INTRODUCTION
**Introduction**

From the times immemorial, drugs have been an inseparable part of mankind’s history since they fulfill one of our most basic necessities. To administer these drugs in an appealing and palatable form and in the required amount and rate, they have to be developed into an acceptable dosage form. Thus the concept of formulation development evolved to alleviate sufferings of the mankind.

1.1 General Overview

Mycophenolate mofetil (Fig. 1) (MMF) 2-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate [Tripodi et al., 2001] is a potent, selective, uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Because T- and B- lymphocytes are critically dependent for their proliferation on novo synthesis of purines, whereas other cell types can utilize salvage pathways, it has potent cytostatic effects on lymphocytes and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection [Bimbaum et al., 2009; Haentzchel et al., 2008]. MMF did not inhibit early events in the activation of human peripheral blood mononuclear cells, such as the production of interleukin-1 (IL-1) and interleukin-2 (IL-2), but did block the coupling of these events to DNA synthesis and proliferation. Due to clinical advantages of MMF, there has been increase in number of MMF formulations (e.g. Cellcept 500 tablets and Cellcept 250 capsules by Roche Laboratories Inc. Nutlly, New Jersey 07110) in market in recent past [Sepe et al., 2007; Tjeertes et al., 2007]. MMF is a non-official ester of mycophenolic acid (Fig. 2) (MPA) so MPA present as synthetic impurity in MMF and believed so act by the inhibition of inosine monophosphate dehydrogenase (IMPDH). It is preferably use
as immunosuppressive drug in organ transplant [Tang et al., 2005]. No official methods were found for the assay of MMF in formulations [Benech et al., 2007]. So there is a need for method development for the assay of MMF in formulations (dosage forms) which can be used for routine analysis [Snyder et al., 1997].

Fig 1.1 Chemical structure of mycophenolate mofetil (MMF)

Fig 1.2 Chemical structure of mycophenolic acid (MPA)

For routine analysis a simple, rapid and cost effective analytical method is preferred. A survey of literature has not revealed any simple HPLC method for estimation of MMF in bulk drug and commercial formulations by PDA detector [Protic et al., 2009]. Several bioanalytical methodologies including HPLC, EMIT have been used for the quantification of MPA in plasma
[Toseland et al., 1986]. But there was no method for the determination of MMF in plasma as well as in formulation has been found in literature.

The main purpose of this investigation is to develop & validate reversed phase HPCL method which is simple, precise, sensitive and selective for quantization of MMF in dosage forms. The developed method can be easily used in routine quality control (QC) studies at very low level of MMF concentration. Suitable statistical tests were applied on validation data [ICH, 1995; Validation of Chromatographic Methods, 1994; The United States Pharmacopoeia, 2000].

1.2 Capsule dosage form

In the course of the 19th century, the discovery of substances in powder form like the alkaloids suddenly opened new therapeutic possibilities. With the new substances, new dosage forms were created (like in 1834 the hard gelatin capsule invented by Mothes and in 1843 the tablet invented by Brockedown). The chance to process powders on a large scale with a prolonged stability compared to liquid or semi solid dosage forms opened all possibilities of industrial production. Nowadays, solid dosage forms are still very popular because they have a high metering accuracy, the application of them is very easy and comfortable and their stability is very good.

Advantages of Capsule dosage form

A capsule has a number of advantages compared to a tablet:

- Developing a capsule formulation is in most cases not as complex as for a tablet formulation.

- A powder mixture can be filled directly into a capsule shell without a granulation and a compression process. For this reason, a capsule formulation often is the first dosage form for early clinical studies in the industry and the filling of capsules by hand is a common practice in pharmacies for an individual medication.
For blinding purposes an active ingredient can be easily encapsulated (Desai et al., 1996). Once the shell is soaked and dissolved in the stomach the active may in some cases be available in a loose, dispersed and, for this reason, in an early dissolvable and well absorbable state if the permeability through a biomembrane is given.

Different colors of the capsule shells allow the patients to distinguish their medications (Mallory et al., 1977).

A bad taste of a substance can be covered by a capsule shell (e.g. chloramphenicol, tetracycline).

When a small sized capsule has to be administered the swallowing may in certain cases be more comfortable because after contact with the saliva it gets more slippery than a tablet.

Disadvantages of Capsule dosage form

If a big amount of a compound has to be administered, the size of the capsule can easily get too big compared to the same amount compressed to an oblong tablet.

Some highly efflorescent and hygroscopic materials should not be filled into capsule shells because efflorescent materials may cause the capsules to soften, whereas hygroscopic powders may dry the capsule shell to excessive brittleness.

A major disadvantage of the capsule, however, is the fact that producing a capsule formulation is more expensive compared to a tablet formulation because the capsule shell has to be bought additionally. Furthermore, a tablet rotary press is able to produce up to one million tablets per hour whereas the maximum production speed of a dosating disk capsule filler reaches about 200,000 capsules per hour.
Thus, there are a number of reasons from the economic and marketing point of view to prefer a capsule or tablet formulation.

Nowadays, formulations are usually developed under high-time pressure on the basis of "trial and error" experiments (Leuenberger et al., 2005). They are complex, variable systems consisting not only of an active substance but also of a number of excipients, which contribute to a great extend that the active is at the right time at the right place in the patient with the right effect. The average cost to develop a new drug has grown to about 800 million US-Dollars. Of 500 to 10'000 screened compounds only about 250 enter pre-clinical testing where just one compound is approved. It takes an average time of 12 to 13 years of development from the discovery of the active substance to its commercialization as a dosage form on the market.

The knowledge, whether to formulate a drug as capsule or tablet, would certainly help to shorten the developing process and as a consequence, time and money could be saved.

Nowadays, the FDA favours the attempt to base manufacturing processes on scientific based knowledge than on empirical standards. The FDA addresses the pharmaceutical industry by the recommendation to introduce the concept of the PAT-initiative (PAT: Process Analytical Technology) for manufacturing processes and quality assurance.

The aim of the PAT-Initiative is the voluntary development and implementation of innovative, pharmaceutical production processes and quality assurance concepts. A guideline published by the FDA (FDA, 2004) concerning the PAT-initiative presents a framework with two components:

(1) A set of scientific principles and tools supporting innovation and

(2) a strategy for regulatory implementation that will accommodate innovation.
The basic idea is not to test quality into products but to build it in or to design it. The FDA emphasizes that the PAT-initiative is a recommendation to the pharmaceutical industry not a compulsory regulation.

The PAT-initiative was introduced because conventional manufacturing is generally accomplished by using batch processing with laboratory testing conducted on collected samples in order to evaluate quality. With this concept pharmaceutical products can be provided to the public, but nowadays, significant time and money saving opportunities exist for improving pharmaceutical development, process analysis, manufacturing and quality assurance through innovation. In other words: the actual drug discovery activity is a high tech business but the means or methods are still low tech. Many pharmaceutical processes are poorly understood, which causes a bad or unpredictable process.

Generally, the performance of a manufacturing process can be described with its Six Sigma Value. The champion is the chip industry with a Six Sigma Value, i.e. having an amount of defective samples > 2ppb which is a prerequisite to guarantee the functioning of our computer hardware. Surprisingly, the pharmaceutical manufacturing performance is only about Two Sigma, which corresponds to 4.6% defectives creating high costs (Leuenberger et al., 2005).

The benefits claimed by the FDA for the industry introducing the PAT-concept are a better understanding of the process, an introduction of real time release, a reduction of cycle times, less batch failure, a better management of change controls and regulatory relief.

The impact of the academia is the possibility to perform basic research without time pressure. Possible reasons for various phenomena can be investigated in detail. At the Institute of Pharmaceutical Technology of Basel a lot of science-based work has been done or is still in process in order to build quality in pharmaceutical processes.
1.2.1 Stability / Stable Formulation: A Preview

The term “stability,” with respect to a drug dosage form, refers to the chemical and physical integrity of the dosage unit and, when appropriate, the ability of the dosage unit to maintain protection against microbiological contamination. The shelf life of the dosage form is the time lapse from initial preparation to the specified expiration date. The monograph specifications of identity, strength, quality, and purity apply throughout the shelf life of the product.

The stability parameters of a drug dosage form can be influenced by environmental conditions of storage (temperature, light, air, and humidity), as well as the package components. Important factors affecting the stability are:

1. Temperature
2. Moisture content (Humidity)
3. Presence of oxygen
4. Light
5. pH

One of the main contributors to degradation of an active drug substance in a pharmaceutical formulation is the presence of moisture. Capsules, which are essentially dry dosage forms containing only minute amounts of water, commonly have a much longer shelf life than other formulations, such as oral and parenteral liquids. Nonetheless, it cannot be taken for granted that all capsules will have a long shelf life. The choice of excipients, for example, is an important factor in this respect. Some excipients are hygroscopic, and even minute amounts of moisture can decrease the stability of the drug. This is especially important for effervescent capsules; the packaging material plays an important role in the protection of this capsule form from moisture.
The importance of stability in the development of pharmaceutical dosage forms is well recognized in the pharmaceutical industry. Increasing filing of ANDA by generic drug manufacturer has resulted in submission of stability data to FDA. To assure quality and safety, stability data are required. The application of certain physiochemical principles in the performance of stability study has proven to be considerable advantage in the development of stable dosage forms.

For a drug substance to be developed into a capsule dosage form, the objective may be achieved by investigating the stability of drug under the following conditions.

1) Solid state stability of drug alone
2) Compatibility studies (stability in the presence of excipients)
3) Solution phase stability (stability in gastrointestinal fluid and granulating solvents used during manufacturing process)

1.2.2 Routes of Degradation:

A. Hydrolysis

In this type of reaction the active drug undergoes decomposition following reaction with the solvent present. Usually the solvent is water, but sometimes the reaction involves pharmaceutical co-solvents such as ethyl alcohol or poly ethylene glycol. These solvents act as nucleophiles attacking the electropositive center in drug molecule.

The examples are esters in Aspirin and Alkaloids, lactones in Pilocarpine and Spironolactone, and malonic ureas in Barbiturates.
B. Oxidation

Oxidation reactions are important pathways of drug decomposition. In pharmaceutical dosage forms, oxidation is usually mediated through reaction with atmospheric oxygen under ambient conditions, a process commonly referred to as autoxidation.

The mechanism of oxidation reactions is usually complex, involving multiple pathways for the initiation, propagation, branching and termination. Acids and bases catalyze many oxidation reactions.

Some functional groups subjected to autoxidation in drugs are, phenols in steroids, thiols in chlorpromazine, and amines in morphine and clozapine.

C. Photolysis

Normal room light or sunlight may cause substantial degradation of drug molecules. The energy from light radiations is absorbed by a molecule to cause a photolytic reaction. If that energy is sufficient to achieve activation, degradation of the molecule is possible. A dramatic example of photolysis is the photodegradation of sodium nitroprusside in aqueous solution.
Table 1.1 Stability of Oxidizable Drug and their Oxidizable functional group

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Oxidizable Functional Group</th>
<th>Observed Stability towards Oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextromethorphan</td>
<td>Tertiary amine</td>
<td>Stable</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Tertiary amine</td>
<td>Stable</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Secondary amine, benzylic ether</td>
<td>Stable</td>
</tr>
<tr>
<td>Flurazepam</td>
<td>Tertiary amine, imine</td>
<td>Stable</td>
</tr>
<tr>
<td>Captopril</td>
<td>Thiol</td>
<td>Unstable</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Thioether</td>
<td>Unstable</td>
</tr>
<tr>
<td>Morphine</td>
<td>Allylic alcohol, phenol</td>
<td>Unstable</td>
</tr>
<tr>
<td>L-Ascorbic acid</td>
<td>Allylic alcohol</td>
<td>Unstable</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Phenol</td>
<td>Unstable</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>3,5-Dioxoprazolidine</td>
<td>Unstable</td>
</tr>
<tr>
<td>Tetracyline(s)</td>
<td>Phenol, enols, tertiary amine</td>
<td>Unstable</td>
</tr>
</tbody>
</table>
D. Dehydration

In dehydration the elimination of water molecule from the active substances takes place. The driving force for this type of dehydration is the formation of a double bond that can then participate in electronic resonance with neighboring functional groups. Water removal does not create new bonds but often changes the crystalline structure of the drug.

Dehydration reactions involving water of crystallization may potentially affect the absorption rate. Prostaglandin E₂ and tetracycline degrade by dehydration.

E. Racemization

The racemization of the pharmacologically active agents is of interest because enantiomers often have significantly different absorption, distribution, metabolism, and excretion. Acids or bases catalyze most racemization reactions. The best-known racemization reactions of drugs are those that involve epinephrine, pilocarpine, ergotamine, and tetracyclines.

F. Incompatibilities

Chemical reactions between two or more drug components in the same dosage form or an active ingredient and a pharmaceutical adjuvant occur frequently.

The example of drug-drug incompatibility is the inactivation of cationic amino glycoside antibiotics, such as kanamycin and gentamycin, by anionic penicillines in IV admixtures.
1.2.3 Stability of Amorphous / Crystalline forms

During the processing of drugs to form a solid dosage form, it is possible to mechanically generate amorphous drugs. Amorphous drug regions have lesser stability and lack crystal lattice stabilization energy, and as a result, oxygen permeability and solubility will be higher.

In addition the amorphous form of a compound is higher in energy, the rate of a reaction to a common product will be faster.

1.2.4 General Guidelines for collection of stability data

In general case stability conditions are defined in three types. The conditions are given in the table that covered, storage condition and time period of the study. Accelerated study was done at 40°C ± 2°C/75% RH ± 5% RH, to know the results of the study in short duration of time. The results of accelerated stability study were then extrapolated to know the stability at ordinary conditions. Long-term study was mainly done at 25°C ± 2°C/60% RH ± 5% RH, and the results were collected after 12 months. Sometimes intermediate stability studies at 30°C ± 2°C/65% RH ± 5% RH, was done and the data was collected after 6 months.
Table 1.2 General cases

<table>
<thead>
<tr>
<th>Study</th>
<th>Storage condition</th>
<th>Minimum time period covered by data at submission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long term*</td>
<td>25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH</td>
<td>12 months</td>
</tr>
<tr>
<td>Intermediate**</td>
<td>30°C ± 2°C/65% RH ± 5% RH</td>
<td>6 months</td>
</tr>
<tr>
<td>Accelerated</td>
<td>40°C ± 2°C/75% RH ± 5% RH</td>
<td>6 months</td>
</tr>
</tbody>
</table>

*It is up to the applicant to decide whether long-term stability studies are performed at 25 ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH.

**If 30°C ± 2°C/65% RH ± 5% RH is the long-term condition, there is no intermediate condition.

1.2.5 Stability guidelines for different climatic zones

For convenience in planning for packaging and storage, and for stability studies, international practice identifies four climatic zones, which are described in. The United States, Europe, and Japan are characterized by zones I and II. The values in are based on observed temperatures and relative humidity’s, both outside and in rooms, from which mean kinetic temperatures and average humidity values are calculated. Derived values are based on inspection of data from individual cities and on allowances for a margin of safety in assignment of these specified conditions.
### Table 1.3 International Climatic Zones

<table>
<thead>
<tr>
<th>Climatic Zone</th>
<th>Calculated Data</th>
<th>Derived Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C*</td>
<td>°C MKT**</td>
</tr>
<tr>
<td><strong>I. Temperate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>United Kingdom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern Europe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Russia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>II. Mediterranean, Subtropical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>21.6</td>
<td>22.0</td>
</tr>
<tr>
<td>Japan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southern Europe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Portugal-Greece)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>III. Hot, Dry</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td>26.4</td>
<td>27.9</td>
</tr>
<tr>
<td>Iraq</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudan</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IV. Hot, Humid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>26.7</td>
<td>27.4</td>
</tr>
<tr>
<td>Ghana</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indonesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicaragua</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Philippines</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data recorded as <19° calculated as 19°.

** Calculated mean kinetic temperature.

*** Partial pressure of water vapor.
1.2.6 Manufacturing

The manufacture of conventional capsules is a cost-effective process. Modern capsule filling machines are able to cater for large-scale production; a modern machine can output over 10000 capsules per minute. This speed of production gives the capsule form its superior edge over other solid oral dosage forms.

![Diagram of hybrid system]

Fig 1.3 An overview of hybrid system

1.2.7 Compliance

The capsule form is convenient to handle and easy and safe for the patient to take. Since capsules are a well known dosage form for most patients, there will be fewer requirements for explanatory information and compliance is assumed to be better. However, there are also compliance
disadvantages; some people, especially the elderly and children, find it difficult to swallow capsules. Further, most people require water to facilitate swallowing capsules. However, several new types of capsules, intended for rapid disintegration and drug release in the oral cavity, have been developed over the last decade. This approach may be useful for increasing patient compliance, since disintegration of the capsule in the mouth facilitates swallowing, and concomitant intake of water can sometimes be omitted.
Fig 1.4 The algorithm of a model expert system (MES)
1.2.8 Capsule formulation excipients (in case of amorphous powder API)

In a capsule formulation, a range of excipient materials is normally required along with the active ingredient in order to give the capsule the desired properties. For example, the reproducibility and dose homogeneity of the capsules are dependent on the properties of the powder mass. The capsule should also be sufficiently strong to withstand handling, but should disintegrate after intake to facilitate drug release. The choice of excipients will affect all these properties.

**Filler:** Fillers are used to make capsules of sufficient size for easy handling by the patient and to facilitate production. Capsules containing a very potent active substance would be very small without additional excipients. Good filler will have good compactability and flow properties, acceptable taste, will be non-hygroscopic and preferably chemically inert. It may also be advantageous to have filler that fragments easily, since this counteracts the negative effects of lubricant additions to the formula.

**Binder:** A material with a high bonding ability can be used as a binder to increase the mechanical strength of the capsule. A binder is usually a ductile material prone to undergo plastic (irreversible) deformation. Typically, binders are polymeric materials, often with disordered solid state structures. Of special importance is the deformability of the peripheral parts (asperities and protrusions) of the binder particles. Thereby, this group of materials has the capacity of reducing interparticulate distances within the capsule, improving bond formation. If the entire bulk of the binder particles undergo extensive plastic deformation during compression, the interparticular voids will, at least partly, be filled and the capsule porosity will decrease. This increases the contact area between the particles, which promotes the creation of interparticular
bonds and subsequently increases the capsule strength. However, the effect of the binder depends on both its own properties and those of the other compounds within the capsule. A binder is often added to the granulation liquid during wet granulation to improve the cohesiveness and compactability of the powder particles, which assists formation of agglomerates or granules. It is commonly accepted that binders added in dissolved form, during a granulation process, is more effective than used in dry powder form during direct compression.

Glidant: Glidants are added to increase the flowability of the powder mass, reduce interparticular friction and improve powder flow in the hopper shoe of the capsule filling machine.

1.3 Generic drug and ANDA

A generic drug is the same as a brand name drug in dosage, safety, strength, how it is taken, quality, performance, and intended use. Before approving a generic drug product, FDA requires many rigorous tests and procedures to assure that the generic drug can be substituted for the brand name drug. The FDA bases evaluations of substitutability, or "therapeutic equivalence," of generic drugs on scientific evaluations. By law, a generic drug product must contain the identical amounts of the same active ingredient(s) as the brand name product. Drug products evaluated as "therapeutically equivalent" can be expected to have equal effect and no difference when substituted for the brand name product.

When the company believes it has a viable product, it obtains a patent, which lasts for 20 years. This means that only that company - the originator of the drug - has the exclusive and legal right to manufacture and market the drug during the life of the patent. Also, any other manufacturer cannot use the brand name of the drug. Many drugs have several patents, which prolong the
exclusivity period beyond 20 years. Once the patent expires, the drug may be manufactured and sold by other companies under a different brand name, or under its generic name. All manufacturing and marketing of the generic drug must be conducted in strict compliance with the guidelines established by the Food and Drug Administration (FDA). The FDA is a federal agency staffed with pharmaceutical and medical experts who approve safe and effective drugs for sale. Most prescription drugs require this approval. Generic drug applications are termed "abbreviated" because they are generally not required to include preclinical (animal) and clinical (human) data to establish safety and effectiveness. Instead, generic applicants must scientifically demonstrate that their product is bioequivalent (i.e., performs in the same manner as the innovator drug). One-way scientists demonstrate bioequivalence is to measure the time it takes the generic drug to reach the bloodstream in 24 to 36 healthy, volunteers. This gives them the rate of absorption, or bioavailability, of the generic drug, which they can then compare to that of the innovator drug. The generic version must deliver the same amount of active ingredients into a patient's bloodstream at the same rate as the innovator drug.

1.3.1 ANDA

An Abbreviated New Drug Application (ANDA) contains data which when submitted to FDA's Center for Drug Evaluation and Research, Office of Generic Drugs, provides for the review and ultimate approval of a generic drug product. Once approved, an applicant may manufacture and market the generic drug product to provide a safe, effective, low cost alternative to the American public. A generic drug product is one that is comparable to an innovator drug product in dosage form, strength, and route of administration, quality, performance characteristics and intended use. All approved products, both innovator and generic, are listed in FDA's Approved Drug Products with Therapeutic Equivalence Evaluations (Orange Book).
1.3.2 FDA guidelines for ANDA filing

Immediate-Release Products: Capsules and Tablets

1. General Recommendations

For product quality BA and BE studies, it is recommended that where the focus is on release of the drug substance from the drug product into the systemic circulation, a single-dose, fasting study be performed. It is also recommended that *in vivo* BE studies be accompanied by in vitro dissolution profiles on all strengths of each product. For ANDA’s, we also recommend that the BE study be conducted between the test product and reference listed drug using the strength(s) specified in Approved Drug Products with Therapeutic Equivalence Evaluations (Orange Book).

![ANDA Patent Certification Options](image)

**Fig 1.5 ANDA patent certification**

2. Waivers of *In Vivo* BE Studies (Biowaivers)

a. Investigational New Drugs (INDs), New Drug Applications (NDAs), and Abbreviated New Drug Applications (ANDAs): Preapproval
When the drug product is in the same dosage form, but in a different strength, and is proportionally similar in its active and inactive ingredients to the strength on which BA or BE testing has been conducted, an *in vivo* BE demonstration of one or more lower strengths can be waived based on dissolution tests and an *in vivo* study on the highest strength.

For an NDA, Biowaivers of a higher strength will be determined to be appropriate based on

1. Clinical safety and/or efficacy studies including data on the dose and the desirability of the higher strength,
2. Linear elimination kinetics over the therapeutic dose range,
3. The higher strength being proportionally similar to the lower strength, and
4. The same dissolution procedures being used for both strengths and similar dissolution results obtained. It is recommended that a dissolution profile be generated for all strengths.

### Table 1.6 Comparison between NDA and ANDA requirements

<table>
<thead>
<tr>
<th>NDA Requirements</th>
<th>ANDA Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chemistry</td>
<td>1. Chemistry</td>
</tr>
<tr>
<td>3. Controls</td>
<td>3. Controls</td>
</tr>
<tr>
<td>4. Labelling</td>
<td>4. Labelling</td>
</tr>
<tr>
<td>5. Testing</td>
<td>5. Testing</td>
</tr>
<tr>
<td>6. Animal Studies</td>
<td></td>
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<tr>
<td>7. Clinical Studies</td>
<td></td>
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</tbody>
</table>

If an appropriate dissolution method has been established, and the dissolution results indicate that the dissolution characteristics of the product are not dependent on the product strength, then dissolution profiles in one medium are usually sufficient to support waivers of *in vivo* testing. Otherwise, dissolution data in three media (pH 1.2, 4.5, and 6.8) are recommended. We
recommend that the $f_2$ test be used to compare profiles from the different strengths of the product. An $f_2$ value $\geq 50$ indicate a sufficiently similar dissolution profile such that further *in vivo* studies are not needed. For an $f_2$ value $< 50$, further discussions with CDER review staff may help to determine whether an *in vivo* study is appropriate. The $f_2$ approach is not suitable for rapidly dissolving drug products (e.g., $\geq 85\%$ dissolved in 15 minutes or less).

b. NDAs and ANDAs: Post approval

Information on the types of *in vitro* dissolution and *in vivo* BE studies for immediate-release drug products approved as either NDAs or ANDAs in the presence of specified post approval changes are provided in an FDA guidance for industry entitled SUPAC-I R. For post approval changes, it is recommend that the *in vitro* comparison be made between the prechange and post change products. In instances where dissolution profile comparisons are suggested, we also recommend an $f_2$ test be used. $f_2$ values of $> 50$ suggest a sufficiently similar dissolution profile and no further *in vivo* studies are needed. When *in vivo* BE studies are called for, it is recommend that the comparison be made for NDAs between the prechange and post change products, and for ANDAs between the post change and reference listed drug products.

1.3.3 *In Vitro* dissolution

Under certain circumstances, product quality BA and BE can be documented using in vitro approaches. For highly soluble, highly permeable, rapidly dissolving, and orally administered drug products, documentation of BE using an *in vitro* approach (dissolution studies) is appropriate based on the biopharmaceutics classification system. This approach may also be suitable under some circumstances in assessing BE during the IND period, for NDA and ANDA submissions, and in the presence of certain post approval changes to approved NDAs and
ANDAs. In addition, in vitro approaches to documenting BE for nonbioproblem drugs approved before 1962 remain appropriate.

<table>
<thead>
<tr>
<th>Class I</th>
<th>Class III</th>
</tr>
</thead>
<tbody>
<tr>
<td>High solubility</td>
<td>High solubility</td>
</tr>
<tr>
<td>High permeability</td>
<td>Low permeability</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Class II</th>
<th>Class IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low solubility</td>
<td>Low solubility</td>
</tr>
<tr>
<td>High permeability</td>
<td>Low permeability</td>
</tr>
</tbody>
</table>

Fig 1.7 Biopharmaceutics Classification System (BCS)

Dissolution testing is also used to assess batch-to-batch quality, where the dissolution tests, with defined procedures and acceptance criteria, are used to allow batch release. We recommend that dissolution testing is also used to

(1) Provide process control and quality assurance, and

(2) Assess whether further BE studies relative to minor post approval changes be conducted, where dissolution can function as a signal of bioinequivalence.

In vitro dissolution characterization is encouraged for all product formulations investigated (including prototype formulations), particularly if in vivo absorption characteristics are being defined for the different product formulations. Such efforts may enable the establishment of an in vitro-in vivo correlation. When an in vitro-in vivo correlation or association is available, the in vitro test can serve not only as a quality control specification for the manufacturing process,
but also as an indicator of how the product will perform in vivo. The following guidance's provide recommendations on the development of dissolution methodology, setting specifications, and the regulatory applications of dissolution testing:

(1) Dissolution Testing of Immediate Release Solid Oral Dosage Forms; and


It is recommended that the following information generally be included in the dissolution method development report for solid oral dosage forms

1.3.4 for ANDAs

For immediate-release drug products, we recommend that the appropriate USP method be submitted. If there is no USP method available, we recommend that the FDA method for the reference-listed drug be used. If the USP and/or FDA methods are not available, we recommend that the dissolution method development report described above be submitted.

1.3.5 How are Generic Drugs Approved

When applying for approval of a generic drug, a pharmaceutical company must supply a great deal of information about the product, its manufacturing process, and its quality control testing to the FDA. In addition, a list of all ingredients, both active and inactive, must be provided. The FDA studies this information and determines whether the drug is acceptable. The major difference between the FDA approval of a brand drug and a generic drug is that the manufacturer of the generic drug is not required to duplicate the original medical studies proving the safety and efficacy of the chemical compound. Since the medical safety and effectiveness of the brand name drug has already been determined, only bioequivalence/therapeutic equivalence testing is
required for a generic drug. The FDA approves only those generic drugs shown to be bioequivalent/therapeutically equivalent to their brand name counterparts. To be considered bioequivalent / therapeutically equivalent" to its brand name counterpart, a generic drug must not only have identical active chemical compounds, but the quantity and speed of absorption of its active ingredients into the bloodstream must also be the same or similar, within ranges designated and approved by the FDA. (Most oral medication must be absorbed into the bloodstream before it can produce the medical results your physician is trying to achieve.) If the FDA is satisfied with the tests submitted by the manufacturers for its review, the agency rates the generic drug as bioequivalent/therapeutically equivalent to the brand, recognizing it as equal to and interchangeable with it. Sometimes the generic version of a drug may have a different color or shape from its brand name counterpart.

1.4 Immunosuppressant Drugs

1.4.1 Definition

Immunosuppressant drugs, which are also called anti-rejection drugs, are used to prevent the body from rejecting a transplanted organ.

1.4.2 Purpose

When an organ, such as a liver, heart or kidney, is transplanted from one person (the donor) into another (the recipient), the immune system of the recipient triggers the same response against the new organ that it would have against any foreign material, setting off a chain of events that can damage the transplanted organ. This process is called rejection. It can occur rapidly (acute rejection), or over a long period of time (chronic rejection). Rejection can occur despite close
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matching of the donated organ and the transplant patient. Immunosuppressant drugs greatly decrease the risks of rejection, protecting the new organ and preserving its function. These drugs act by blocking the recipient's immune system so that it is less likely to react against the transplanted organ. A wide variety of drugs are available to achieve this aim but work in different ways to reduce the risk of rejection.

In addition to being used to prevent organ rejection, immunosuppressant drugs are also used to treat such severe skin disorders as psoriasis and such other diseases as rheumatoid arthritis, Crohn's disease (chronic inflammation of the digestive tract), and patchy hair loss (alopecia areata). Some of these conditions are termed "autoimmune" diseases, indicating that the immune system is reacting against the body itself.

1.4.3 Description

Immunosuppressant drugs can be classified according to their specific molecular mode of action. The four main categories of immunosuppressant drugs currently used in treating patients with transplanted organs are the following:

- **Cyclosporins** (Neoral, Sandimmune, SangCya). These drugs act by inhibiting T-cell activation, thus preventing T-cells from attacking the transplanted organ.
- **Azathioprin**es (Imuran). These drugs disrupt the synthesis of DNA and RNA as well as the process of cell division.
- **Monoclonal antibodies**, including basiliximab (Simulect), daclizumab (Zenpax), and muromonab (Orthoclone OKT3). These drugs act by inhibiting the binding of interleukin-2, which in turn slows down the production of T-cells in the patient's immune system.
Such corticosteroids as prednisolone (Deltasone, Orasone). These drugs suppress the inflammation associated with transplant rejection.

Immunosuppressants can also be classified according to the specific organ that is transplanted:

- Basiliximab (Simulect) is also used in combination with such other drugs as cyclosporin and corticosteroids in kidney transplants.
- Daclizumab (Zenapax) is also used in combination with such other drugs as cyclosporin and corticosteroids in kidney transplants.
- Muromonab CD3 (Orthoclone OKT3) is used along with cyclosporin in kidney, liver and heart transplants.
- Tacrolimus (Prograf) is used in liver and kidney transplants. It is under study for bone marrow, heart, pancreas, pancreatic island cell, and small bowel transplantation.

Some immunosuppressants are also used to treat a variety of autoimmune diseases:

- Azathioprine (Imuran) is used not only to prevent organ rejection in kidney transplants, but also in treatment of rheumatoid arthritis. It has been used to treat chronic ulcerative colitis, although it has proved to be of limited value for this use.
- Cyclosporin (Sandimmune, Neoral) is used in heart, liver, kidney, pancreas, bone marrow, and heart/lung transplantation. The Neoral form of cyclosporin has been used to treat psoriasis and rheumatoid arthritis. The drug has also been used to treat many other conditions, including multiple sclerosis, diabetes, and myasthenia gravis.
- Glatiramer acetate (Copaxone) is used in the treatment of relapsing-remitting multiple sclerosis. In one study, glatiramer reduced the frequency of multiple sclerosis attacks by 75% over a two-year period.

- Sirolimus (Rapamune) is used in combination with other drugs, including cyclosporin and corticosteroids, in kidney transplants. The drug is also used to treat patients with psoriasis.

1.4.4 Mechanism

![Mechanism of Action of Various Immunosuppressants](image)

**Fig 1.8 Mechanism of Action of Various Immunosuppressants**

1.4.5 Recommended Dosage

Immunosuppressant drugs are available only with a physician's prescription. They come in capsule, liquid, and injectable forms. The recommended dosage depends on the type and form of
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immunosuppressant drug and the purpose for which it is being used. Doses may be different for
different patients.

1.4.6 Special Conditions

People who have certain diseases or disorders, or who are taking certain other medicines may
have problems if they take immunosuppressant drugs. Before taking these drugs, patients should
inform the prescribing physician about any of the following conditions:

Allergies

Anyone who has had unusual reactions to immunosuppressant drugs in the past should let his or
her physician know before taking the drugs again. The physician should also be told about any
allergies to foods, dyes, preservatives, or other substances.

Pregnancy

Azathioprine has been considered a cause of birth defects. The British National Formulary,
however, states: "Transplant patients immunosuppressed with azathioprine should not
discontinue it on becoming pregnant; there is no evidence that azathioprine is teratogenic. There
is less experience of cyclosporin in pregnancy but it does not appear to be any more harmful than
azathioprine. The use of these drugs during pregnancy needs to be supervised in specialist units.
Any risk to the offspring of azathioprine-treated men is small." Nonetheless, patients who are
taking any immunosuppressive drug should consult with their physician before conceiving a
child, and they should notify the doctor at once when there is any indication of pregnancy.

Basiliximab should not be used during pregnancy. The manufacturer recommends using
adequate contraception during use of this drug, and for eight weeks following the final dose.
The manufacturers warn against the use of tacrolimus during pregnancy, on the basis of findings from animal studies. They recommend using adequate contraception while taking these drugs, and for six weeks after the last dose.

The safety of corticosteroids during pregnancy has not been absolutely determined. There is some evidence that use of these drugs during pregnancy may affect the baby's growth; however, this result is not certain, and may vary with the medication used. Patients taking any steroid drug should consult with their physician before starting a family, and should notify the doctor at once if they think they are pregnant.

Most of these medicines have not been studied in humans during pregnancy. Women who are pregnant or who may become pregnant and who need to take immunosuppressants should consult their physicians.

Lactation

Immunosuppressant drugs pass into breast milk and may cause problems in nursing babies whose mothers take it. Breastfeeding is not recommended for women taking immunosuppressants.

1.4.7 Other Medical Conditions

People with any of the following conditions may have problems if they take immunosuppressant drugs:

- People who have shingles (herpes zoster) or chickenpox, or who have recently been exposed to chickenpox, may develop severe disease in other parts of their bodies when they take these medicines.

- Immunosuppressants may produce more intense side effects in people with kidney disease or liver disease, because their bodies are slow to get rid of the medicine.
Oral forms of immunosuppressants may be less effective in people with intestinal problems, because the medicine cannot be absorbed into the body.

1.4.8 Side Effects

Increased risk of infection is a common side effect of all immunosuppressant drugs. The immune system protects the body from infections; when the immune system is suppressed, infections are more likely. Taking such antibiotics as co-trimoxazole prevents some of these infections. Immunosuppressant drugs are also associated with a slightly increased risk of cancer because the immune system plays a role in protecting the body against some forms of cancer. For example, the long-term use of immunosuppressant drugs carries an increased risk of developing skin cancer as a result of the combination of the drugs and exposure to sunlight.

Other side effects of immunosuppressant drugs are minor and usually go away as the body adjusts to the medicine. These include loss of appetite, nausea or vomiting, increased hair growth, and trembling or shaking of the hands. Medical attention is not necessary unless these side effects continue or cause problems.

The treating physician should be notified immediately if any of the following side effects occur:

- unusual tiredness or weakness
- fever or chills
- frequent need to urinate

1.4.9 Interactions

Immunosuppressant drugs may interact with other medicines. When interactions occur, the effects of one or both drugs may change or the risk of side effects may be greater. Other drugs may also have adverse effects on immunosuppressant therapy. It is particularly important for
patients taking cyclosporin or tacrolimus to be careful about the possibility of drug interactions. Other examples of problematic interactions are:

- The effects of azathioprine may be greater in people who take allopurinol, a medicine used to treat gout.

- A number of drugs, including female hormones (estrogens), male hormones (androgens), the antifungal drug ketoconazole (Nizoral), the ulcer drug cimetidine (Tagamet), and the erythromycins (used to treat infections), may intensify the effects of cyclosporine.

- When sirolimus is taken at the same time as cyclosporin, the blood levels of sirolimus may be increased to a level that produces severe side effects. Although these two drugs are usually used together, the dose of sirolimus should be taken four hours after the dose of cyclosporin.

- Tacrolimus is eliminated through the kidneys. When this drug is used with other medications that may harm the kidneys, such as cyclosporin, the antibiotics gentamicin and amikacin, or the antifungal drug amphotericin B, the blood levels of tacrolimus may rise. Careful kidney monitoring is essential when tacrolimus is given with any drug that might cause kidney damage.

- The risk of cancer or infection may be greater when immunosuppressant drugs are combined with certain other drugs that also lower the body's ability to fight disease and infection. These drugs include corticosteroids, especially prednisone; the anticancer drugs chlorambucil (Leukeran), cyclophosphamide (Cytoxan), and mercaptopurine (Purinethol); and the monoclonal antibody muromonab-CD3 (Orthoclone), which is also used to prevent transplanted organ rejection.