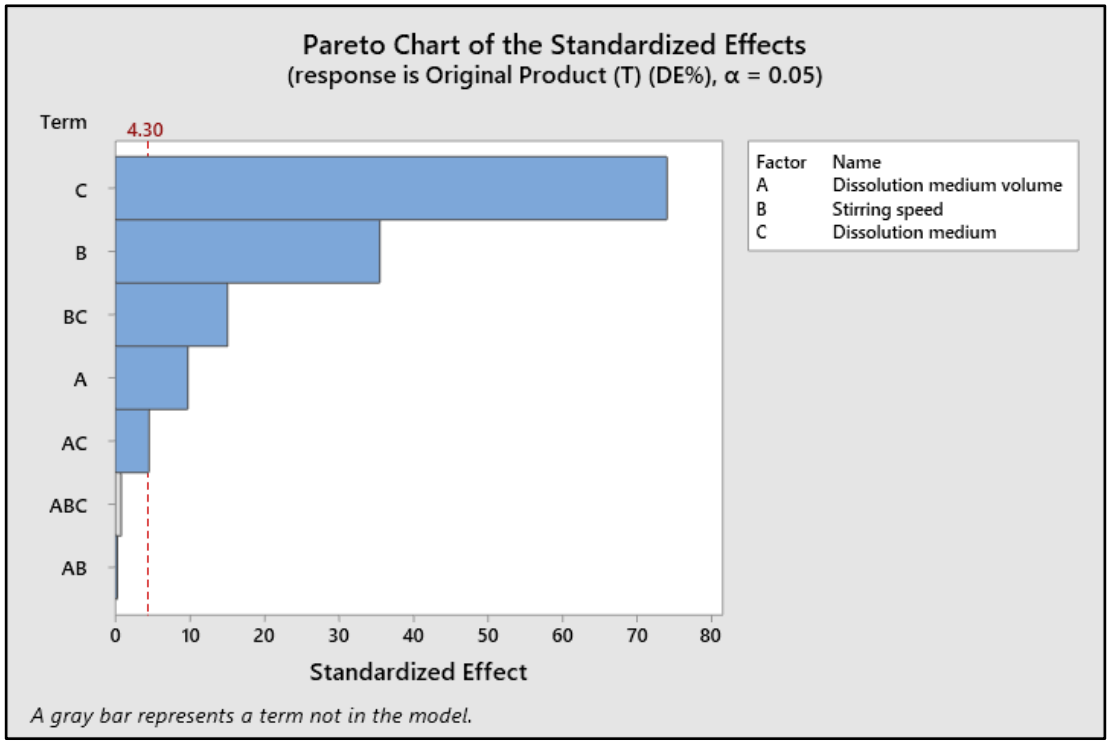


Figure 4.23: Pareto diagram for the analysis of standardized effects for original product

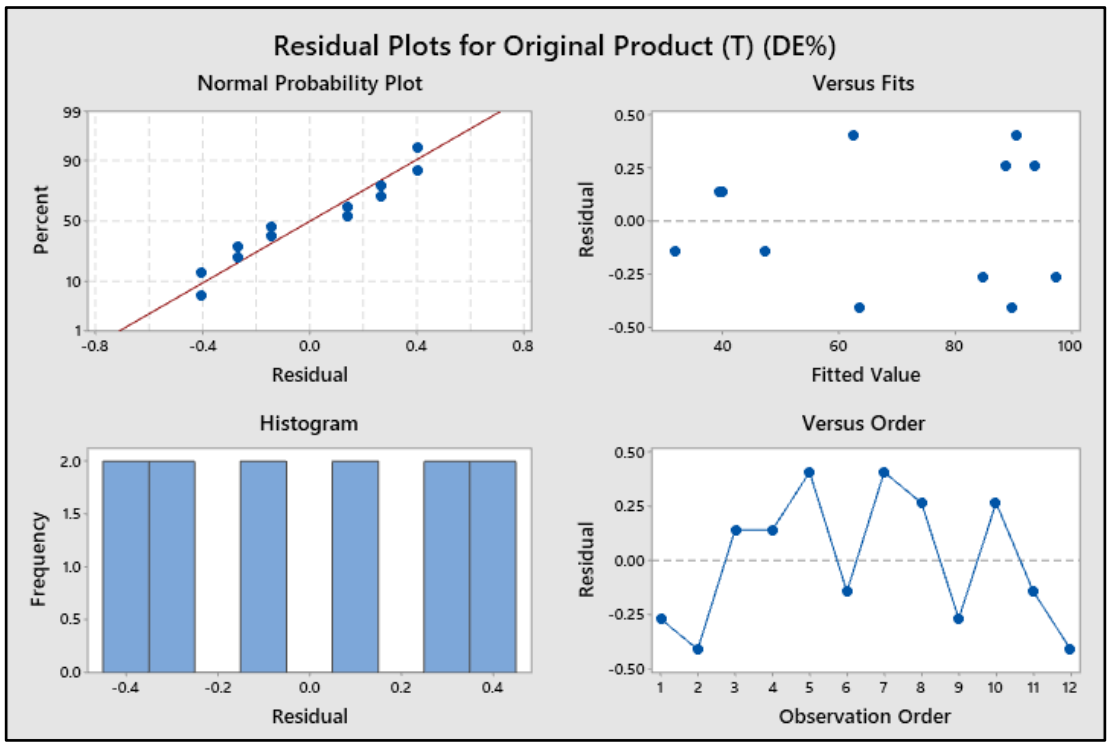


Figure 4.24: Residual plots for the DoE for original product

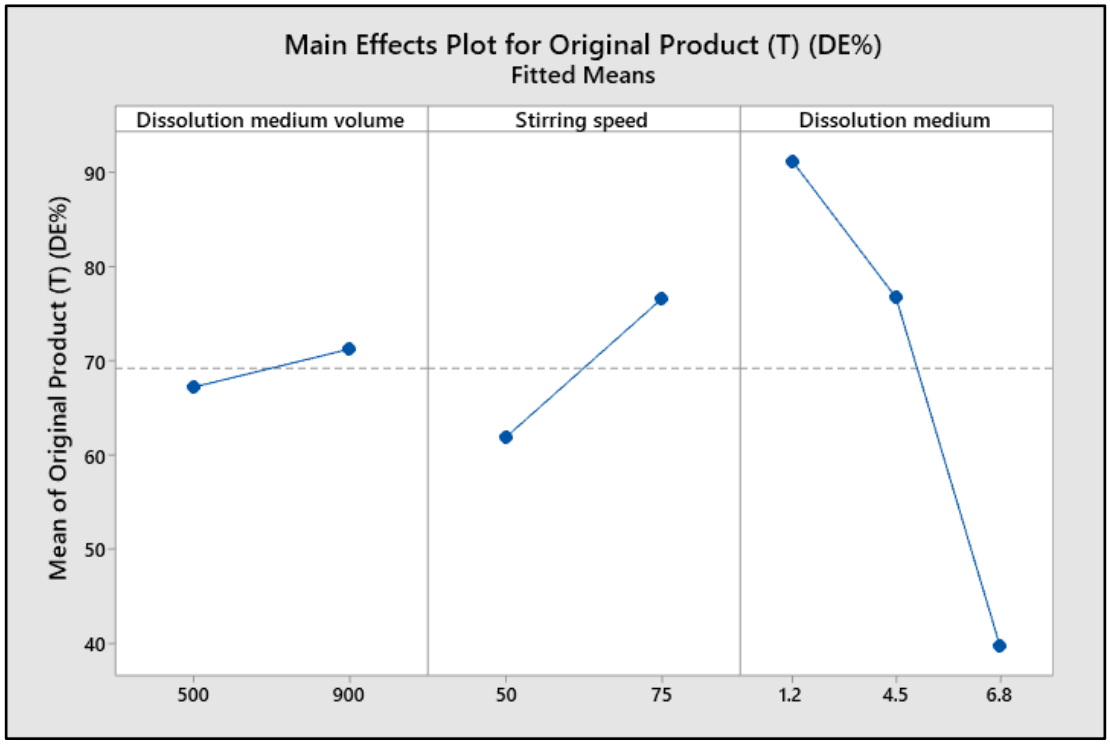


Figure 4.25: Main effects diagram for original product

Table 4.41. Response Optimization: Original Product (T) (DE%)

Solution

Solution	Dissolution medium volume	Stirring speed	Dissolution medium	Original Product (T) (DE%) Fit	Composite Desirability
1	500	75	1.2	93.8333	0.981569

Multiple Response Prediction

Variable	Setting
Dissolution medium volume	500
Stirring speed	75
Dissolution medium	1.2

Response	Fit	SE Fit	95% CI	95% PI
Original Product (T) (DE%)	93.833	0.656	(91.012, 96.654)	(89.649, 98.018)

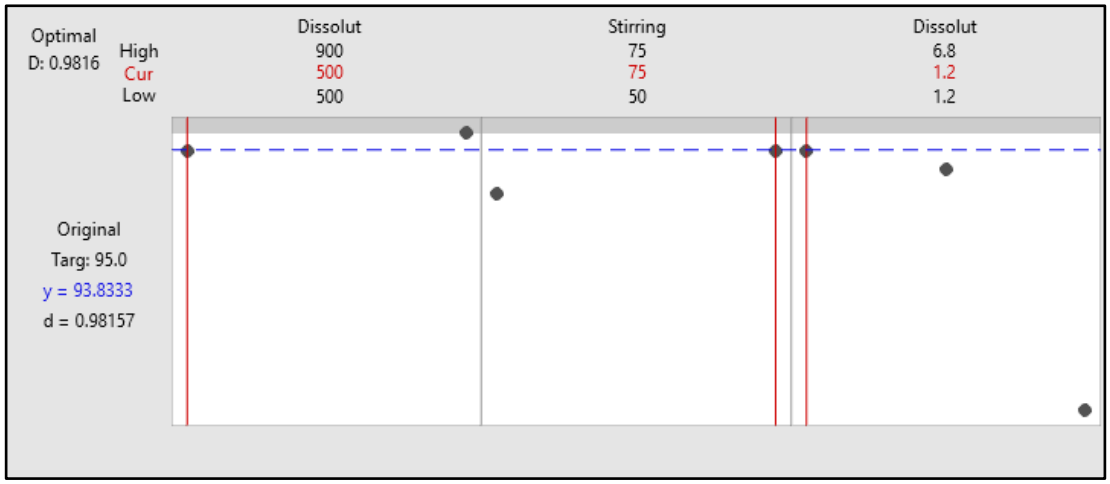


Figure 4.26: Response Optimization: Original Product (T) (DE%)

In the below, regression model was given for generic and original product.

For generic product;

$$y = 73{,}864228 + 0{,}5486667 * x_2 - 8{,}3824148 * x_3 \quad (4.1)$$

For original product;

$$y = 62{,}2780956 + 0{,}010124 * x_1 + 0{,}58866667 * x_2 - 8{,}867743 * x_3 \quad (4.2)$$

5. DISCUSSION

To evaluate the significance that each parameter/factor has on the dissolution of Axitinib and to determine the optimum conditions for inclusion of the method in routine laboratorial tests, a 3 × 22 factorial design with mixed levels containing factors at three (–1, 0, +1 for the dissolution medium) and two (–1 and +1 for the factor dissolution volume and stirring speed) levels was randomly conducted in a total of 12 experiments with six replicates in each experimental level.

The analysis results were analyzed in two parts as experimental and statistical. When the experimental part is examined, we can interpret the analysis results as follows; For both the generic product and the original product, the pH of the dissolution medium is the parameter with the greatest influence on the solubility of the drug. Dissolution volume and stirring speed did not make an effective difference on the results. We can see it graphically in Figures 4, 5, and 6. For the results of experiments 2, 3, 6, 8, 9 and 12, the DE % value remained below 80% and these experiments were deemed invalid. It is seen that the most suitable test for DE% and f2 factor is Experiment 4.

ANOVA analysis results were obtained by applying the proposed experimental design. Here, the p value is important and whether the parameter is effective or not is determined by the p value. If the p value is less than 0.05, we can interpret that parameter as having an effect on the analysis results. For generic product, when we look at the p value in the Table 4. 38 for the dissolution medium volumes used in the test, we can see that the dissolution medium volume parameter does not make a significant difference on the results, since the p value is greater than the significance level (0.621> 0.05). However, it was found that there were differences for the pH of the medium and the mixing speed because the p-value corresponds to 0.024 for the mixing speed and 0.005 for the dissolution medium, which is less than the determined significance level. In the same way, this table was created for the original drug. However, it is seen that all of the parameters in the original drug are effective, albeit at different levels (Table 4.39). We can interpret the fact that different parameters are effective in two products with the same active ingredient, as it is due to the way the drug is produced.

The Pareto diagram allows to determine the magnitude and significance of standardized effects, which are the changes observed in the analysis results due to the level change in the parameters. If we examine Figure 4.19, the corresponding bar for the dissolution medium and mixing speed manages to exceed the reference line for generic product. Therefore, only this factor is statistically significant at the level of $\alpha = 0.05$, according to the conditions of the established model. But when we look at Figure 4.23, we see that the three parameters(dissolution medium, stirring speed and dissolution medium volume) and the parameters in the binary interactions (stirring speed*dissolution medium and dissolution medium volume*dissolution medium) cross the reference line. We see that the above inference is supported by the Pareto diagram.

Figure 4.21 and Figure 4.25 are a graphical representation of the main effects for generic and original product. While you can see the levels on the horizontal axis for each parameter examined, there is the average of the responses observed for each level on the vertical axis. In this case, the main influence of the pH of the medium is greater than the volume of the dissolution medium and the mixing speed, since the difference between the averages of its three levels is greater.

Finally, as a requirement for any analysis of variance, it is necessary to do a verification of the assumptions of normality, equality of variances and independence of residuals. Figure 4.20 and Figure 4.24 is representative of a sample of residues that have a normal distribution. Also, this figure allows to check the independence of the residuals, since the represented points do not follow a specific pattern and also allows satisfying the constant variance assumption, because the points are distributed in a random way on the horizontal band.

When the experimental data (Table 37) and statistical data(Table 4.40 and Table 4.41) are examined together, we see that the most suitable dissolution method parameters that meet our criteria are as follows;

Table 5.1: Optimal dissolution method parameters

Experiment	**Dissolution medium volume, x_1**	**Stirring speed (rpm), x_2**	**Dissolution medium, x_3**
4	500 ml	75 rpm	pH 1.2

The results obtained according to the above parameters are listed below.

Table 5.2: Results of analysis for optimal method parameters

Experiment Number	**Generic Product (T) (DE%) at 45th min.** ***(>80.0%)***	**f2 (Similarity factor)** ***(>50)***
Experiment 4	94,7	70,5

Finally, a regression model was created using excel (Experiment 4-1 and Experiment 4-2). The model equations we have created will guide us in future studies. Based on these models, we can find out how we will get results in the experimental design we will create.

REFERENCES

[1] Malerba F., Orsenigo L., (2015), The evolution of the pharmaceutical industry, Business History.

[2] Mahato R.I., Narang A.S., (2012), "Pharmaceutical Dosage Forms an Drug Delivery", 2nd Edition, Pharmacy Education.

[3] Silva B.C., Campos L.M.M., Nunan E.A., Soares C.D.V., Araujo-Alves B.L., Cesar I.C., Pianetti G.A., (2004), Optimization and statistical evaluation of dissolution tests for indinavir sulfate capsules, IL FARMACO, 59 921–927.

[4] Polonini H.C., Oliveira M.A.L., Ferreira A.O., Raposo N.R.D., Grossic L.N., Brandã M.A.F., (2011), Optimization of a New Dissolution Test for Oxcarbazepine Capsules using Mixed-Level Factorial Design, J. Braz. Chem. Soc., Vol. 22, No. 7, 1263-1270.

[5] Henríquez L.C., Zúñiga R.V., Berrocal G.C., Redondo G.M., Guzmán B.C., Viales E. B., (2019), Development of immediate release Rupatadine fumarate 10 mg tablets: A Quality by Design (QbD) approach, Drug Development and Industrial Pharmacy, 1520-5762.

[6] Keating G.M., (2015), Axitinib: A Review in Advanced Renal Cell Carcinoma, Adıs Drug Evaluatıon.

[7] Chen Y., Tortorici M.A., Garrett M., Hee B., Klamerus K.J., Pithavala Y.K., (2013), Clinical Pharmacology of Axitinib, Clin Pharmacokinet.

[8] Wang Q., Fotaki N., Mao Y., (2009), Biorelevant Dissolution: Methodology and Application in Drug Development, Dissolution Technologies.

[9] Lue B.-M., Nielsen, F.S., Magnussen, T., Schou, H.M., Kristensen, K., Jacobsen, L.O., Müllertz, A. 2008. Using biorelevant dissolution to obtain IVIVC of solid dosage forms containing a poorly soluble model compound, European Jouranl of Pharmaceutics and Biopharmaceutics, 69, 648-657.

[10] Siepmann J., Siepmann F.,(2013), Mathematical modeling of drug dissolution, International Journal of Pharmaceutics, 453 12– 24.

[11] Uddin R., Saffoon N., Sutradhar K.B., (2011), Dissolution and Dissolution Apparatus: A Review, International Journal of Current Biomedical and Pharmaceutical Research, 1(4): 201 -207.

[12] Ghayas S., Sheraz M.A., Anjum F., Baıg M.T., (2013), Factors Influencıng The Dıssolutıon Testıng Of Drugs, Pak. J. Health Research, 1(1): 01-11.

[13] Kincl M., Turk S., Vrecer F., (2005), Application of experimental design methodology in development and optimization of drug release method, International Journal of Pharmaceutics 291 (2005) 39–49.

[14] Galia, E., Nicolaides, E., Horter, D., Lobenberg, R., Reppas, C., Dressman, J.B., 1998. Evaluation of various dissolution media for predicting in vivo performance of class I and class II drugs. Pharmaceutical Research, 15, 698–705.

[15] Aungst B.J., (2017), Optimizing oral bioavailability in drug discovery: an overview of design and testing strategies and formulation options, J. Pharm. Sci, 106 (4), 921-929.

[16] Wagner J.G., (1969), Interpretation of percent dissolved-time plots derived from in vitro testing of conventional tablets and capsules, J. Pharm. Sc,. 58 (10), 1253-1257.

[17] Raman N.V.V.S.S., Mallu U.R., Bapatu H.R., (2015), Analytical Quality by Design Approach to Test Method Development and Validation in Drug Substance Manufacturing, Journal of Chemistry, Volume 2015.

[18] Lan Z, Shirui M., (2017), Application of quality by design in the current drug development, Asian J Pharm., 12(1):1-8.

[19] Tome T., Zigart N., Casar Z., Obreza A., (2019), Development and Optimization of Liquid Chromatography Analytical Methods by Using AQbD Principles: Overview and Recent Advances, Organic Process Research & Development.

[20] Lawrence X.Y., Amidon G., Khan M.A., Hoag S.W., Polli J., Raju G., (2014), Understanding pharmaceutical quality by design, AAPS Journal, 16 (4), 771-783.

[21] Monks, K., Molnar, I., Rieger, H. J., Bogati, B., Szabo, E., (2012), Quality by Design: Multidimensional Exploration of the Design Space in High Performance Liquid Chromatography Method Development for Better Robustness Before Validation. J. Chromatogr. A, 1232, 218–230.

[22] Fukuda I.M., Pinto C.F.F., Moreira C.S., Saviano A.M., LourençoF.R., (2018), Design of Experiments (DoE) applied to Pharmaceutical and Analytical Quality by Design (QbD), Brazilian Journal of Pharmaceutical Sciences, 54.

[23] Kolla S.B., Vallabhaneni M.R., Puttagunta S.B., Venkata M.S., (2019), Design of Experiments Approach to Discriminatory Dissolution Method Development of Poorly Soluble Drug in Immediate Release Dosage Form, Indian Journal of Pharmaceutical Education and Research, Vol 53.

[24] Peraman R., Bhadraya K., Reddy Y.P., (2015), Analytical Quality by Design: A Tool for Regulatory Flexibility and Robust Analytics, International Journal of Analytical Chemistry.

[25] Yu L.X., Amidon G., Khan M.A., Hoag S.W., Polli J., Raju G. K., Woodcock J., (2014), Understanding Pharmaceutical Quality by Design, American Association of Pharmaceutical Scientists, Vol. 16.

CURRICULA VITAE

Hülya Özer successfully completed the Department of Chemical Engineering, Faculty of Engineering, Ege University, which she started in 2009 and ended in 2014. She started her master's education at Gebze Technical University, graduate school of natural and applied sciences, Department of Chemical Engineering in the spring semester of 2019. She worked as a Stability Analyst in the Quality Control department at Mustafa Nevzat Pharmaceuticals between May 2015 and November 2017, worked as an Analytical Specialist in the R&D and Technology Transfer department at Onko Koçsel Pharmaceuticals between November 2017 and January 2021, since February 2021 she works as an Regulatorry Affairs Specialist in the department.