Development and Evaluation of Modified Release Oral Dosage Form of Mycophenolate Sodium

THESIS

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CERTIFICATE

This is to certify that the thesis entitled **"Development and Evaluation of Modified Release Oral Dosage Form of Mycophenolate Sodium"** submitted by Mr. Sukhjeet Singh, I.D. No. 2002 PHXF045 for award of Ph. D. Degree of the Institute, embodies original work done by him under my supervision.

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ABSTRACT

The field of transplantation remains one of the most innovative and pioneering areas in medicine today. The goal of transplantation is to prolong life. However, transplantation itself is not enough; there is a need to balance the amount of immunosuppression necessary to assure graft survival with the potential toxicity of the immunosuppressive agents. The last three decades have seen many advances in immunosuppressive treatment options for both immediate, post-transplant therapy and ongoing maintenance. The immunosuppressant therapy regimen for kidney transplantation comprises of a calcineurin inhibitor, an anti-proliferative agent, and a corticosteroid, although local protocols vary but almost every regimen includes mycophenolic acid MPA. The other drugs *viz.,* tacrolimus and steroids are available as once daily formulation. Only MPA is available as twice daily dosing. There are two agents available for mycophenolic acid *i.e.,* as a prodrug mycophenolate mofetil (Cellcept, Roche Inc.) and the other mycophenolate sodium (Myfortic, Novartis). A patient needs to take 2-3 tablets of either Mycophenolate Mofetil or Mycophenolate Sodium each time. In current clinical practice the oral formulations of available Mycophenolate Sodium, at least two tablets, are generally administered on a twice daily basis which leads to patient compliance concerns. Hence, developing a patient compliant dosage form of selected immunosuppressant is the need of the hour. Moreover, gastric adverse events of MPS seem to be related to C_{max}, whereas immunosuppressant activity is related to total exposure *i.e.,* AUC. Therefore, it is envisaged that with MR product, C_{min} levels will be maintained for longer duration of time suggesting very low probability of rejection. It is envisaged to prepare a new oral formulation of Mycophenolate Sodium with prolonged-release characteristics compared to the currently available immediate release dosage form**:** In the present investigation, 720 mg was selected as the strength to be developed (Equivalent to two tablets of MPS or Mycophenolate Mofetil at a time). This strength was selected on the basis of the FDA-approved dosing schedule for mycophenolic acid wherein 720 mg MPA is administered twice daily.

It has been reported in the literature that an AUC value of 30-60 µg.h/mL (for 720 mg of MPA) is sufficient to prove efficacy. Patients with low AUC for MPA appear to be at high risk for experiencing graft rejection whereas high target concentration can increase toxicity. The maintenance of C_{min} levels between 1-3 µg/mL for prolonged period is also related to efficacy. The abovementioned parameters were selected as

acceptance criteria for the product. In order to obtain these pharmacokinetic parameters, the dissolution profile of the proposed product was defined. The main objective of the study was development of patient compliant once a day dosage form for MPS with patentable and non infringing composition. The formulation should have safety and efficacy comparable with that of immediate release formulation while it should reduce side effects related to C_{max} *viz.,* GI adverse effects. In the present investigation, it was intended to exploit lipid as well as polymer matrix system to achieve controlled release of Mycophenolate Sodium. The study aims at examining the range of polymers/combinations for preparation of Mycophenolate Sodium tablets with various evaluation parameters. In addition to the formulation development, the plan of the study includes: solubility determination, preformulation studies, analytical method development and validation for the quantitation of Mycophenolate Sodium for assay and dissolution samples, formulation development, *in vitro* dissolution tests and effects of diluents on drug release profile, fitting of the dissolution profile into mathematical models to ascertain the mechanism of drug release and stability studies of the optimized formulations. Based on the values of r^2 obtained for first order, Korsmeyer Peppas and Hixon Crowell it could be interpreted that two mechanistic phenomenon take place: the swelling and erosion of the polymer. The release kinetics of the drug are dependent upon the relative magnitude of the rate of polymer swelling at the moving rubbery/glassy front and the rate of polymer erosion at the swollen polymer/ dissolution medium front. The results are consistent with a release process where the fickian release mechanism plays an important role along with the matrix erosion, which is a considerable characteristics of system based on hydrophilic polymers.

The investigational biostudy was conducted on the formulations one each from lipid matrix and polymer matrix technology. It was observed that lipid matrix technology led to more bioavailability loss than that of polymer matrix technology when compared with that of immediate release formulation.

Therefore, further optimization of formulation was conducted on polymer matrix technology by a simple process which could be scaled up. Two more biostudies were performed with a fast release and slow release formulations based on polymer matrix technology. Effect of food on bioavailability of MPS MR dosage form was also established in these studies. It was found that the slow release polymer matrix formulation met all the acceptance criteria for pharmacokinetic end points viz. $Auc_{0-\infty}$, C_{max}, C_{min} and fluctuation.

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LIST OF ABBREVIATIONS/SYMBOLS

ACE	:	Angiotensin-Converting Enzyme
ADME	:	*Absorption*, *Distribution*, *Metabolism* (Biotransformation) and *Excretion*
ALG	:	Anti-lymphocyte globulin (ALG)
ALG	:	Antilymphocyte Globulin
API	:	Active Pharmaceutical Ingredients
ASD	:	Accelerated Stability Data
ATG	:	Anti-thymocyte globulin (ATG)
ATG	:	Anti Thymocyte Globulin
ATGAM	:	Lymphocyte Immune Globulin
AUC	:	Area Under Curve
AUC_∞	:	AUC to time infinity
AUC_t	:	AUC to time t
AUMC	:	Area Under Moment Curve
BCS	:	Biopharmaceutical Classification System
BMI	:	Body Mass Index
CABG	:	Coronary Artery Bypass Graft surgery
CC	:	Calibration Curve
CDR	:	Complementarity Determining Region
C_{max}	:	Maximum Concentration
CMI	:	Cell Mediated Immunity
C_{min}	:	Minimum plasma conc. Showing therapeutic effect
CNS	:	Central Nervous System
COX	:	Cyclo Oxygenase
C_p	:	Plasma Drug Concentration
CR	:	Controlled Release
CR DDS	:	Controlled Release Drug Delivery System
CsA	:	Cyclosporine
CTL	:	cytotoxic T lymphocyte
DDS	:	Drug Delivery System
DNA	:	Deoxyribo Nucleic Acid
DSC	:	Differential Scanning Colorimetry
ECG	:	Electro Cardio Gram
EDTA	:	Ethylene Diamine Tetra Acetate
ELISA	:	Enzyme-Linked Immunosorbent Assay
FKBP-12	:	FK506-bindin~protein-12
GC	:	Gas Chromatography
GI	:	Gastrointestinal
HbsAg	:	Hepatitis B surface antigen
HCV	:	Hepatitis C Virus
HIV	:	Human Immuno Virus
HLA	:	Human Leukocyte Antigen
HMGCoA	:	3-Hydroxy-3-Methyl Glutaryl Coenzyme A
HPLC	:	High Performance Liquid Chromatography
HPLC	:	High-Performance Liquid Chromatography
HQC	:	High Quality Control
ICH	:	International Conference on Harmonization
IgG_{Za}	:	Immunoglobin Za

IL	:	Inter Lukin
IMPDH	:	Inosine Monophosphate Dehydrogenase
IMPDH	:	Inosine Monophsophate Dehydrogenase
IR	:	Immediate Release
IVIVC	:	*In Vivo* In Vitro Correlation
LBD	:	Loose Bulk Density
LDL	:	Low Density Lipoprotein
LFA	:	Lymphocyte Function Associated Antigen
LLOQ	:	Lower Limit of Quantitation
LOQ	:	Limit of Quantitation
LOD	:	Limit of Detection
LOC	:	Low Quality Control
mAbs	:	Monoclonal Antibodies
MAT	:	Mean Absorption Time
MDT	:	Mean Dissolution Time
MHC	:	Histocompatibility Complex
MMF	:	Mycophenolate Mofetil
MPA	:	Mycophenolic Acid
MPS	:	Mycophenolate Sodium
MQC	:	Medium Quality Control
MR	:	Modified Release
MRT	:	Mean Residence Time
mTOR	:	mammalian target of rapamycin
MTT	:	Mean Transit Time
ND	:	Not Detected
NDA	:	New Drug Application
NEORAL	:	Microemulsion Formulation.
NFAT	:	Nuclear Factor of Activated T
NFAT	:	Nuclear Factor of Activated T Cell
NSAIDs	:	Non Steroidal Anti Inflammatory Drugs
PD	:	Pharmacodynamic
PE	:	Prediction Error
PEO	:	Poly Ethylene Oxide
PK	:	Pharmacokinetic
PTDM	:	Post Transplant Diabetes Mellitus
QC	:	Quality Control
RPR	:	Rapid Plasma Reagin
SIP-R	:	Sphingosine I-phosphate Receptor
TBD	:	Tapped Bulk Density
TCR	:	T Cell Receptor
TGF-β	:	transforming growth factor
T_{max}	:	Time of Maximum Concentration
TOR	:	Target of Rapamycin
UNOS	:	United Network for Organ Sharing
USFDA	:	United States Food and Drug Administration
USP	:	United States Pharmacopeias
VRT	:	Variance of Residence Time

1.0 Introduction

1.0 INTRODUCTION

1.1 Organ Transplantation: An Overview

The word 'transplantation' is defined as, “the operation with which some organs or tissues are placed from one part of the body to another”. In such cases people classify transplantations from dead donors and from live donors. Kidney failure, heart disease, lung disease and cirrhosis of the liver are all conditions that might be effectively treated by a transplant. For problems with the heart, the lungs and other highly sensitive organs, a transplant is typically the course of the last resort. But if all other avenues have been explored and the patient is willing and able, transplantation is a good, viable option. Indeed, it is more wonderful than to replace organs that function badly or not at all, by new healthy ones [1, 2].

Transplantation occurs because the recipient's organ has failed or has been damaged through illness or injury. Table 1 describes the major organs and tissues transplanted along with donor type. The transplantations of heart and some other organs which cannot be transplanted when a person is alive are transplanted from dead donors. In cases with live donors, very often, such organs as skin, lung, and kidneys are transplanted as they are paired or big organs or sometimes organs or tissues that easily regenerate. In such types of organ transplantation, one person can be a donor even for several times, for example in case of blood transplantation.

The number of organs being transplanted is constantly growing and is probably equal with the speed of general medicine development as well as of surgery innovations. The first attempt of transplantation was made in 1870 by Swiss Surgeon Jacques Reverdin [3]. After him a number of surgeons tried to cope up with organ transplantation and in most cases such attempts were rather successful. It can be said that organ transplantation has survived till the present time and is increasingly developing. But it can also be said that there are some problems in different types of organ transplant. Sometimes it happens so that the organ being transplanted is not able to regenerate in a new place and makes the situation even worse. In most cases such kind of operations are rather expensive and it is a strange fact that with the course of time the number of people who are to come across such an operation has grown greatly.

Nevertheless, transplantation is rather successful and popular now a days. Thousands of people become volunteers and become donors of their blood even several times a

year not because it is needed by their relatives or friends but to help unknown people. Some people ask to use their organs for transplantation after their death to help a needy person.

Table 1: Major organs and tissues transplantation and donor type

Organs	Donor
Thoracic organs	
Heart	Deceased-donor only (http://www.healthsystem.virginia.edu/uvahealth/peds_cardiac/hearttran.cfm)
Lung	Deceased-donor and Living-Donor (http://www.healthsystem.virginia.edu/uvahealth/peds_respire/lungstran.cfm)
Heart/Lung	Deceased-donor and Domino transplant
Other organs	
Kidney	Deceased-donor and Living-Donor
Liver	Deceased-donor and Living-Donor
Pancreas	Deceased-donor only
Intestine	Deceased-donor only
Tissues, cells, fluids	
Hand	Deceased-donor only
Cornea	Deceased-donor only
Skin graft Face replant Face transplant	Autograft Extremely rare
Penis	Deceased-donor only
Islets of Langerhans	Pancreas Islet Cells (Deceased-donor and Living-Donor)
Bone marrow/Adult stem cell	Living-Donor and Autograft
Blood transfusion/Blood Parts Transfusion	Living-Donor and Autograft
Blood vessels	Autograft and Deceased-Donor
Heart valve	Deceased-Donor, Living-Donor and Xenograft [Porcine/bovine]
Bone	Deceased-Donor, Living-Donor, and Autograft
Skin	Deceased-Donor, Living-Donor, and Autograft

Transplantation is important for thousands of people in the world. The improvement of today's medicine in the field of grafting has raised a new hope for patients and opened new perspectives for the vital and urgent needs of the patients. Key subjects such as mechanisms of chronic rejection in liver transplantation, tolerance in bone marrow transplantation, infection in transplantation like cardiac, small bowel, lung and renal transplantation are thirsty areas of research in the present scenario [4].

Table 2: Growth in Number of Transplanted Organs, 2005-2006

Transplanted Organs	2005	2006	Percent Change
Kidney	16,076	16,646	3.5%
Deceased donor	9,508	10,212	7.4%
Living donor	6,568	6,434	-2.0%
Pancreas	1,368	1,304	-4.7%
Pancreas alone	129	98	-24.0%
Pancreas after kidney	343	292	-14.9%
Kidney-pancreas	896	914	2.0%
Liver	6,000	6,136	2.3%
Deceased donor	5,679	5,849	3.0%
Living donor	321	287	-10.6%
Intestine	68	60	-11.8%
Deceased donor	63	57	-9.5%
Living donor	5	3	-40.0%
Heart	2,062	2,147	4.1%
Deceased donor	2,062	2,146	4.1%
Living donor	-	1	-
Lung	1,403	1,401	-0.1%
Deceased donor	1,402	1,397	-0.4%
Living donor	1	4	300.0%
Heart-lung	34	31	-8.8%
Total	27,530	28,291	2.8%
Deceased donor	20,633	21,561	4.5%
Living donor	6,897	6,730	-2.4%

Source: ***2007 OPTN/SRTR Annual Report***

Among all the transplantation that takes place world wide, the kidneys and livers may be transplanted from a *living donor*, since people are born with an extra kidney and the liver is regenerative. Even a lung can be transplanted from a living donor, but this is still very rare. For these procedures, a patient will generally find a willing donor in a friend or family member. If the donor is a match, they can proceed directly to the surgery stage. A smaller number of living transplants come from charitable people donating for the general good. The Table 2 represents the growth in number of transplanted organs in year 2005-2006 performed in the USA.

1.1.1 Types of Transplants

There are a big number of different types of organ transplant. The most popular are autograft, allograft and xenograft *etc.*, which are described as follows:

1.1.2 Autograft

It is tissue transplanted from one part of the body to another in the same individual. Sometimes this is done with surplus tissue, or tissue that can regenerate, or tissues

more desperately needed elsewhere {examples include skin grafts, vein extraction for Coronary Artery Bypass Graft surgery (CABG) *etc.,*} [5]. Sometimes this is done to remove the tissue and then treat it or the person, before returning it (examples include stem-cell autograft and storing blood in advance of surgery).

1.1.3 Allograft

An allograft is a transplanted organ or tissue from a genetically non-identical member of the same species. Most human tissue and organ transplants are allografts. This type of transplantation requires immunosuppressive drugs to prevent the body's antibodies from rejecting and destroying the new organ. This dramatically affects the entire immune system making the body vulnerable to pathogens [6].

1.1.4 Isograft

It is subset of allografts in which organs or tissues are transplanted from donor to genetically identical recipient (such as an identical twin). Isografts are differentiated from other types of transplants because while they are anatomically identical to allografts, they are closer to autografts in terms of the recipient's immune response.

1.1.5 Xenograft and Xenotransplantation

Xenotransplantation is transplantation of organ or tissue from one species(s) to another. Xenotransplantation is often an extremely dangerous type of transplant. Examples include porcine heart valves, which are quite common and successful, a baboon (African and Asian monkeys)-to-human heart (failed), and piscine-primate (fish to non-human primate) islet (*i.e.,* pancreatic or insular tissue), the latter research study directed for potential human use if successful.

1.1.6 Split Transplants

Sometimes, a deceased-donor organ (specifically the liver) may be divided between two recipients, especially an adult and a child [7, 8].

1.1.7 Domino Transplants

This operation is usually performed for cystic fibrosis as both lungs need to be replaced and it is a technically easier operation to replace the heart and lungs. As the recipient's native heart is usually healthy, this can then itself be transplanted into someone needing a heart transplant. That term is also used for a special form of liver transplant, in which the recipient suffers from familial amyloidotic polyneuropathy in

which the liver (slowly) produces a protein that damages other organs; their liver can be transplanted into an older patient who is likely to die from other causes before a problem arises [9].

1.1.8 History

Successful human allotransplants have a relatively long history; the operative skills were present long before the necessities for post-operative survival. Rejection and the side effects of preventing rejection (especially infection and nephropathy) were, are, and may always be the key problem [10].

Several apocryphal accounts of transplants exist well prior to the scientific understanding and advancements that would be necessary for them to have actually occurred. The Chinese physician Pien Chi'ao [11], reportedly exchanged hearts between a man of strong spirit but weak will with one of a man of weak spirit but strong will in an attempt to achieve balance in each man. Roman Catholic accounts report the third-century saints Damian and Cosmas as replacing the gangrenous leg of the Roman Deacon Justinian with the leg of a recently deceased Ethiopian [12]. Most accounts have the saints performing the transplant in the fourth century, decades after their deaths; some accounts have them only instructing living surgeons who performed the procedure.

The more likely accounts of early transplants deal with skin transplantation. The first reasonable account is of the Indian surgeon Sushruta in the second century BC, who used autografted skin transplantation in nose reconstruction rhinoplasty [13]. Success or failure of these procedures is not well documented. Centuries later, Italian surgeon Gaspare Tagliacozzi [14] performed successful skin autografts; he also failed consistently with allografts, offering the first suggestion of rejection centuries before that mechanism could possibly be understood. He attributed it to the "force and power of individuality" in his 1596 work *De Curtorum Chirurgia per Insitionem* [15].

The first successful corneal allograft transplant was performed in 1837 in a gazelle model [16]; the first successful human corneal transplant, a keratoplastic operation, was performed by Eduard Zirm in Austria in 1905 [17]. Pioneering work in the surgical technique of transplantation was made in the early 1900s by the French surgeon Alexis Carrel and Charles Guthrie for the transplantation of arteries or veins [18, 19]. Their skilful anastomosis operations, the new suturing techniques, laid the

groundwork for later transplant surgery and won Carrel the 1912 Nobel Prize for Medicine or Physiology [20]. Dr. Carrel was the first to identify the problem of rejection, which remained insurmountable for decades.

Major steps in skin transplantation occurred during World War I, notably in the work of Harold Gillies at Aldershot [21]. Among his advances was the tube pedicle graft, maintaining a flesh connection from the donor site until the graft established its own blood flow. Gillies' assistant, Archibald McIndoe, carried on the work into World War II as reconstructive surgery [22].

The first attempted human deceased-donor transplant was performed by the Ukrainian surgeon Yu Yu Voronoy in the 1930s; rejection resulted in failure. Joseph Murray performed the first successful transplant, a kidney transplant between identical twins, in 1954, as no immunosuppression was necessary in genetically identical twins [23].

In the late 1940s Peter Medawar [24], working for the National Institute for Medical Research, improved the understanding of rejection. Identifying the immune reactions in 1951 Medawar suggested that immunosuppressive drugs could be used. Cortisone discovered and the more effective azathioprine was identified in 1959 [25], but it was not until the discovery of cyclosporine in 1970 that transplant surgery found a sufficiently powerful immunosuppressive [26].

Dr. Murray's success with the kidney led to attempts with other organs [27]. There was a successful deceased-donor lung transplant into a lung cancer sufferer in 1963 by James Hardy in Jackson, Mississippi [28]. The patient survived for eighteen days before dying of kidney failure. Thomas Starzl of Denver attempted a liver transplant in the same year, but was not successful until 1967 [29].

The heart was a major prize for transplant surgeons. But, as far as rejection issues are concerned, the heart deteriorates within minutes of death so any operation would have to be performed at great speed. The development of the heart-lung machine was also needed. Lung pioneer James Hardy attempted a human heart transplant in 1964 [30], but a premature failure of the recipient's heart caught Hardy with no human donor, he used a chimpanzee heart which failed very quickly. The first success was achieved on December 3rd 1967 by Christiaan Barnard in Cape Town, South Africa. Louis Washkansky, the recipient, survived for eighteen days [31].

It was the advent of cyclosporine that altered transplants from research surgery to life-saving treatment. In 1967, the International Surgical Society awarded Dr. Cooley its highest honor, the Renée Lebiche Prize. In its citation, the society called him "the most valuable surgeon of the heart and blood vessel anywhere in the world." [32]. Fourteen of his patients died within six months. By 1984 two-thirds of all heart transplant patients survived for five years or more. With organ transplants becoming commonplace, limited only by donors, surgeons moved onto more risky fields, multiple organ transplants on humans and whole-body transplant research on animals. On March 9th 1981 the first successful heart-lung transplant took place at Stanford University Hospital. The surgeon, Bruce Reitz, credited the patient's recovery to cyclosporine-A [33].

Recently, researchers are looking into steroid-free immunosuppression. This would avoid the side-effects of steroids. Short-term outcomes are outstanding, long-term outcomes are still unknown. In addition, calcineurin-inhibitor-free immunosuppression is currently undergoing extensive trialing, the result of which would be to allow sufficient immunosuppression, without the nephrotoxicity associated with standard regimens that include calcineurin inhibitors. Positive results have yet to be demonstrated in any trial. An FDA approved immune function test from Cylex has shown effectiveness in minimizing the risk of infection and rejection in post-transplant patients [34] by enabling doctors to tailor immunosuppressant drug regimens. By keeping a patient's immune function within a certain window, doctors can adjust drug levels to prevent organ rejection while avoiding infection. Such information could help physicians reduce the use of immunosuppressive drugs, lowering drug therapy expenses while reducing the morbidity associated with liver biopsies, improve the daily life of transplant patients, and could prolong the life of the transplanted organ.

1.1.8.1 Bench Marks of Successful Transplants

- 1905: First successful cornea transplant by Eduard Zirm
- 1954: First successful kidney transplant by Joseph Murray (Boston, U.S.A.)
- 1966: First successful pancreas transplant by Richard Lillehei and William Kelly (Minnesota, U.S.A.)
- 1967: First successful liver transplant by Thomas Starzl (Denver, U.S.A.)

- 1967: First successful heart transplant by Christiaan Barnard (Cape Town, South Africa)
- 1970: First successful monkey head transplant by Robert White (Cleveland, U.S.A.) [35].
- 1981: First successful heart/lung transplant by Bruce Reitz (Stanford, U.S.A.)
- 1983: First successful lung lobe transplant by Joel Cooper (Toronto, Canada)
- 1986: First successful double-lung transplant (Ann Harrison) by Joel Cooper (Toronto, Canada)
- 1987: First successful whole lung transplant by Joel Cooper (St. Louis, U.S.A.) [36].
- 1995: First successful Laproscopic live-donor nephrectomy by Lloyd Ratner and Louis Kavoussi (Baltimore, U.S.A.)
- 1998: First successful live-donor partial pancreas transplant by David Sutherland (Minnesota, U.S.A.)
- 1998: First successful hand transplant (France)
- 2005: First successful partial face transplant (France)
- 2006: First successful penis transplant (China)

1.1.9 Issues with Organ Transplantation

1.1.9.1 Availability of Donors

Clinical organ transplantation provides a way of giving the gift of life to patients with terminal failure of vital organs, which requires the participation of other fellow human beings and of society by donating organs from deceased or living individuals. The increasing incidence of vital organ failure and the inadequate supply of organs, especially from cadavers, have created a wide gap between organ supply and organ demand, which has resulted in very long waiting times to receive an organ as well as an increasing number of deaths while waiting. These events have raised many ethical, moral and societal issues regarding supply, the methods of organ allocation, use of living donors as volunteers including minors. It has also led to the practice of organ sale by entrepreneurs for financial gains in some parts of the world through exploitation of the poor, for the benefit of the wealthy.

1.1.9.2 Comparative Costs

One of the driving forces for illegal organ trafficking and "transplantation tourism" is the price differences for organs and transplant surgeries in different areas of the world. According to the New England Journal of Medicine, a human kidney can be purchased in Manila for $1000- $2000, but in urban Latin America a kidney may cost more than $10,000. Kidneys in South Africa have sold for as high as $20,000 [37]. Price disparities based on donor race are a driving force of attractive organ sales in South Africa, as well as in other parts of the world. The Voluntary Health Association of India reports the prospect of such a small fortune has enticed about 2,000 impoverished Indians to sell a kidney every year as the price in India is substantially lower compared to the price in the West. In China, a kidney transplant operation runs for around $70,000, liver for $160,000, and heart for $120,000 [38].

1.1.9.3 Safety

Safety is the major concern for both donor, if alive and recipients in case of organ transplantation. Likewise compensation for donors increases the risk of introducing diseased organs to recipients because these donors often yield from poorer populations, unable to receive health care regularly and organ dealers may evade disease screening processes. In most cases one major payment been issued and no follow up care for the donor. There has been a possibility of transfer of the fatal diseases to recipients with the organ which has to be transplanted. Experts say that the diseases did not show up on screening tests is probably because they were contracted within three weeks before the donor's death, so antibodies wouldn't have existed in high enough numbers to detect. Therefore, always sensitive screening tests should be required, which could pick up antibodies sooner.

1.1.9.4 Organ Transplant Laws

Both developing and developed countries have forged various policies to try to increase the safety and availability of organ transplants to their citizens. Brazil, Italy, Poland and Spain have ruled all adults potential donors with the "opting out" policy, unless they attain cards specifying not to be. Iran is the only country in the world where it is lawful for one citizen to sell an organ to another for transplantation [39]. The Indian government is facing difficulty in tracking the flourishing organ black market in the country. Other countries victimized by illegal organ trade have

implemented legislative reactions. Despite these efforts, illegal organ trafficking continues to thrive and can be attributed to corruption in healthcare systems, which has been traced as high up as the doctors themselves [39].

1.1.9.5 Ethical Concerns

The existence and distribution of organ transplantation procedures in developing countries, while almost always beneficial to those receiving them, raise many ethical concerns. Both the source and method of obtaining the organ to transplant are major ethical issues to consider, as well as the notion of distributive justice. The World Health Organization argues that transplantations promote health, but the notion of "transplantation tourism" has the potential to violate human rights or exploit the poor, to have unintended health consequences, and to provide unequal access to services, all of which ultimately may cause harm. Regardless of the "gift of life", in the context of developing countries, this might be coercive [40]. The practice of coercion could be considered exploitative of the poor population, violating basic human rights memorandum. Even within developed countries there is a concern that enthusiasm for increasing the supply of organs may trample on the respect for the right to life.

1.1.10 Transplant Rejection

Transplant rejection occurs when the immune system of the recipient of a transplant attacks the transplanted organ or tissue. This is because a normal healthy human immune system can distinguish foreign tissues and attempts to destroy them, just as it attempts to destroy infective organisms such as bacteria and viruses [41].

1.1.10.1 Types of Rejection

The immune system comprises all the elements in the body that keep bacteria, microbes, viruses, toxins and parasites from destroying your organs and tissues. In other words, the immune system works to destroy any harmful foreign matter that ends up in the body. When the system is working correctly, it can distinguish most foreign cells from cells produced by the body. A transplanted organ is made entirely of foreign cells, of course, which means the body will attack it if left to its own devices. To minimize the immune response, transplant teams make sure donors and recipients have matching blood and tissue types. But even with a good match, the body will see the new cells as foreign matter and reject the organ (destroy it cell by cell). The tissues from identical twins can be fully accepted.

There are three types of rejection that might occur following a transplant:

1.1.10.2 Hyperacute Rejection

Hyperacute rejection is a complement-mediated response in recipients with pre-existing antibodies to the donor (for example, ABO blood type antibodies). Hyperacute rejection occurs within minutes and the transplant must be immediately removed to prevent a severe systemic inflammatory response. Rapid agglutination of the blood occurs. This is a particular risk in kidney transplants, and so a prospective cytotoxic cross match is performed prior to kidney transplantation to ensure that antibodies to the donor are not present. For other organs, hyperacute rejection is prevented by transplanting only ABO-compatible grafts. Hyperacute rejection is the likely outcome of xenotransplanted organs.

1.1.10.3 Acute Rejection

Acute rejection is generally acknowledged to be mediated by T cell responses to proteins from the donor organ which differ from those found in the recipient (unlike antibody-mediated hyperacute rejection, development of T-cell responses first occurs several days after a transplant if the patient is not taking immunosuppressant drugs. Since the development of powerful immunosuppressive drugs such as cyclosporine, tacrolimus and rapamycin, the incidence of acute rejection has been greatly decreased. However, organ transplant recipients can develop acute rejection episodes months to years after transplantation. Acute rejection episodes can destroy the transplant if it is not recognized and treated appropriately. Episodes occur in around 60-75% of first kidney transplants, and 50 to 60% of liver transplants. A single episode is not a cause for concern if recognized and treated promptly and rarely leads to organ failure, but recurrent episodes are associated with chronic rejection of grafts. The bulk of the immune system response is to the Major Histocompatibility Complex (MHC) proteins. MHC proteins are involved in the presentation of foreign antigens to T-cells, and receptors on the surface of the T-cell (TCR) are uniquely suited to recognition of proteins of this type. MHC are highly variable between individuals, and therefore the T-cells from the host recognize the foreign MHC with a very high frequency leading to powerful immune responses that cause rejection of transplanted tissue. Identical twins and cloned tissue are MHC matched, and are therefore not subject to T-cell mediated rejection [42, 43].

The diagnosis of acute rejection relies on the clinical data including patient signs and symptoms, laboratory testing and ultimately a liver biopsy. The biopsy is interpreted by a pathologist who notes changes in the tissue that suggest rejection. Histologically acute rejection is characterized by three main features. First, a predominately T-cell rich lymphocytic infiltrate is often present and may be accompanied by a heterogeneous infiltrate including eosinophils, scattered plasma cells and neutrophils. An abundance of eosinophils within the mixed infiltrate is a helpful feature of acute rejection. Secondly, evidence of injury to the bile ducts is often seen, manifested by the presence of intraepithelial lymphocytes and loss of epithelial cell polarity. Lastly, injury to the vessels may be seen as endothelialitis. Typically this involves portal vein branches, but may include central veins and sinusoids. For a pathologist who evaluates biopsies for liver disease following transplantation, it is important to be aware of the disorders that commonly occur in this setting and their histologic differences. These include autoimmune hepatitis, which will often have a large number of plasma cells; post-transplant lymphoproliferative disorder with its characteristic monotonous infiltrate and primary biliary cirrhosis which may have focal injury to bile ducts unlike the more monotonous process seen in acute rejection.

1.1.10.4 Chronic Rejection

Chronic rejection is used to describe all long term loss of function in organ transplants associated with fibrosis of the internal blood vessels of the transplant, but this is now termed chronic allograft vasculopathy and the term chronic rejection is reserved for those cases where the process is shown to be due to a chronic alloreactive immune response [44]. It can be caused by a member of the Minor Histocompatibility Complex such as the H-Y gene of the male Y chromosome. This usually leads to need for a new organ after a decade or so.

1.1.11 Rejection Mechanisms

Rejection is an adaptive immune response and is mediated through both T cell and humoral immune (antibodies) mechanisms. The number of mismatched alleles determines the speed and magnitude of the rejection response. Different grafts usually have a proclivity to a certain mechanism of rejection (Table 3).

Table 3: Organ and corresponding Rejection mechanism

Organ/tissue	Mechanism
Blood	Antibodies (isohaemagglutinins)
Kidney	Antibodies, Cell Mediated Immunity (CMI)
Heart	Antibodies, Cell Mediated Immunity (CMI)
Skin	Cell Mediated Immunity (CMI)
Bone marrow	Cell Mediated Immunity (CMI)
Cornea	Usually accepted unless vascularised, Cell Mediated Immunity (CMI)

1.1.11.1 Prevention of Rejection

Rejection is prevented with a combination of drugs including:

- **Calcineurin inhibitors**
  - Cyclosporine
  - Tacrolimus
- **mTOR inhibitors**
  - Sirolimus
  - Everolimus
- **Anti-proliferatives**
  - Azathioprine
  - Mycophenolic acid
- **Corticosteroids**
  - Prednisolone
  - Hydrocortisone
- **Antibodies**
  - Monoclonal anti-IL-2Rα receptor antibodies
    - Basiliximab
    - Daclizumab
  - Polyclonal anti-T-cell antibodies
    - Anti-thymocyte globulin (ATG)
    - Anti-lymphocyte globulin (ALG)

Generally a *triple therapy* regimen of a calcineurin inhibitor, an anti-proliferative, and a corticosteroid is used, although local protocols vary. mTOR inhibitors can be used to provide calcineurin-inhibitor or steroid-free regimes in selected patients. An FDA approved immune function test from Cylex has shown effectiveness in minimizing the

risk of infection and rejection in post-transplant patients by enabling doctors to tailor immunosuppressant drug regimens [34].

1.1.11.2 Treatment of Rejection

Acute rejection is normally treated initially with a short course of high-dose methylprednisolone, which is usually sufficient to treat successfully. If this is not enough, the course can be repeated or ATG can be given. Acute rejection refractory to these treatments may require plasma exchanges to remove antibodies to the transplant. The monoclonal anti-T cell antibody OKT3 was formerly used in the prevention of rejection, and is occasionally used in treatment of severe acute rejection [45]. Acute rejection usually begins after the first week of transplantation, and most likely occurs to some degree in all transplants (except between identical twins). It is caused by mismatched Human Leukocyte Antigen (HLA) antigens that are present on all cells. The reason that acute rejection occurs a week after transplantation is because the T-cells involved in rejection must differentiate and the antibodies in response to the allograft must be produced before rejection is initiated. These T-cells cause the graft cells to lyse or produce cytokines that recruit other inflammatory cells, eventually causing necrosis of allograft tissue. Endothelial cells in vascularized grafts such as kidneys are some of the earliest victims of acute rejection. The risk of acute rejection is highest in the first 3 months after transplantation, and is lowered by immunosuppressive agents in maintenance therapy. The onset of acute rejection is combated by episodic treatment.

Chronic rejection is irreversible and cannot be treated effectively. The only definitive treatment is re-transplantation, if necessary. This would typically be ten years after a transplant, and this may entail returning to a transplant queue [46-48].

1.1.12 General Approach to Organ Transplantation Therapy

The *first principle* of transplantation is careful patient preparation and selection of the best available ABO blood type-compatible HLA match for organ donation.

Second, a multitiered approach to immunosuppressive drug therapy, similar to that in cancer chemotherapy, is employed. Several agents are used simultaneously, each of which is directed at a different molecular target within the allograft response. Synergistic effects permit use of the various agents at relatively low doses, thereby limiting specific toxicities while maximizing the immunosuppressive effect.

The *third principle* is the greater immunosuppression is required to gain early engraftment and/or to treat established rejection than to maintain long-term immunosuppression. Therefore, intensive induction and lower-dose maintenance drug protocols are employed.

Fourth, careful investigation of each episode of transplant dysfunction is required, including evaluation for rejection, drug toxicity, and infection, keeping in mind that these problems can and often do coexist. Organ-specific problems (e.g. obstruction in the case of kidney transplants) must also be considered.

The *fifth principle*, which is common to all drugs, is that a drug should be reduced or withdrawn if its toxicity exceeds its benefit [49].

1.2 Immunosupressents and Transplantation

In transplantation, the major classes of immunosuppressive drugs used today are:

- Glucocorticicoids,
- Calcineurin inhibitors,
- Antiproliferative / Antimetabolic agents, and
- Biologics (antibodies).

These drugs have met with a high degree of clinical success in treating conditions such as acute immune rejection of organ transplants and severe autoimmune diseases [50]. However, such therapies require life long use and nonspecifically suppress the entire immune system, exposing patients to considerable higher risks of infection and cancer. The calcineurin inhibitors and glucocorticoids, in particular, are nephrotoxic and diabetogenic, respectively, thus restricting their usefulness in a variety of clinical settings [51,52].

Monoclonal and polyclonal antibody preparations directed at reactive T cells are important adjunct therapies and provide a unique opportunity to target specifically immune-reactive cells. Finally, newer small molecules and antibodies have expended the arsenal of immunosuppressives. In particular, mTOR (mammalian target of rapamycin) inhibitors (sirolimus, everolimus) and anti-CD25 [interleukin (IL)-2 receptor] antibodies (basiliximab, daclizumab) target growth factor pathways, substantially limiting clonal expansion and thus potentially promoting tolerance. Immunosuppressive drugs are used more commonly and the goal of

immunosuppressive therapy is to find a balance between prevention of graft rejection, long-term graft survival, and frequency of adverse events [53].

1.2.1 Biologic Induction Therapy

Induction therapy with polyclonal and monoclonal antibodies (mAbs) has been an important component of immunosuppression dating back to the 1960s [54]. Over the past 40 years, several polyclonal antilymphocyte preparations have been used in renal transplantation; however, only 2 preparations are currently FDA approved: lymphocyte immune globulin (ATGAM) and antithymocyte globulin (thymoglobulin) [55]. Another important milestone in biologic therapy was the development of mAbs and the introduction of the murine anti-CD3 mAb (muromonab-CD3 or OKT3) [56]. These are widely used to delay the use of the nephrotoxic calcineurin inhibitors or to intensify the initial immunosuppressive therapy in patients at high risk of rejection (*i.e.,* repeat transplants, broadly presensitized patients). Table 4 depicts the site of action of the selected immunosuppression agent on the T cell activation [57].

Table 4: Sites of action of selected immunosuppression agents on T cell activation

Drug	Site of Action
Glucocorticoids	Glucocorticoid response elements in DNA (regulate gene transcription)
Muromonab-CD3	T-cell receptor complex (blocks antigen recognition)
Cyclosporine	Calcineurin (inhibits phosphatase activity)
Tacrolimus	Calcineurin (inhibits phosphatase activity)
Azathioprine	Deoxyribonucleic acid (faIse nucleotide incorporation)
Mycophenolate Mofetil	Inosine monophosphate dehy drogenase (inhibits activity)
Daclizumab, Basiliximab	IL-2 receptor (block IL-2-mediated T-cell activation)
Sirolimus	Protein kinase involved in cellcycle progression (mTOR) (inhibits activity)

Biologic agents for induction therapy in the prophylaxis of rejection currently are used in approximately 70% of de novo transplant patients and have been propelled by several factors, including the introduction of the safe anti-IL-2R antibodies and the emergence of antithymocyte globulin as a safer and more effective alternative to lymphocyte immune globulin or muromonab-CD3 mAb. Biologics for induction can be divided into 2 groups:

Depleting agents consist of lymphocytes immunoglobulin, antithymocyte globulin and muromonab-CD3 (the latter also produces immune modulation); their efficacy

derives from their ability to deplete the recipient's CD3-positive cells at the time of transplantation and the antigen presentation [58, 59]. *Immune modulators* compromise, the anti-IL-2R mAbs, does not deplet T lymphocytes, but rather blocks IL-2-mediated T-cell activation by binding to the α chain of IL-2R [49].

1.2.2 Maintenance Immunotherapy

The basic immunosuppressive protocols include administration of multiple drugs simultaneously. Therapy typically involves a calcineurin inhibitor, glucocorticoids, and mycophenolate mofetil (a purine metabolism inhibitor), each directed at a discrete site in T-cell activation [60, 61]. Glucocorticoids, azathioprine, cyclosporine, tacrolimus, mycophenolate mofetil, sirolimus, and various monoclonal and polyclonal antibodies are approved for transplantation. Glucocorticoid-free regimens have achieved special prominence in recent successes in using pancreatic islet transplants to treat patients with type I diabetes mellitus. Protocols employing steroid withdrawal or steroid avoidance are being evaluated in many transplant centers. Short-term results are good, but the effects on long-term graft function are unknown [62]. Recent data suggests that calcineurin inhibitors may shorten graft half-life by their nephrotoxic effects [63, 64]. Protocols under evaluation include calcineurin dose reduction or switching from calcineurin to sirolimus-based immunosuppressive therapy at 3 to 4 months [65].

1.2.3 Therapy for Established Rejection

Although low doses of prednisolone, calcineurin inhibitors, purine metabolism inhibitors, or sirolimus are effective in preventing acute cellular rejection, they are less effective in blocking activated T lymphocytes, and thus are not very effective against established, acute rejection or for the total prevention of chronic rejection [55]. Therefore, treatment of established rejection requires the use of agents directed against activated T cells. These include glucocorticoids in high doses (pulse therapy), polyclonal antilymphocyte antibodies, or muromonabcm mAb [66].

1.3 Modified Release Dosage Forms: Pharmaceutical Overview

Oral solid dosage forms are the preferred route for many drugs and are still the most widely used formulations for new and existing modified release (MR) products. However, this creates a challenge for drug discovery and development since many drugs do not have suitable pharmacokinetics to achieve this goal via a conventional, immediate release (IR) dosage form.

IR dosage forms are, by definition, designed to release the drug immediately after ingestion and not to delay or slow the disintegration or dissolution process. For such formulations development relies upon a variety of pharmacokinetic (PK) and pharmacodynamic (PD) measurements in order to establish the dose and frequency required to achieve efficacy. This approach works because the performance of the dosage form is consistent and can be accurately predicted. Drug is delivered rapidly to the same site of absorption (*i.e.,* upper small intestine) every time. Consequently, the key performance variables are limited to those associated with administration (e.g. drug loading, number of units, fed / fasting state etc). Formulation performance and regional variation within the gastrointestinal (GI) tract are effectively removed from the equation [67].

The United States Pharmacopoeia definition of an MR system is that: "the drug release characteristics of time, course and/or location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage forms..." [68].

The challenge for the successful development of a modified release (MR) dosage form is that formulation performance and regional variation within the GI tract are the key performance variables. The initial development of prototypes is often based on *in silico* predictions and *in vitro* profiles. But these must then be correlated with *in vivo* performance and often initial prototypes fail to behave as predicted [69]. There is a variety of types of MR dosage forms (Table 5) [70], but for all the PK profile is the product of multiple factors including transit of the dosage form through the GI tract, rate of drug delivery and regional differences in bioavailability which are influenced by permeability, fluid volume and composition, contractile activity, pH and gut wall enzymes and transporters.

Table 5: Definitions: Types of Modified Release Drug Delivery Systems

Modified release	Umbrella term for all non-conventional dosage forms: Dosage forms whose drug release characteristics of time course and / or location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage forms.
Delayed release	Release of drug at a time other than immediately following oral administration. (e.g. enteric coated).
Extended/ prolonged release	Formulated to make the drug available over an extended period after ingestion. This allows a reduction (at least 2-fold) in dosing frequency compared to a drug presented in a conventional dosage form.
Controlled release	Extended release dosage form, formulated such that drug is released at a planned, predictable and slower than normal rate.
Sustained release	Extended release dosage form, formulated such that initial plasma concentrations are sufficient to achieve therapeutic effect, and are then sustained.
Gastroretentive	A dosage form that is retained in stomach (*i.e.,* above the absorption window) to deliver drug to upper intestine.

As a result, there are many missing links between MR dosage form administration and the PK profile and hence, interpreting the PK data can be extremely challenging. Even the best informed scientist with detailed knowledge of the systems involved ultimately has to speculate as to the reasons for failure. This speculative approach can result in many iterations of the same process as the development team follows a "modify – test – modify – test" pattern with no guarantee of success.

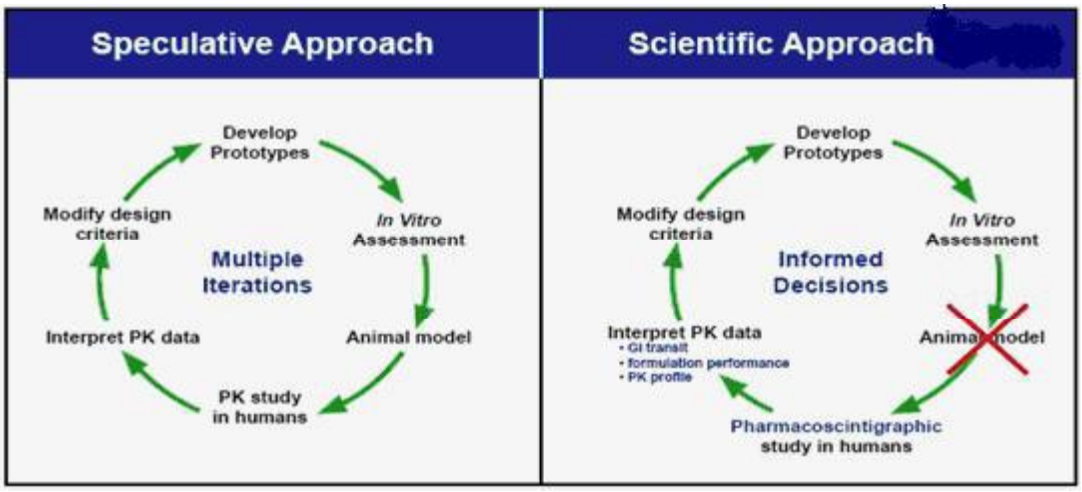


Figure 1: Design, evaluation and validation of MR formulations

Consequently, the traditional approach of developing prototype dosage forms and testing via PK assessments is suboptimal for MR dosage forms (Figure 1) [71]. The alternative, data-driven approach is based on understanding the factors that influence the PK profile. This approach provides detailed information on formulation

performance and facilitates scientifically valid, informed decisions regarding the improvements needed to achieve the target PK profile.

The IR drug delivery system is not suitable for all the drugs as modification is required for patient's compliance as well as drug characteristics point of view. On this context the modified-release preparation might be beneficial. MR dosage form offers following major advantages over IR dosage form.

- Reduce the dosing frequency and improve patient compliance;
- Reduce fluctuations (peaks and troughs) in drug plasma concentrations, in order to reduce concentration-related side effects or improve effectiveness;
- Control the site of drug delivery in the gastrointestinal (GI) tract.

Generally, the use of an MR preparation cannot be justified unless it offers clear clinical advantages over, often less expensive, conventional-release preparations.

1.3.1 Improving patient compliance

By slowing the rate of drug release, MR preparations allow drugs with short half-lives to be administered less frequently. It is generally well accepted that, for the majority of patients, reducing the dosing frequency to once or twice daily improves compliance [72]. However, there is little good quality evidence to suggest that once daily dosing has a clear clinical advantage over twice daily dosing. Whilst this improvement has reached statistical significance in some studies [73, 74, 77, 78].

1.3.2 Reducing fluctuations in drug plasma concentrations

By slowing the rate of drug release, and hence absorption, MR preparations aims to provide close to constant plasma concentrations over a prolonged period of time [75].

Leveling out the plasma profile can be advantageous, but only for drugs where there is a close correlation between plasma concentration and either therapeutic effect or toxicity. Reducing high peak plasma concentrations can reduce concentration-related side-effects, particularly for rapidly absorbed drugs such as nifedipine [76]. Minimizing the trough may improve effectiveness, for example in maintaining 24-hour blood pressure control with certain antihypertensive agents [77]. MR preparations are often used for drugs with a narrow therapeutic index, such as theophylline and lithium. This may help to maintain the plasma concentration within the limits of effectiveness and toxicity [75].

1.3.3 Controlling the site of delivery

MR preparations can be developed to deliver a drug to a specific site in the GI tract. For example, enteric coated preparations are a direct delivery to the small intestine, preventing drug release in the stomach. This aims to either protect the stomach from the drug, or protect the drug from the degrading environment of the stomach. Other preparations, such as those containing aminosalicylates for inflammatory bowel disease, are formulated to allow site specific delivery to the colon or small intestine to exert local effects.

For some drugs, an MR preparation can offer clinical advantages. If theophylline is prescribed for nocturnal asthma and early morning wheezing, an MR preparation given as a single dose at night is advisable [78]. The slow release of theophylline decreases side-effects seen with rapid absorption and ensures therapeutic levels are maintained throughout the night, provided a suitable dose is prescribed. If nifedipine is prescribed for angina or hypertension, an MR preparation is recommended. Short-acting preparations have been associated with large variations in blood pressure and reflex tachycardia [78]. A recent, randomized double-blind trial in 6321 patients with hypertension found *Adalat LA* (a once daily MR nifedipine preparation) to be as effective as co-amilozide (amiloride/hydrochlorothiazide) in preventing overall cardiovascular or cerebrovascular complications [79]. PRODIGY guidance for prescribing nifedipine in angina and hypertension only offers the drug as an MR preparation prescribed by brand name. Conventional-release carbamazepine is often prescribed three or four times a day for epilepsy. MR preparations allow twice daily dosing and may also reduce the incidence of dose-related side-effects [72, 78]. Drugs with a narrow therapeutic index, those which are rapidly absorbed, and those with a short duration of action are often formulated into MR preparations.

1.3.4 Shortcomings of MR preparation

The *'once a day is best'* belief is heavily promoted but can have drawbacks. Patients may forget that a dose has already been taken and repeat it later in the day [74]. They may also miss a dose completely. Missing a dose is a particular problem with a once daily preparation as it can result in long periods where drug plasma concentrations are sub-therapeutic. All the drugs can not be delivered as MR preparations. Apart from formulations that control the site of drug delivery, most MR preparations slow the rate

of drug release. To ensure maximum absorption from these preparations, it is essential that the drug is well absorbed throughout the entire GI tract. Drugs which are absorbed only at specific sites, such as iron [78], folic acid and vitamin B12, are not suitable as MR preparations [75]. The release of a drug from an MR preparation is dependent on changes in GI transit time. In patients with 'GI hurry' some of the dose may be lost if the preparation passes through the body before drug release is complete. Conversely, if the transit time is delayed, excessive release of the drug or 'dose dumping' can occur. This may cause local GI damage (e.g. with NSAIDs), or acute systemic toxicity. Breaking, chewing or crushing an MR preparation can result in the immediate release of possibly toxic amounts of drug. Therefore, patients should be told to swallow MR preparations whole. Drugs with a long duration of action, such as amitriptyline, do not need to be given frequently and an MR preparation is unnecessary. MR analgesic preparations with a slow onset of action are of little value when immediate pain relief is required, the therapeutic area should be considered for the use of MR preparations. In context to patients compliance many reports shown that compliance is either the same, or slightly improved, with a once daily preparation [73, 74, 80-83].

1.3.5 Current Oral Modified Release Technologies

Over many years, approaches and technologies in the area of MR oral drug delivery have been developed to:

- Extend the release of drug over a number of hours, an effect accomplished either by combining the drug with release-retardant materials to form a matrix core, or applying release-modifying film coatings to cores containing the drug; and,
- Delay the release of drug for a period of time, usually through the application of an externally applied enteric coating.

Technologies are available for the formulation, development and production of MR tablets and multi-particulates such as drug-loaded pellets and granules, mini-tablets and drug crystals. Over the last decade, the approach to MR oral drug delivery systems has changed from a line extension strategy to a clinically superior approach for marketed drugs as well as for new chemical entities. The benefits offered by MR systems include reduced dosing frequency with improved patient compliance, better

and more uniform clinical effects with lower incidence of side effects and possible enhanced bioavailability. The rational design of MR systems, where biological and physicomechanical considerations have been taken into account during formulation of MR dosage form has alleviated the risk of 'dose dumping' *in vivo*. In addition to the pharmacological and patient benefits, MR dosage forms offer commercial opportunity through intellectual property, brand differentiation and recognition, plus the potential to license technologies to other companies.

Although there have been a number of technologies reported (Table 6) [70], only a limited number have been commercialized due to high raw material and/or specialist production requirements. The most successful MR oral delivery systems are those that include readily available raw materials and manufacturing processes.

Table 6: Current Oral Modified Release Technologies

Pharmaceutical modification	The rate of drug release is reduced by increasing particle size or forming insoluble crystals e.g., Tegretol Retard or Adalat Retard.
Coated pellets	Drug pellets are coated with a slowly dissolving polymer of varying thickness for varied release. The pellets can either be compressed into a tablet or put in a gelatin capsule e.g., Fenbid, Slo-Phyllin or Inderal-LA.
Insoluble matrix	The drug is dispersed within an insoluble porous matrix. As fluid enters the matrix, the drug is dissolved and diffuses out slowly e.g., Slow-K, Imdur or Betaloc-SA.
Eroding matrix	The drug is dispersed within a soluble matrix. As the matrix is eroded, the drug is slowly released e.g., MST Continus or Phyllocontin Continus.
Osmotic pump	The drug and an osmotic agent are enclosed by a semi permeable membrane. As water is drawn into the tablet, dissolved drug is released in a controlled way through a laser-drilled hole e.g., Volmax, Adalat LA.
pH sensitive coating	The formulation is coated with a polymer of pH dependent solubility for site specific delivery. This can either avoid drug release in the stomach (enteric coating) e.g., Nu-Seals Aspirin, or specifically deliver drug to the colon e.g., Asacol.

This includes technologies that modify the site of drug delivery. The successful formulation of an MR device requires a comprehensive understanding of the mechanisms of drug release from the macroscopic effects of size, shape and structure through to chemistry and molecular interactions. Multiparticulate dosage forms have been shown to be less prone to food effects than monolithic [84] and are often the preferred formulation for extended and/or delayed release. Film coating is an ideal process for the production of extended release multiparticulate dosage forms. For application in extended release delivery systems, film coats with well-characterized permeability properties are essential.

Ethyl cellulose is the most widely used water insoluble polymer in extended release coating applications. The advantageous properties of this polymer are that it is tasteless, odorless and has the ability to form tough, flexible coatings.

While ethyl cellulose was initially used in organic solvent-based solutions, the application of water based dispersions of ethyl cellulose (such as Surelease® aqueous ethylcellulose dispersion) is now commonplace in the pharmaceutical industry and is the preferred method of choice for extended release coating.

Several studies have investigated the parameters that influence the rate of drug release from ethyl cellulose-coated pellets. The effects of drug solubility, coating equipment, coating process and core characteristics have been demonstrated previously. Study on release of metoclopramide hydrochloride (a very water soluble cationic drug) and diclofenac sodium (a sparingly soluble anionic drug) from pellets coated with Surelease containing hydroxypropylmethylcellulose (HPMC) at different coating loads was investigated by Rajabi-Siahboomi et. al., [85]. Results reveal that release rates of either drug at each coating composition decreased as the coating load increased and addition of HPMC E15 increased the release rates of both drugs compared to pellets coated only with Surelease. This happening may be due to the leakage of the soluble part of the film (HPMC E15) during dissolution, which left pores for drug release. The drug's aqueous solubility is of utmost importance to the formulation of coated pellets when the mechanism of release is mainly by transport of the dissolved drug via diffusion through the film or through water-filled pores or channels within the coating [85].

Aqueous solubility is also a major factor affecting the osmotic pressure inside coated pellets when they are in contact with the dissolution medium. The difference between the osmotic pressure inside the pellets and the dissolution medium plays an important role in the release of drug from coated pellets [86]. An important MR technology is delayed release through application of gastro-resistant coatings. In this case, a coating layer is applied to the dosage form, either multiparticulate or monolithic, providing protection to the stomach from the drug or protecting the drug from exposure to acidic gastric fluids. The majority of modern enteric coatings rely on polymers containing carboxylic acid groups as the functional moiety. These groups remain unionized in the low pH environment of the stomach but start to ionize as the dosage form passes into

the small intestine. As the pH level rises above the point of dissolution, the polymer is ionized and the drug is released.

In the past, enteric coating systems have required the use of non-aqueous solvents for application; however, the majority of new enteric coating developments are based on aqueous enteric polymeric systems. The advantages offered by aqueous systems include:

- lower raw material costs;
- avoidance of capital cost for solvent recovery and explosion-proof equipment, with a safer working environment in development and production;
- environmentally friendly;
- faster processing time, while still providing reliable enteric performance; and
- Faster development and scale-up process.

For the monolithic matrix approach for slow/controlled drug delivery, cellulose ethers and, more specifically, hypromellose (HPMC) are the most widely used polymers. When HPMC polymers within the matrix are exposed to an aqueous medium, they undergo rapid hydration and chain relaxation to form a viscose gelatinous layer, which is commonly termed 'gel layer', at the surfaces of the tablet [87]. Failure to generate a uniform and coherent gel may cause a rapid drug release. It is the subsequent physicochemical characteristics of this gel layer that control water uptake and the drug release mechanism from the matrix.

Hydration of HPMC polymers is not affected by natural variation in pH level throughout the gastrointestinal tract. They hydrate rapidly to form a gel layer in the acid conditions of the stomach. However, pH of the dissolution fluid has been reported to affect drug release rate [88]. These effects are primarily due to pH influence on the solubility of the drug, which, in turn, may affect the mechanism of drug release from HPMC matrices.

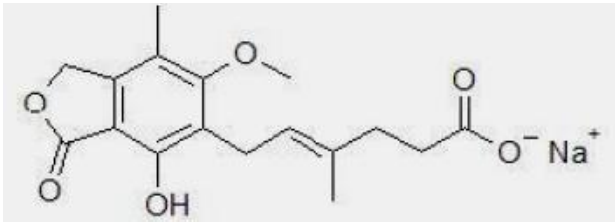
The benefits offered by MR systems include reduced dosing frequency with improved patient compliance, better and more uniform clinical effects with lower incidence of side effects and possible enhanced bioavailability.

Although a rapid burst release of soluble drugs from the external layer may occur, drug release is controlled either by diffusion of the drugs through the gel layer or by

gradual erosion of the gel, exposing fresh surfaces containing drug to the dissolution medium. Diffusion is the dominant mechanism controlling the dissolution of water-soluble drugs, and erosion of the matrix is the dominant mechanism controlling the release of water-insoluble drugs [89]. However, generally, release of drugs will occur by a mixture of these two mechanisms. Although interactions between water, polymer, excipients and drug are the main factors determining drug release, formulation variables such as drug and polymer levels and types, drug/polymer/excipients ratios and polymer and drug particle size have been reported to influence the rate of drug release.

Future trends in the development of MR delivery systems may involve employing novel strategies in terms of defining drug release characteristics, utilizing non-traditional materials or using geometric design techniques as well as material science to achieve specific release characteristics. To achieve commercial success, the systems must provide an opportunity for intellectual property to maintain exclusivity (be patentable), the product should be cost-effective to ensure healthy return on investment.

1.4 Drug Profile

Drug Name	:	Mycophenolate Sodium
Molecular formula	:	$C_{17}\ H_{19}\ O_6\ Na$.
Chemical Structure	:	
Chemical Name	:	Sodium 4(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate
CAS No.	:	37415-62-6
Molecular wt of MPS	:	343.32
Molecular wt of MPA	:	320.34
Category	:	Immunosuppressant
Physicochemical Properties		
Solubility	:	Highly soluble in aqueous media at physiological pH and practically insoluble in 0.1N HCl
Nature	:	White to off-white crystalline powder.

1.4.1 Mechanism of Action

MPA is an uncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the *de novo* pathway of guanosine nucleotide synthesis without incorporation to DNA. Because T- and B-lymphocytes are critically dependent for their proliferation on *de novo* synthesis of purines, whereas other cell types can utilize salvage pathways, MPA has potent cytostatic effects on lymphocytes.

Mycophenolate sodium has been shown to prevent the occurrence of acute rejection in rat models of kidney and heart allotransplantation. Mycophenolate sodium also decreases antibody production in mice.

1.4.2 Pharmacokinetics

Absorption

In-vitro studies demonstrated that the enteric-coated Mycophenolate Sodium tablet does not release MPA under acidic conditions (pH < 5) as in the stomach but is highly soluble in neutral pH conditions as in the intestine. Following Mycophenolate Sodium oral administration without food in several pharmacokinetic studies conducted in renal transplant patients, consistent with its enteric-coated formulation, the median delay (T_{lag}) in the rise of MPA concentration ranged between 0.25 and 1.25 hours and the median time to maximum concentration (T_{max}) of MPA ranged between 1.5 and 2.75 hours. In comparison, following the administration of Mycophenolate Mofetil, the median T_{max} ranged between 0.5 and 1.0 hours. In stable renal transplant patients on cyclosporine, USP (MODIFIED) based immunosuppressant, gastrointestinal absorption and absolute bioavailability of MPA following the administration of Mycophenolate Sodium delayed-release tablet was 93% and 72%, respectively. Mycophenolate Sodium pharmacokinetics is dose proportional over the dose range of 360 to 2160 mg.

Distribution

The mean (± SD) volume of distribution at steady state and elimination phase for MPA is 54 (± 25) L and 112 (± 48) L, respectively. MPA is highly protein bound to albumin, > 98%. The protein binding of its metabolite, mycophenolic acid glucuronide (MPAG) is 82%. The free MPA concentration may increase under

conditions of decreased protein binding (uremia, hepatic failure, and hypoalbuminemia).

Metabolism

MPA is metabolized principally by glucuronyl transferase to glucuronidated metabolites. The phenolic glucuronide of MPA, mycophenolic acid glucuronide (MPAG), is the predominant metabolite of MPA and does not manifest pharmacological activity. The acyl glucuronide is a minor metabolite and has comparable pharmacological activity to MPA. In stable renal transplant patients on cyclosporine, USP (MODIFIED) based immunosuppressant, approximately 28% of the oral Mycophenolate Sodium dose was converted to MPAG by presystemic metabolism. The AUC ratio of MPA:MPAG:acyl glucuronide is approximately 1:24:0.28 at steady state. The mean clearance of MPA was 140 (± 30) mL/min.

Elimination

The majority of MPA dose administered is eliminated in the urine primarily as MPAG (>60%) and approximately 3% as unchanged MPA following Mycophenolate Sodium administration to stable renal transplant patients. The mean renal clearance of MPAG was 15.5 (±5.9) mL/min. MPAG is also secreted in the bile and available for deconjugation by gut flora. MPA resulting from the deconjugation may then be reabsorbed and produce a second peak of MPA approximately 6–8 hours after Mycophenolate Sodium dosing. The mean elimination half-life of MPA and MPAG ranged between 8 and 16 hours, and 13 and 17 hours, respectively.

Food Effect

Compared to the fasting state, administration of Mycophenolate Sodium 720 mg with a high-fat meal (55 g fat, 1000 calories) had no effect on the systemic exposure (AUC) of MPA. However, there was a 33% decrease in the maximal concentration (Cmax), a 3.5-hour delay in the Tlag (range, -6 to 18 hours), and 5.0-hour delay in the T_{max} (range, -9 to 20 hours) of MPA. To avoid the variability in MPA absorption between doses, Myfortic should be taken on an empty stomach.

Indication

Mycophenolic acid is indicated for the prophylaxis of organ rejection in patients receiving allogeneic renal transplants, administered in combination with cyclosporine and corticosteroids.

Adverse Effects

The principal adverse reactions associated with the administration of Mycophenolic acid include constipation, nausea, and urinary tract infection in de novo patients and nausea, diarrhea and nasopharyngitis in maintenance patients.

Available Dosage Form

Mycophenolic acid is available for oral use as delayed-release tablets containing either 180 mg or 360 mg of mycophenolic acid as Sodium Salt (Myfortic)®

1.5 Polymers Used in the Investigation

1.5.1 Hydroxypropylmethylcellulose

Nonproprietary Names

BP : Hypromellose

JP : Hydroxypropylmethylcellulose

PhEur : Methylhydroxypropylcellulosum

USP : Hydroxypropyl methylcellulose

Synonyms

Benecel MHPC; Cellulose, hydroxypropyl methyl ether; E464; HPMC; *Methocel*; methylcellulose propylene glycol ether; methyl hydroxypropylcellulose; *Metolose*; *Pharmacoat*.

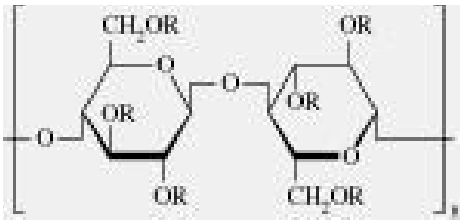
Chemical Name and CAS Registry Number

Cellulose, 2-Hydroxypropyl methyl ether [9004-65-3]

The PhEur describes hydroxypropyl methylcellulose as a partly *O*-methylated and *O*-(2-hydroxypropylated) cellulose. It is available in several grades which vary in viscosity and extent of substitution. Grades may be distinguished by appending a number indicative of the apparent viscosity, in mPa s, of a 2% w/w aqueous solution at 20°C. Hydroxypropyl methylcellulose defined in the USP specifies the substitution

type by appending a four digit number to the nonproprietary name, e.g., hydroxypropyl methylcellulose 1828. The first two digits refer to the approximate percentage content of the methoxy group (OCH_3). The second two digits refer to the approximate percentage content of the hydroxypropoxy group ($OCH_2CHOHCH_3$), calculated on a dried basis. Molecular weight is approximately 10 000-1 500 000.

Structural Formula



Where R is H, CH_3, or $[CH_3CH(OH)CH_2]$.

Functional Category

Coating agent; film-former; rate-controlling polymer for sustained release; stabilizing agent; suspending agent; tablet binder; viscosity-increasing agent.

Applications in Pharmaceutical Formulation or Technology

In oral products, hydroxypropyl methylcellulose is primarily used as a tablet binder, in film-coating, and as an extended-release tablet matrix. Concentrations of between 2-5% w/w may be used as a binder in either wet- or dry-granulation processes. High viscosity grades may be used to retard the release of drugs from a matrix at levels 10-80% w/w in tablets and capsules.

Depending upon the viscosity grade, concentrations between 2-20% w/w are used as film-forming solutions to film-coat tablets. Lower viscosity grades are used in aqueous film-coating solutions while higher viscosity grades are used with organic solvents.

In addition, hydroxypropyl methylcellulose is used in the manufacture of capsules, as an adhesive in plastic bandages and as a wetting agent for hard contact lenses. It is also widely used in cosmetics and food products.

Description

Hydroxypropyl methylcellulose is an odorless and tasteless, white or creamy-white colored fibrous or granular powder.

Pharmacopeial Specifications

Test	JP	Ph.Eur.	USP
Identification	+	+	+
Appearance of solution	+	+	—
pH (1% w/w solution)	5.0-8.0	5.5-8.0	—
Apparent viscosity	+	+	+
Loss on drying	<5.0%	<10.0%	<5.0%
Residue on ignition			
For viscosity grade > 50 mPa s	<1.5%	—	<1.5%
For viscosity grade £ 50 mPa s	<1.5%	—	<3.0%
For type 1828 of all viscosities	<1.5%	—	<5.0%
Sulfated ash	—	<1.0%	—
Chlorides	—	<0.5%	—
Heavy metals	—	<20 ppm	<0.001%
Methoxy content			
Type 1828	—	—	16.5-20.0%
Type 2208	19.0-24.0%	—	19.0-24.0%
Type 2906	27.0-30.0%	—	27.0-30.0%
Type 2910	28.0-30.0%	—	28.0-30.0%
Hydroxypropoxy content			
Type 1828	—	—	23.0-32.0%
Type 2208	4.0-12.0%	—	4.0-12.0%

Typical Properties

Acidity/alkalinity:

pH = 5.5-8.0 for a 1% w/w aqueous solution.

Ash: 1.5-3.0%, depending upon the grade.

Autoignition temperature: 360°C

Density (*bulk*): 0.341 g/cm^3

Density (*tapped*): 0.557 g/cm^3

Density (*true*): 1.326 g/cm^3

Melting point: browns at 190-200°C; chars at 225-230°C. Glass transition temperature is 170-180°C.

Moisture content: hydroxypropyl methylcellulose absorbs moisture from the atmosphere, the amount of water absorbed depending upon the initial moisture content and the temperature and relative humidity of the surrounding air.

Solubility: soluble in cold water, forming a viscous colloidal solution; practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol. Certain grades of hydroxypropyl methylcellulose are soluble in aqueous acetone solutions, mixtures of dichloromethane and propan-2-ol, and other organic solvents. *See also* Section 11.

Specific gravity: 1.26

Viscosity (*dynamic*): a wide range of viscosity types are commercially available. Aqueous solutions are most commonly prepared although hydroxypropyl methylcellulose may also be dissolved in aqueous alcohols such as ethanol and propan-2-ol provided the alcohol content is less than 50% w/w. Dichloromethane and ethanol mixtures may also be used to prepare viscous hydroxypropyl methylcellulose solutions. Solutions prepared using organic solvents tend to be more viscous; increasing concentration also produces more viscous solutions.

1.5.2 Polyethylene oxide

Nonproprietary Names

USP: Polyethylene oxide

Synonyms

Polyox; polyoxirane; polyoxyethylene.

Chemical Name and CAS Registry Number

Polyethylene oxide [25322-68-3]

Molecular Weight

***Polyox* grade**	**Approximate number of repeating units**	**Approximate molecular weight**
WSR 301	90 000	4 000 000

Structural Formula: $(CH_2CH_2O)_n$

Polyethylene oxide is a nonionic homopolymer of ethylene oxide, where *n* represents the average number of oxyethylene groups (about 2000 to over 100 000). It may contain up to 3% of silicon dioxide.

Functional Category

Hydrophilic matrix formation; mucoadhesive; tablet binder; thickening agent.

Applications in Pharmaceutical Formulation or Technology

Polyethylene oxide can be used as a tablet binder at concentrations from 5-85%. The higher molecular weight grades provide delayed drug release via the hydrophilic matrix approach.

Polyethylene oxide has been shown to be an excellent mucoadhesive polymer. Low levels of polyethylene oxide are effective thickeners. Polyethyleneoxide films demonstrate good lubricity when wet. Polyethylene oxide can be radiation crosslinked in solution to produce a hydrogel. The hydrogels so produced have been used in wound-care applications.

Description

White to off-white, free-flowing powder. Slight ammoniacal odour.

Pharmacopoeial Specifications

Test	USP
Identification	+
Loss on drying (105°C for 45 min)	< 1%
Nonsilicon dioxide loss on ignition	< 2%
Silicon dioxide	< 3%
Heavy metals	< 0.001%
Free ethylene oxide	< 10 ppm
Organic volatile impurities	+
Trichloroethylene	< 100 ppm
Viscosity	+

Typical Properties

Angle of repose: 34°

Density (*true*): 1.3 g/cc

Melting point: 65-70°C

Moisture content: < 1%.

Solubility: polyethylene oxide is soluble in water and a number of common organic solvents such as acetonitrile, chloroform, and methylene chloride. It is insoluble in aliphatic hydrocarbons, ethylene glycol, and most alcohols.

1.5.3 Glyceryl behenate (Compritol 888)

Synonyms

Compritrol 888; docosanoic acid; 1,2,3-propanetriyl ester; glyceryl tribehenate

Chemical Name

Glyceryl behenate: $C_{69}H_{134}O_6$

Empirical Formula

Glyceryl behenate: $C_{69}H_{134}O_6$

Appearance

Fine white powder with a faint odor.

Typical Properties

Acid value: ≤ 4

Color: < 5 (Gardner scale)

Free glycerin: ≤ 1.0%

Heavy metals: ≤ 0.001%

Iodine value: ≤ 3

Melting point: about 70°C

1-Monoglycerides: 12.0-18.0%

Residue on ignition: ≤ 0.1%

Saponification value: 145-165

Solubility: soluble, when heated, in chloroform and dichloromethane, practically insoluble in ethanol (95%), hexane, mineral oil, and water.

Safety: LD_{50} (mouse, oral); 5 g/kg [12]

Comments: glyceryl behenate is a mixture of glycerides of fatty acids, mainly behenic acid, and is used as a tablet and capsule lubricant.

Functional Category

Tablet and capsule diluent; tablet and capsule lubricant, sustained release formulation

Applications in Pharmaceutical Formulation or Technology

Glyceryl behenate is used in oral solid-dosage pharmaceutical formulations as a lubricant. It is used as a lipophilic matrix for sustained-release tablet and capsule formulations. Tablet formulations may be prepared by granulation or a hot melt technique, the former producing tablets that have the faster-release profile.

Use Concentration (%w/w)

Matrix for sustained release 10-50

Tablet lubricant 0.5-5

1.5.4 Xanthan gum

Nonproprietary Names

BP: Xanthan gum

PhEur: Xanthani gummi

USP: Xanthan gum

Synonyms

Corn sugar gum; E415; *Keltrol*; *Merezan*; polysaccharide B-1459; *Rhodigel*; xantham gum.

Chemical Name and CAS Registry Number

Xanthan gum [11138-66-2]

Empirical Formula and Molecular Weight

The USP describes xanthan gum as a high molecular weight polysaccharide gum. It contains D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid, and is prepared as the sodium, potassium, or calcium salt. The molecular weight is approximately 2×10^6.

Structural Formula

Each xanthan gum repeat unit contains five sugar residues: two glucose; two mannose, and one glucuronic acid. The polymer backbone consists of four b-D-

glucose units linked at the 1 and 4 positions, and is therefore identical in structure to cellulose. Trisaccharide side chains on alternating anhydroglucose units distinguish xanthan from cellulose. Each side chain comprises a glucuronic acid residue between two mannose units. At most of the terminal mannose units is a pyruvate moiety; the mannose nearest the main chain carries a single group at C-6. The resulting stiff polymer chain may exist in solution, as a single, double, or triple helix which interacts with other xanthan gum molecules to form complex, loosely bound networks.

Functional Category

Stabilizing agent; suspending agent; viscosity-increasing agent.

Applications in Pharmaceutical Formulation or Technology

Xanthan gum is widely used in oral and topical pharmaceutical formulations, cosmetics, and foods as a suspending and stabilizing agent. It is also used as a thickening and emulsifying agent.

It is nontoxic, compatible with most other pharmaceutical ingredients, and has good stability and viscosity properties over a wide pH and temperature range.

When mixed with certain inorganic suspending agents, such as magnesium aluminum silicate, or organic gums, synergistic rheological effects occur. In general, mixtures of xanthan gum and magnesium aluminum silicate in ratios between 1:2 and 1:9 produce the optimum properties. Similarly, optimum synergistic effects are obtained with xanthan gum:guar gum ratios of between 3:7 and 1:9.

Although primarily used as a suspending agent xanthan gum has also been used to prepare sustained-release matrix tablets.

Description

Xanthan gum occurs as a cream or white-colored, odorless, free-flowing, fine powder.

Typical Properties

Acidity/alkalinity: pH = 6-8 for a 1% w/v aqueous solution.

Freezing point: 0°C for a 1% w/v aqueous solution.

Heat of combustion: 14.6 J/g (3.5 cal/g)

Melting point: chars at 270°C

Particle size distribution: 100% less than 250 µm, 95% less than 177 µm in size for Rhodigel; 100% less than 177 µm, 92% less than 74 µm in size for Rhodigel 200.

Refractive index:

n_D^{20} = 1.333 for a 1% w/v aqueous solution.

Solubility: practically insoluble in ethanol and ether; soluble in cold or warm water.

Specific gravity: 1.600 at 25°C

Viscosity (dynamic): 1200-1600 mPa s.

Pharmacopeial Specifications

Test	PhEur	USP
Identification	+	+
Characters	+	—
pH	6.0-8.0	—
Viscosity	+	+
Microbial limits	+	+
Loss on drying	< 15.0%	< 15.0%
Ash	6.5-16.0%	6.5-16.0%
Arsenic	—	< 3 ppm
Heavy metals	—	< 0.003%
Lead	—	< 5 ppm
Isopropyl alcohol	< 750 ppm	< 0.075%
Other polysaccharides	+	—
Pyruvic acid	1.5%	—
Organic volatile impurities	—	+

1.6 Bioavailability: In-Terms of Modified Release Dosage Forms

Over the last 25 years, Pharmacokinetics (application of kinetics to a Pharmakon, the Greek word used to specify drugs and poisons) has emerged as an integral part of drug development, especially when identifying a drug's biological properties. The term, pharmacokinetics, thereby implies the time course and fate of drugs in the body. This general definition broadly embraces *absorption*, *distribution*, *metabolism*

(biotransformation) and *excretion* (ADME). The linking of Pharmacodynamics (response) and pharmacokinetics offers a composite understanding both about how the drug affects the body and how the body affects the drug. The most comprehensive insight about a drug's inherent pharmacokinetic properties is gained by studying an intravenous dose. This route of administration has the greatest quantitative potential, as it permits a mass balance approach to be applied to distribution, clearance and the body processes associated with excretion and metabolic elimination (e.g., renal, hepatic). The administration of a drug by other routes, notably oral, introduces an uncertainty that reflects the unknown fraction that is actually absorbed.

Consequently, such doses alone cannot accurately identify the distribution and clearance processes. The most important property of any non-intravenous dosage form, intended to treat a systemic condition, is the ability to deliver the active ingredient to the bloodstream in an amount sufficient to cause the desired response. This property of a dosage form has historically been identified as physiologic availability, biologic availability or *bioavailability*. Bioavailability captures two essential features, namely *how fast* the drug enters the systemic circulation (rate of absorption) and *how much* of the nominal strength enters the body (extent of absorption). Given that the therapeutic effect is a function of the drug concentration in a patient's blood, these two properties of non-intravenous dosage forms are (e.g., oral, inhalation, topical, patch, rectal, *etc.,*) in principle, important in identifying the response to a drug dose. Onset of response is linked to the *rate* of drug absorption whereas the time-dependent extent of response is linked to the *extent* of drug absorption. Orally administered products certainly represent the major pharmaceutical class in drug development and patient treatment. Bioavailability following oral doses may vary because of either patient-related or dosage-form-related factors. Patient factors can include the nature and timing of meals, age, disease, genetic traits and gastrointestinal physiology. While dosage form factors includes; chemical form of drug (e.g., salts. acid), physical properties (e.g., crystal structure, particle size) and an array of formulation (e.g., non-active ingredients) and manufacturing (e.g., tablet hardness) variables. Hence, at the time of formulation and development of the IR or MR preparations the formulator should consider above mentioned factors.

1.6.1 Comparative bioavailability: A universal approach

Most bioavailability studies, whether for a new or generic product, possess a common theme. A test is conducted to identify the quantitative nature of a specific product comparison. This comparison for a new drug may be, for example, to assess the performance of an oral formulation relative to that of an intravenous dose, or the performance of a modified-release formulation in comparison to a conventional one. For a generic product, it is typically a comparison of a competitive formulation with a reference product. Such commonality surrounding comparative bioavailability studies suggests a universal experimental approach.

Figure 2 shows the two primary metrics for such concentration versus time profiles are the area under the curve (AUC) and the maximum observed concentration (C_{max}); the former customarily includes the AUC to the last sampling time in a trial (AUC_t) and the extrapolated total AUC to time infinity (AUC_∞). The time at the maximum concentration (T_{max}) is also of some minor interest. This is an attempt to establish a drug's concentration versus time profile following product administration in some form of comparative test.

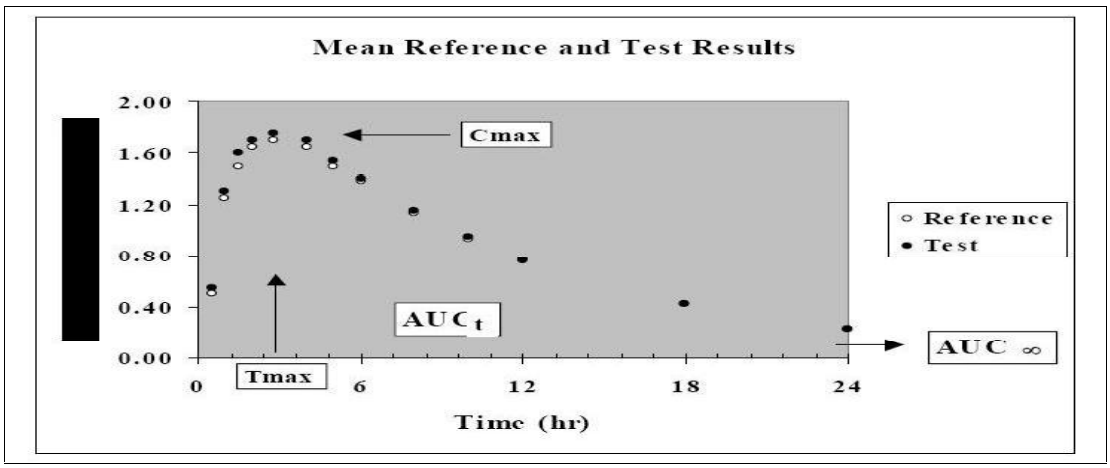


Figure 2: Illustration of the key metrics in a comparative bioavailability trial.

The maximum concentration (C_{max}) occurs at the T_{max}. The AUC_t is the total area under the concentration versus time profile to the last sampling time. The area to time infinity (AUC_∞) is the extrapolated area based on the AUCt and the terminal constant (λz).

After obtaining the profiles in a comparative trial, and computing the metrics, conclusions need to be reached regarding the comparison. Statistical methods are applied to test if the metrics are sufficiently similar to be considered equivalent. When the metrics are deemed equivalent, the drug concentration profiles are regarded as

fundamentally the same. To achieve this equivalence, the study products' geometric mean ratios (e.g. AUC test/AUC reference), as well as their projected 90% confidence intervals for the population mean ratio, must be located within an 80 to 125% window. For the maximum concentration (C_{max}) some regulatory agencies consider it adequate if only the mean ratios are within the interval (Figure 3).

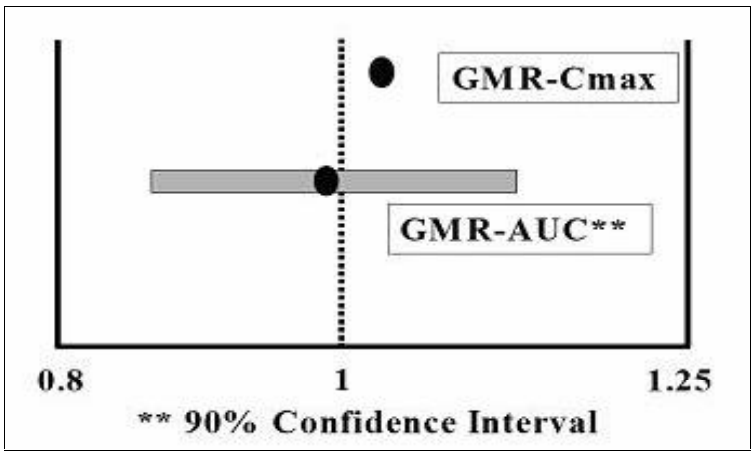


Figure 3: An illustration of the statistical criteria

Figure 3 shows an illustration of the statistical criteria to be satisfied to gain equivalence status in a comparative bioavailability assessment. For example in a bioequivalence trial, the geometric mean ratio for the test/reference Cmax (GMR Cmax) must be located between 0.8 and 1.25 the GMR AUC's (whether AUC or AUC) and their computed 90 % confidence intervals must reside completely within the 0.8 to 1.25.

1.6.2 Development of a new formulation (e.g. MR product)

The development of modified-release preparations have a clinical rationale as it may reduce dose related side effects, improve efficacy and add to- compliance to drug therapy.

Modified release products may be developed to reduced dose frequency which adds to convenience of use which in turn may facilitate compliance. Another rationale for developing modified release preparations is to smoothen the peaks of the plasma concentration curves in order to prevent peak concentration related adverse events.

Rarely a modified release preparation has been developed solely in order to mimic a TID or QID dosage schedule. In these cases the modified release preparation should be bioequivalent with the immediate release formulation given in dose schedule that is imitated.

In general modified-release formulations are not bioequivalent to their immediate release form. Consequently it might be difficult to assess whether the benefit/risk of the modified release is comparable to the corresponding doses of the immediately release form. Depending on the clinical setting additional clinical data will be required.

The first modified-release product requires an NDA. The purpose of the required studies is to determine if the following conditions are met:

- The drug product meets the controlled release claims made for it;
- The bioavailability profile rules out the occurrence of what is called "dose dumping", which is the premature release of the drug from the dosage form;
- The formulation provides consistent performance between individual dosage units;
- The steady state performance, in comparison to an available conventional product, is equivalent. If, based on accumulated evidence between circulating concentrations of the drug and response, the modified-release product is different, clinical studies will be needed to show the impact of such differences.

The pharmacokinetic studies required for modified-release products permit some flexibility, but shall include the following:

- A single dose crossover comparison of a conventional, immediate release, product and the modified release product (ideally, the study would also include a solution or suspension of the same drug in the same strength);
- A single dose food-effect study;
- A steady-state study.

1.6.2.1 Primary endpoints (Single Dose PK study)

Determine the time-dependent concentrations of the administered drug in the collected blood (or plasma/serum) of each subject following administration of the modified-release and immediate-release products.

1.6.2.2 Exploratory endpoints (Single Dose PK study)

Determine the Cmax, AUCt, AUC∞, Tmax, λz and half-life of the primary (and secondary) endpoints following the modified-release and immediate-release products, for each subject. Some agencies will also require the area over the usual dosing interval for the modified-release product.

Study elements (Single Dose PK study)

The fasting study shall be designed in such a way that the effects of the formulation can be distinguished from other factors. When two formulations are compared, a randomized two-period, two-sequence crossover study is considered the design of choice. An adequate washout period between periods is needed to avoid drug carryover effects. Doses are given to subjects following an overnight fast.

However, it should be recognized that differences in Cmax can be anticipated because the fundamental drug release properties for the modified-release and immediate-release products are different. The potential impact of such differences needs to be weighed in the light of concentration versus response evidence.

PK studies determine the effect of food upon the bioavailability of a modified-release product.

1.6.2.3 Primary endpoints (Single Dose PK study with food effects)

Determine the time-dependent concentrations of the administered drug in the collected blood (or plasma/serum) of each subject following administration of the product under fasting and fed conditions.

1.6.2.4 Exploratory endpoints (Single Dose PK study with food effects)

Determine the Cmax, AUCt, AUC∞, Tmax, λz and half-life of the primary (and secondary) endpoints following administration of the modified-release product in each period, for each subject.

1.6.2.5 Study elements (Single Dose PK study with food effects)

This fasting/fed study shall be designed in such a way that the potential effects of the meal upon the formulation can be distinguished from other factors. When the formulation is tested as required, a randomized two-period, two-sequence, crossover study is considered the design of choice. An adequate washout period between

periods is needed to avoid drug carryover effects. Doses are given to subjects following an overnight fast. In one period, the fast is continued, whereas in the other period a meal is given before dose administration. Typically, both fasting and fed periods become common four hours after dose administration when normal food ingestion cycles are permitted.

1.7 *In Vitro In Vivo* Correlation

1.7.1 *In Vitro-In Vivo* Correlations (IVIVC)

Development of an oral Drug Delivery System (DDS) usually starts with the establishment of a theoretical drug release profile for the formulation based on desirable target blood concentration and pharmacokinetic characteristics of the drug [90]. Based upon previous experience and the information available in the literature, various prototype formulations utilizing different ingredients and processes are developed, and the formulations are characterized for pharmacokinetics. The desirable in *vivo* performance of this formulation, thus calculated, is used to determine *in vitro* targets for the formulation. Subsequently, the formulations are evaluated for real experimental drug release performance [91]. Drug release profiles thus obtained, however, may not reflect the actual behavior of the drug dosage form *in vivo* during a bioavailability study in human volunteers (*i.e., biostudy).* Hence, to ascertain its true behavior *in vivo,* biostudy is usually obligatorily required [92]. Nonetheless, carrying out such a biostudy is not only arduous, expensive and extensive, but involves several ethical issues too.

1.7.2 Significance of IVIVC

To circumnavigate the hassles of biostudy, the concept of *in vitro-in vivo* correlation (IVIVC) has been gaining increased attention. The IVIVC is defined as "a quantitative rational relationship between a biological property and a parameter desired from a biological property produced by a dosage form and a physicochemical property or characteristic of the same dosage form" [93]. In simpler terms, IVIVC is a relationship between *in vitro* test property and *in vivo* response. The biological property most commonly used is one or more pharmacokinetic parameters, like plasma drug concentration (e.g., C_{max}) or amount of drug absorbed (e.g., AUC) obtained following administration of the dosage form [94]. The physicochemical property most commonly used is the rate and extent of drug release from a dosage form [92-94].

The main objective of developing an IVIVC is to allow dissolution testing to serve as a surrogate for human bioequivalence studies during development, scale-up or manufacturing site changes of dosage forms. The IVIVC's help to reduce costs speed up the product development process and reduce the need to perform costly bioavailability /bioequivalence studies [92, 94]. The IVIVC's can be required to demonstrate bioequivalence, when certain pre-approval changes are made in formulation, equipment, manufacturing process or in manufacturing site.

1.7.3 IVIVC Methodology

An important aspect of the development of a pharmaceutical product is to find an *in vitro* characteristic of potential formulations that reflects their *in vivo* performance. Suitable dissolution methods, therefore, are developed and validated to predict the *in vitro* performance of drug products. The intent in developing dissolution methods include establishing best IVIVC, sensitive enough to detect any changes in the *in vivo* performance due to small alterations in formulation, critical method/ process parameters or drug release patterns due to fluctuations associated with the environmental conditions to which it is exposed. Robustness of the dissolution method to various factors that may affect the results of the drug release study, and hence the drug release *in vivo,* are also established. The information obtained is combined and used to develop better performing prototype formulations. The best performing formulation amongst the prototype preparations is further optimized using validated dissolution methods and established IVIVC's. Subsequently, during the scale-up, post-approval use and post-approval changes in formulation, drug release studies are used to ensure bioequivalence of the product.

IVIVC models are developed to explore the relationships between *in vitro* dissolution/ release and *in vivo* absorption profiles. Upon the successful establishment of IVIVC, the model relationship facilitates the rational development and evaluation of immediate/extended-release dosage forms as a tool for formulation screening, and in setting up the dissolution specifications. Besides, the model can prove as a surrogate to the extensive, expensive and time consuming *in vivo* bioavailability testing on humans [95], whenever certain pre-approval and post-approval changes are made in the formulations, equipment, and manufacturing process or in the manufacturing site. IVIVC modeling involves three stages, which are model development, model validation, and model application to different scenarios. Broadly, three types of

IVIVC have been suggested [93-97] viz.:

- **Level A,** where a point to point correlation exists between the entire *in vitro* dissolution or drug release time course and the entire *in vivo* response time course, e.g., the time course of plasma drug concentration or amount of drug absorbed or *in vivo* dissolution of the drug from the dosage form.
- **Level B,** between single-point summary parameters that characterize *in vitro* and *in vivo* time courses, e.g., *in vitro* MDT with *in vivo* MDT or MRT or MAT.
- **Level C**, which establishes a single point relationship between *in vitro* dissolution parameter (e.g., $t_{50\%}$ or percent dissolved in 4 h) and a pharmacokinetic parameter (AUC, T_{max} or C_{max}).

The best IVIVC is explored through the application of convolution and deconvolution techniques which predict plasma drug concentration using a mathematic model as stated in Eqn. 01 based on convolution integral.

$$C_t = \int_0^t C_\delta (t-\mu)\, r_{abs}(\mu)\, du \qquad \text{... (01)}$$

The function C_δ represents the concentration time course that would result from the instantaneous absorption of a unit amount of drug, typically estimated from intravenous injection bolus data or reference oral solution data. C(t) is the plasma drug concentration from the oral drug delivery device at a specific time and r_{abs} is drug input rate of the oral solid dosage form and u is variable of integration. Besides the above convolution-deconvolution approach, IVIVC can be explored by correlating (i) stochastic moments like mean residence time (MRT) or mean absorption time (MAT) with *in vitro* mean dissolution time (MDT) or *in vivo* mean dissolution time (ii) cumulative relative fraction absorbed calculated by Wagner Nelson method with cumulative fraction dissolved *in vitro* [93-96]. Generally, Level A correlations are linear and are most useful and informative from regulatory perspective esp. for the CR DDS. However, it involves complex computations [98]. Albeit the level B and C correlations are easier to compute, they are based only on single point measures. Hence, they reveal miniscule information about the overall plasma level curve, the most important factor for drug performance in the patient, and accordingly are of very little value in CR formulation development from regulatory point of view.

2.0
Research Envisaged

2.0 RESEARCH ENVISAGED

Background: The field of transplantation remains one of the most innovative and pioneering areas in medicine today. The goal of transplantation is to prolong life. It is the most appropriate therapy for several conditions of end-stage organ failure *viz.,* renal, hepatic or cardiac failure. Renal transplantation rescues patients from the fate of chronic dialysis and improves patient quality of life to near normality [99-102]. In the recent years, the number of organ transplants performed has increased globally. With the advent of new therapeutic agents, transplantation has become a very successful procedure with success rates greater than 90% at one year post-transplant, regardless of the kind of transplanted organ [103, 104]. However, transplantation itself is not enough; there is a need to balance the amount of immunosuppression necessary to assure graft survival with the potential toxicity of the immunosuppressive agents. Long-term immunosuppressive therapy is essential, and advances in this area have resulted in a dramatic reduction in the frequency of transplant rejection. The last three decades have seen many advances in immunosuppressive treatment options for both immediate, post-transplant therapy and ongoing maintenance.

Regimen for immunosuppressants after Organ transplantation: Calcineurin inhibitors like cyclosporine and tacrolimus form the basis for immunosuppressive regimens [105], and great success has been obtained in combination with the other immunosuppressants *viz.,* inosine monophosphate dehydrogenase (IMPDH) inhibitors [106], as Mycophenolic acid and corticosteroids. In current clinical practice the oral formulations of available immunosuppressants are generally administered on a twice daily basis. Poor compliance has been shown to be one of the factors associated with late graft loss. This has demonstrated a statistically significant association for adherence to medication regimen with once daily dosing versus twice daily dosing in adult kidney transplant recipients. The efficacy of these immunosuppressants has also been associated with adverse effects. Hence, there is a need to develop formulations for these agents which not only increase patient compliance but also reduce the adverse effects [107].

Rationale for selection of immunosuppressant: Immunosuppressant treatment continues for lifetime. There is an upward trend in the market of immunosuppressants used in transplantation. On the basis of business potential, immunosuppressant drug was chosen for development. The immunosuppressant therapy regimen for kidney

transplantation comprises of a calcineurin inhibitor, an anti-proliferative agent, and a corticosteroid, although local protocols vary but almost every regimen includes mycophenolic acid MPA. The other drugs *viz.,* tacrolimus, steroids are available as once daily formulation. Only MPA is available as twice daily dosing. If MPA is also available once a day dosing then all the drugs will be administered once a day.

Rationale for selection of Mycophenolate Sodium: There are two agents available for mycophenolic acid i.e., as a prodrug mycophenolate mofetil (Cellcept, Roche Inc.) and the as mycophenolate sodium (Myfortic, Novartis). A patient needs to take 2-3 tablets of either Mycophenolate Mofetil or Mycophenolate Sodium each time. Out of these two agents, MPS was selected for the following reasons:

- Using sodium salt of MPA led to low tablet weight while mycophenolate mofetil leads to high weight. About 720 mg of MPA is euqivalent to approx.1000 mg and 770 mg of Mycophenolate Mofetil and Mycophenolate Sodium respectively.
- The MR formulation of mycophenolate mofetil may lead to bioavailability loss as the solubility of mycophenolate mofetil is low at higher pH.
- It has been reported in the literature that side effects due to MPS are less when compared with that of mycophenolate mofetil.

Mycophenolate Sodium is a relatively new immunosuppressive drug. It inhibits inosine monophosphate dehydrogenase, a key enzyme in the *de novo* pathway of purine synthesis, and thus causes lymphocyte-selective immunosuppression [110]. Large clinical trials have revealed the efficacy of Mycophenolate Sodium in the prevention of allograft rejection when administered together with cyclosporine or tacrolimus and corticosteroids [108]. Although the adverse effect profile of Mycophenolate Sodium is comparatively benign (anemia, leucopenia), the gastrointestinal adverse effects *viz.,* constipation, diarrhea, nausea, dyspepsia, vomiting, urinary tract infections and cytomegalo virus infections are of major concern [107]. These effects are partially explained by the increased immune suppression, by the mode of action and by interactions, particularly with other immunosuppressants [111]. The aetiology of the gastrointestinal adverse effects is still not completely clear. It has been proposed that the enhanced immunosuppression caused by the addition of Mycophenolate Sodium to other immunosuppressive

regimens increases the general susceptibility to common infectious diseases. Since the regimen involves at least 2-3 drugs to be given concomitantly, every additional immunosuppressant increases the risk, not only of specific and opportunistic infections, but also of the common infectious diseases [112-114]. Therapy depends upon the clinical gravity of the adverse effects and is therefore a case of waiting and observing. An adjustment of dosage of immunosuppressant according to the clinical situation and, particularly in the case of Mycophenolate Sodium, spreading the total dosage over more than 2 daily doses are often sufficient [115]. If adverse effects persist for a longer period of time and are of a more serious nature, a comprehensive invasive diagnostic process is necessary, including endoscopy and biopsy and the search for opportunistic infections. The side effects are often managed using dose reduction, temporary dose interruption or dose discontinuation [115, 116].

Rationale for MR dosage form of MPS

In current clinical practice, Mycophenolate Sodium is available as; an enteric formulation that delivers the active moiety mycophenolic acid (MPA) and as delayed-release tablets containing either 180 mg or 360 mg of mycophenolic acid. With current formulation, at least two tablets are generally administered on a twice daily basis which leads to patient compliance concerns. Hence, developing a patient compliant once a day dosage form of selected immunosuppressant is the need of the hour. Additionally, MPS is given in combination with other drugs *viz.,* tacrolimus which is available as once a day formulation. The modified release product of MPS will help in making the whole therapy as once a day obviating the need for second daily dose. Moreover, some of the adverse events of MPS seem to be related to C_{max}, whereas immunosuppressant activity is related to total exposure *i.e.,* AUC. Therefore, it is envisaged that with MR product where C_{max} levels would be lower than the conventional product, will also lead to reduction in adverse effects. The C_{min} levels will be maintained for longer duration of time suggesting very low probability of rejection. It is envisaged to prepare a new oral formulation of Mycophenolate Sodium with prolonged-release characteristics compared to the currently available immediate release dosage form.

Selection of Strength for MPS MR Tablets: In the present investigation, 720 mg was selected as the strength to be developed (Equivalent to two tablets of MPS or Mycophenolate Mofetil). This strength was selected on the basis of the FDA-

approved dosing schedule for mycophenolic acid wherein 720 mg MPA administered twice daily. Accordingly, we have recommended that we should develop strength of Mycophenolate sodium MR tablets equivalent to 720 mg of MPA so as to shift patients taking Myfortic 720 mg / Mycophenolate Mofetil 1000 mg administered twice daily (daily dose of 1440 mg MPA) to 2 tablets of Mycophenolate sodium MR 720mg tablets administered once a day (daily dose of 1440 mg MPA). The proposed indication for the modified release Mycophenolate Sodium tablet is in the (1) prophylaxis of transplant rejection (primary immunosuppression and maintenance therapy) in adult kidney or liver allograft recipients; (2) conversion from immediate release dosage form taken twice daily to prolonged-release formulation of Mycophenolate Sodium taken once daily in adult allograft recipients; (3) treatment of allograft rejection resistant to treatment with other immunosuppressive drugs in adult patients. The proposed dosage will be based on starting dose that depends on type of indication and transplanted organ and followed by therapeutic drug monitoring including measurement of Mycophenolate Acid in whole blood concentrations.

Objective of the study: The objectives of the present investigation were:

- Development of patient compliant once a day dosage form
- Safety and efficacy comparable with that of immediate release formulation
- Reduction in side effects related to C_{max} *viz.,* GI adverse effects
- Patentable and non infringing composition

The proposed indication for the modified release Mycophenolate Sodium tablet is in the (1) prophylaxis of transplant rejection (primary immunosuppression and maintenance therapy) in adult kidney or liver allograft recipients; (2) conversion from immediate release dosage form taken twice daily to prolonged-release formulation of Mycophenolate Sodium taken once daily in adult allograft recipients; (3) treatment of allograft rejection resistant to treatment with other immunosuppressive drugs in adult patients. The proposed dosage will be based on starting dose that depends on type of indication and transplanted organ and followed by therapeutic drug monitoring including measurement of Mycophenolate Acid in whole blood concentrations.

Target Product Profile: It has been reported in the literature that an AUC value of 30-60 μg.h/mL (for 1gm of MPA) is sufficient to prove efficacy. Patients with low AUC for MPA appear to be at high risk for experiencing graft rejection whereas high

target concentration can increase toxicity. The maintenance of C_{min} levels between 1-3 µg/mL for prolonged period is also related to efficacy. The pharmacokinetic parameters mentioned in the following table were selected as acceptance criteria for the product.

Target Product profile for proposed product

Target	Objectives to be achieved
The modified release product should have comparable total systemic exposure with the immediate release product. AUC 30 to 60 µg.h/mL AUC: T/R > 0.8 Lower level of 90% CI for AUC > 0.7	To ensure comparable efficacy with that of reference product.
The modified release product should produce similar or less fluctuations as the immediate release product Fluctuation: T/R preferably ≤ 1 but not more than 1.25 C_{max}: T/R preferably ≤ 1 but not more than 1.25	To reduce side effects related to high C_{max}
Inter individual variation for test: less than that of reference for all pharmacokinetic parameters	To achieve consistency in efficacy.
C_{min}: 0.8µg/mL or more T/R > 0.8	To prevent rejection

Based on the above mentioned target product profile, following dissolution profile was selected for the proposed product.

Target Dissolution Profile for the proposed product:

Percentage of Drug Dissolved	Time (Hours)
~20%	2±1
~50%	6±2
~85%	12±2

Formulation Strategies for MPS MR Dosage form: It is intended to exploit lipid as well as polymer matrix system to achieve controlled release of Mycophenolate Sodium over a period of 24 hrs. The study aims at examining the range of

polymers/combinations for preparation of Mycophenolate Sodium tablets with evaluation parameters *viz.*, hardness, assay, release profile. Of particular interest to the formulator is the comparative evaluation study of the drug release characteristics from such matrices and to quantify the differential release behavior and specific formulation requirement. In addition to the formulation development, the plan of the study included:

- Solubility determination of Mycophenolate Sodium
- Preformulation studies of the Mycophenolate Sodium with excipients
- Analytical method development and validation for the quantitation of Mycophenolate Sodium for assay and dissolution samples
- Formulation development for Mycophenolate Sodium
- *In vitro* dissolution tests and effects of diluents on drug release profile
- Fitting of the dissolution profile into mathematical models to ascertain the mechanism of drug release.
- Stability studies of the optimized formulations.
- Analytical method development and validation for quantitation of metabolite of Mycophenolate Sodium in plasma
- Preparation of scale up batches as per GMP requirement for phase I clinical trials
- Since it's a new drug, submission of documents as per schedule Y to seek permission of Drugs Controller General of India for Phase I clinical trials
- Performing *in vivo* bioavailability studies of the selected formulations in human volunteers and comparison of pharmacokinetic parameters with that of immediate release formulations
- Statistical evaluation of data obtained from pharmacokinetic studies is done using software
- Calculation of pharmacokinetic parameters using data obtained from the biostudy using WinNonlin version 5.2
- To establish an *in vitro in vivo* correlation

3.0
Materials and Methods

3.0 MATERIALS AND METHODS

3.1 Materials

Reagents and equipments used in the experiments are enlisted below:

3.1.1 Reagents Used

Sr. No.	Reagents	Batch No.	Make
1.	Sodium acetate	50670	Rankem
2.	Aerosil 200 (Silicon Di Oxide)	08248	Evonik
3.	Compritol 888 (Glyceryl Behenate)	03101 C	Colorcon
4.	Dibutyl sebacate	13052314170	Fluka Analytical
5.	Dichloromethane	B07 A/0107/3101	
6.	Dihydrogen Potassium Phosphate	MF7M571474	Merck
7.	Ethanol	A440G90	Merck
8.	Eudragit® RLPO (Meth-/ acrylates copolymers with trimethyl-ammonioethylmethacrylate)	G040836114	Rohm GmbH & Co. KG
9.	Eudragit® RS 30D 30% aqueous dispersion of Methacrylates copolymers with trimethyl-ammonioethylmethacrylate	G080718145	Evonik industries
10.	Eudragit® RSPO Meth-/ acrylates copolymers with trimethyl-ammonioethylmethacrylate	G060138003	Evonik industries
11.	Eudragit NE 30D (Neutral polymer of methacrylates)	B051012085	Evonik industries
12.	Hydrochloric Acid	E061/02026	S D Fine Chemical Ltd.
13.	Methocel K-15 M CR (Hydroxypropylmethylcellulose K 15)	SA03012	Colorcon
14.	Methocel K-100 M CR (Hydroxypropylmethylcellulose K 100)	VC29012N32	Colorcon
15.	Benecel MP 874	VK6899	Aqualon
16.	Isopropyl alcohol	B125K07	Rankem
17.	Kollicoat IR (Polyvinyl alcohol-polyethylene glycol graft copolymer)	72580716KO	BASF
18.	Kollicoat SR 30D (27% polyvinyl acetate as a filmformer and with 2.7% povidone and 0.3% sodium Iauryl	11385275LO	BASF

Sr. No.	Reagents	Batch No.	Make
	sulfate as stabilizers.)		
19.	DCL 21 (Lactose anhydrous)	1025611	DMV Pharma
20.	Magnesium stearate	C51225	Signet
21.	Mannitol	E2342	Signet
22.	Sodium hydroxide	88956612	Qualingens
23.	Orthophosphoric Acid	IO1A-0101-1809-13	S D Fine Chemical Ltd.
24.	PEG 400	01450°	Signet
25.	Sodium dihydrogen phosphate	MD6M552997	Merck
26.	Polyvinylpyrollidone K 30	G12156PTO	BASF Corp.
27.	Polyox WSR 301 (Nonionic, high molecular weight water-soluble poly (ethylene oxide) polymers)	SG0155S5B1	DOW Chem.
28.	Saturated Potassium nitrate	F123H45	Merck
29.	Sodium Hydroxide	88956612	Merck
30.	Triethylamine	R12345G78	S D Fine Chemical Ltd.
31.	Xantural 75 (Xanthan gum)	6L3980K	CP Kelco

3.1.2 Instruments

Sr. No.	Instruments	Batch No.	Make
1.	Clit Rotary Station Tablet Compression Machine	CPM 3-10	Clit
2.	Column Kromacil 4.6x125mm, 5µ	-	Lichosphere
3.	Differential Scanning Calorimeter (DSC)	DSC821	Mettler Toledo
4.	Dissolution Apparatus	TDT 08 L	Electrolab
5.	Electro lab Friabilator (USP)	EF-1W	Electrolab
6.	Electro lab Tab Density Apparatus (USP 1)	ETD-1020	Electrolab
7.	Erweka Hardness Tester	TBH 220D	Erweka
8.	HELOS Particle size analyzer	BF HELOS-Magic	Sympatec
9.	High Performance Liquid Chromatography (HPLC)	515 plus	Waters
10.	HPLC column Lichrosphere RP 18 (4.0 X 250 mm)	-	Lichrosphere
11.	Hypodermic Syringe	-	-
12.	Indwelling Venous Cannula	-	-
13.	Magumps Tray Dryer	GM12	Magumps
14.	Micro Centrifuge Tubes	MJI23	Tarsen
15.	Millipore HVLP type membrane filter	-	Millipore
16.	Portable Dehumidifier	TNV 3000SS	Tropical Nortec
17.	Retsch Rapid Dryer	TG100	Retsch
18.	Sartorius Loss On Drying Tester	MA45	Sartorius
19.	Sartorius Weighing Balance	TE 3135-D5	Sartorius
20.	Slug Punches	-	Pacific
21.	Stainless sieve (No: 24,30,40)	-	Pharma Spares
22.	UV-visible spectrometer	Evolution 300	Nicolet
23.	Vernier Calliper	CD-8	LS X Mitutoyo

3.2 Experimental

3.2.1 Preformulation Studies

3.2.1.1 Organoleptic properties

The state and crystal structure of the drug was recorded using descriptive terminology.

3.2.1.2 pH dependent solubility studies

For determining the saturation solubility study, 10 mg of the API (active pharmaceutical ingredients) was added into 100 mL of water and sonicated for 10 min. Additional quantity of drug was added and sonicated until undissolved particles were seen visually. The resultant dispersion was filtered through a 0.22µ filter and analyzed by HPLC. Then the drug was estimated in mg/mL. The same procedure was repeated for 0.1N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer.

3.2.1.3 Physical characteristics

3.2.1.3.1 Melting point:

The melting point of drug was measured by recording the temperature from the point where the melting starts until it ends using Digital Melting point apparatus.

3.2.1.3.2 Loss on drying

Loss on drying of the drug was determined using Infrared moisture balance to find out moisture content.

3.2.1.3.3 Bulk density

About 25.0 g of Mycophenolate Sodium was weighed and transferred in graduated measuring cylinder and the surface of the Mycophenolate Sodium was leveled by manual loose tapping and the loose volume was recorded [120]. Then, the study was carried out using tap density apparatus as per USP-I method with 500 taps. The final volume was noted down after completion of the test. Again the study was performed for another 750 taps. The test was continued with another 1250 taps till the difference between initial volume (V_a) and final volume (V_b) was not more than 2%.

The bulk density (BD) and the tapped density (TD) were determined using the following formula:

$$BD = \frac{Mass\ \ of\ \ Drug}{Bulk\ \ \ Volume} \qquad \text{(i)}$$

$$TD = \frac{Mass\ \ of\ \ Drug}{Tapped\ \ \ \ Volume} \qquad \text{(ii)}$$

3.2.1.3.4 Hausner ratio

The hausner ratio was calculated using the following formula [120]:

$$Hausner \quad Ratio = \frac{TD}{BD} \qquad \text{(iii)}$$

3.2.1.3.5 Compressibility index

The compressibility index for Mycophenolate Sodium was determined by using the following formula [120]:

$$Compressibility \quad index = \left(\frac{BD - \quad TD}{BD} \right) \times 100 \qquad \text{(iv)}$$

3.2.1.3.6 Angle of repose

Weighed quantity of Mycophenolate Sodium was taken and placed on to the glass funnel which was fitted with stand which was kept on to the graph paper [120]. The top of flat side of funnel was gently tapped such that Mycophenolate Sodium fell on to the paper in the form a heap. Experiment was performed with out any vibration on an even surface and the distance between tip of the funnel and top of the powder pile was maintained from 2 to 4 cm.

Finally, the angle of repose was calculated using the following formula

$$Tan\theta = \frac{H}{R} \qquad \text{(v)}$$

Where, θ = is the angle of repose

H = Height of heap

R = Radius of heap

3.2.1.3.7 Particle size

Powder drug particle size distribution was determined by using SYMPATEC Particle Size Analyzer with dry powder system (Rodos assembly) which runs on laser light scattering technique and the percentage distribution of drug powder was recorded.

3.2.1.4 Chemical characteristics

3.2.1.4.1 Drug-excipients compatibility study

The compatibility study was performed by using Differential Scanning Calorimetry (DSC). Final prototype formulations as well as placebo blends were subjected to DSC analysis. Final prototype formulation was crushed in mortar and pestle and about 10 mg was put in the aluminum crucible. The crucible was sealed and subjected to calorimetric analysis by DSC, the temperature scale of which had been calibrated with high purity zinc and indium standards. The rate of increase in temperature was 5 ºC per minute. Same procedure was repeated for Mycophenolate Sodium as well as for placebo. The process conditions were as follows:

Scanning temperature range: 25ºC-350ºC

Gas: N_2, 20.0 mL /min

Beside this the drug excipients compatibility study was also performed with individual excipients by HPLC method. In this study the related substances (known impurity, Single highest impurity and total impurity) were determined after keeping the samples in 50ºC for 1 month and 2 months.

3.2.2 Analytical Method Development and validation for Assay, Related Substance and dissolution

3.2.2.1 Determination of λ_{max}

A known concentration of drug solution was prepared and was scanned against blank by UV-Visible spectrophotometer to determine the λ_{max} of 25 mcg/mL solution of Mycophenolate Sodium prepared in following media.

i) Phosphate buffer having pH 6.8.
ii) 0.1N HCl.

Prepared solutions were scanned for UV absorbance in the range of 200-400 nm to determine the λ_{max}.

3.2.2.2 Analytical method for assay

Standard Preparation

About 39 mg of Mycophenolate Sodium was weighed and transferred accurately to a 100 mL volumetric flask, dissolved in methanol and volume was made up to 100 mL.

Test Preparation

Five intact tablets were weighed and transferred to a 500 mL volumetric flask, 350 mL of diluent (methanol) was added, sonicated for 10 min to dissolve and kept on rotary shaker for 3 h , again sonicated for 30 min with intermittent vigorous shaking. The sample was cooled and made up to the mark with the diluent. Five millilitre of the final solution was diluted to 100 mL with methanol.

Mobile Phase preparation

Exactly 350 mL of acetonitrile was mixed with a mixture of 650 mL of water and 10 mL of triethylamine previously adjusted to pH 5.30±0.05 with dilute orthophosphoric acid. The mobile phase was filtered and degassed.

Chromatographic Conditions

HPLC column used was Lichrosphere RP 18e (4.0 x 250 mm), 5 µm at temperature of 45°C. The flow rate was set at 1.5 mL/min. The detection of the analyte was done by UV detector at 250 nm. Five microlitre of injection volume made in methanol was injected for analysis.

Procedure for System Suitability

Standard preparation was injected in five replicate injections, after filtering through 0.45 µm membrane filter (Millipore HVLP type), and the chromatograms were recorded. The system was considered suitable for analysis if the theoretical plates, determined on Mycophenolate Sodium were not less than 2000. The tailing factor determined on Mycophenolate Sodium peak was not more than 1.5 and the relative standard deviation of five replicate injections, in terms of area, was not more than 2.0%.

Procedure for sample analysis

The test solution (in duplicate), after filtering through 0.45 µm-membrane filter (Millipore HVLP type) was injected in to the system and Mycophenolate Sodium was calculated in mg per tablet using following formula.

$$\underset{mg/tablet}{Mycophenolate\ Sodium} = \frac{A_T}{A_S} \times \frac{Ws}{100} \times \frac{500}{5} \times \frac{100}{5} \times \frac{P}{100} \times \frac{320.4}{343.4} \qquad \text{(vi)}$$

A_T = Area obtained for test

A_s = Area obtained for standard

P = Potency of standard

W_s = Weight of standard taken

320.4 = Molecular weight of Mycophenolate Sodium

343.4 = Molecular weight of Mycophenolate Sodium

3.2.2.3 Analytical Method Validation for Assay

Specificity

A blank solution (*i.e.* mobile phase), a standard solution, unstressed test sample and unstressed placebo were subjected to analysis on high-pressure liquid chromatography equipped with photodiode array.

Forced Degradation study

Further, placebo and the test samples of Mycophenolate Sodium were subjected to the stress treatment for studying the elution of prospective degradation products. After stress treatment the samples were analyzed for peak purity and contents of Mycophenolate Sodium. The procedure for stress is represented in Table 7.

Table 7: Forced Degradation study

Stress Condition	Procedure
Unstressed	Not Applicable
Acidic	0.1 N Methanolic HCl /2 hrs at 60°C
Alkaline	0.1 N Methanolic NaOH/ 2 hr at 60°C.
Oxidative	50% H_2O_2 for 2 hrs
Humidity	Humidity (Saturated Potassium nitrate solution) 24 hrs
Heat	50°C/2Hrs
Light	UV cabinet for 12 hrs

Linearity

Linearity of the method was studied from 50%, 60%, 80%, 100%, 120%, 140%, 150% and 160% of Mycophenolate Sodium of the test concentration in the test as per the proposed method. The injections were made in duplicate. Required solutions were prepared from the stock solution after serial dilutions.

Precision

Precision of the method was determined under the following heads.

- Repeatability
- Intermediate Precision

Repeatability

Repeatability of the proposed assay method was assessed by making six determinations at 100% of test concentration. Six aliquots of the test sample were analyzed as per the proposed method using 5 tablets/analysis and 39.50 mg of standard.

Intermediate Precision

Intermediate precision of the proposed assay method was assessed by making six determinations at 100% of test concentration by different analysts on different instruments. Six aliquot of the test sample were prepared in similar way as described for repeatability and were analyzed using proposed method. Weight of the Mycophenolate Sodium working standard taken-39.08 mg

Accuracy

Accuracy of the method was studied at three levels (80%, 100% and 120% of assay concentration) in triplicate. Data is summarized in the Table 8 which represents the procedure at three different levels. Weight of the working standard taken was 37.97 mg.

Table 8: Concentration range for Accuracy determination

Concentration	80%			100%			120%		
Placebo taken (in mg)	371.67	371.17	371.28	371.12	371.32	371.08	372.13	371.66	371.85
Mycophenolate Sodium added (mg)	617.96	617.51	617.54	771.23	770.92	770.94	927.42	925.67	926.67

Robustness

Change in Flow rate

Robustness was studied by altering the flow rate of the mobile phase from 1.5 mL to 1.5 ± 0.1 mL/min.

Change in Column Oven Temperature

Temperature of the column oven was changed from 45°C to 45±5°C to see the impact of this change on robustness of method.

3.2.2.4 Analytical Method development for Related substances

Specificity: Following solutions were prepared and analyzed on high-pressure liquid chromatography equipped with photodiode array detector.

Sample Preparation:

Blank Solution

Sample Diluent (methanol)

Mycophenolate Sodium equivalent to Mycophenolic Acid 720 mg (NAXMP19):-

Weighed and finely powdered not less than 10 tablets, selected randomly. Weighed and transferred 297.72 mg of the tablet powder equivalent to about 200 mg of Mycophenolic Acid to a 100 mL volumetric flask, add about 60 mL of methanol, sonicate for about 45 minutes with intermittent shaking, cool and make up the volume with methanol and mix.

Test Preparation (Tablet)

Mycophenolate Sodium equivalent to Mycophenolic Acid 720 mg (NAXMP20):-

Weighed and finely powdered not less than 10 tablets, selected randomly. Weighed and transferred 301.28 mg of the tablet powder equivalent to about 200 mg of Mycophenolic Acid to a 100 mL volumetric flask, add about 60 mL of methanol, sonicate for about 45 minutes with intermittent shaking, cool and make up the volume with methanol and mix.

Mycophenolate Sodium equivalent to Mycophenolic Acid 720 mg (NAXMP19):-

The placebo tablets were powdered finely. Weighed and transferred 96.82 mg of the placebo tablet powder to a 100 mL volumetric flask, add about 60 mL of methanol, sonicate for about 45 minutes with intermittent shaking, cool and make up the volume with methanol and mix.

Placebo Preparation

Mycophenolate Sodium equivalent to Mycophenolic Acid 720 mg (NAXMP20):-

The placebo tablets were powdered finely. Weighed and transferred 101.86 mg of the placebo tablet powder to a 100 mL volumetric flask, add about 60 mL of methanol, sonicate for about 45 minutes with intermittent shaking, cool and make up the volume with methanol and mix.

Forced Degradation study: Further, placebo and test samples were given the stress treatment to study the elution of prospective degradation products. After stress treatment the samples were analyzed for peak purity (diluted 10 times) and content of Mycophenolic Acid.

Precision

Determined the Precision of the method as-**Repeatability.**

The repeatability of the method was studied through repeated analysis of the test sample (Mycophenolate Sodium Tablets) in which the single highest Impurity and total impurities precision was evaluated.

Accuracy

Accuracy study was carried out by performing recovery studies at five levels (80%, 100%, 120%, 150% and 200% of 5, 7-Dihydroxy-4-methylphthalide concentration) in triplicate. The data given in the table represents the percentage recovery at five different levels.

Determination of LOQ & LOD limits

The Limit of quantification (LOQ) and Limit of detection (LOD) of the method was studied through repeated analysis of the diluted solution in which the precision was evaluated.

Stability of solution

Stability of solution of the proposed related substances method was studied by analyzing aliquot of a homogenous test sample after keeping the sample solution for 12 hrs at room temperature. The results obtained with each parameter are discussed below:

Filter Standardization

The filter standardization was tested in test sample as per the Related Substances method. The results are presented in the table.

3.2.2.4.1 Description of Analytical method (Related Substances)

Instrument

HPLC system with UV detector

Reagents*: *Reagent

Same as mentioned in the assay

Chromatographic system

Column	:	Phenomenex Luna, C_{18} (4.6 X 250) mm, 5µm.
Wavelength	:	250 nm
Flow rate	:	1.5 mL per minute
Injection volume	:	10 µL
Column temperature	:	45ºC
Auto sampler temperature	:	10ºC
Run time	:	50 minutes
Mobile phase	:	**Buffer Solution:** To 650 mL of Water, add 10 mL of Triethylamine and adjust the pH to 5.30 ± 0.05 with diluted orthophosphoric acid. Buffer solution: Acetonitrile (650: 350)

Note: *The tentative retention time of Mycophenolate Sodium peak is about 7.0 minutes. Protect the solution from light. Prepare the solutions immediately before use; allow the temperature of the solution to equilibrate in the vials for 15 minutes before injection.*

System suitability solution

Weigh and transfer accurately about 10 mg of each Mycophenolate Mofetil WS and Mycophenolate Sodium WS standard to a 25 mL volumetric flask, add 10 mL of methanol, sonicate to dissolve and make up the volume with methanol and mix.

Placebo preparation

Select 10 placebo tablets randomly. Finely powder the tablets. Weigh and transfer accurately the placebo tablet powder (equivalent to formulation tablet powder taken for the analysis by subtracting the weight of active ingredients) to a 100 mL volumetric flask, add about 60 mL of methanol, sonicate for about 45 minutes with intermittent shaking, cool and make up the volume with methanol and mix.

Test preparation

Select 10 tablets randomly and calculate the average weight. Let it be Av. Finely powder the tablets and transfer the powdered test sample equivalent to about 200 mg of Mycophenolic Acid to a 100 mL volumetric flask, add about 60 mL of methanol, sonicate for about 45 minutes with intermittent shaking, cool and make up the volume with methanol and mix.

System suitability

Inject each of methanol as blank, placebo preparation, system suitability solution after filtering through 0.45µm membrane filter (Millipore HVLP type) and record the chromatograms.

The system is not suitable until the following parameters are met (determined on system suitability solution):

- The resolution between Mycophenolate Sodium standard peak and Mycophenolate Mofetil standard peak is not less than 15.
- The theoretical plates, determined on Mycophenolate Sodium peak, are not less than 2000.
- The tailing factor determined on Mycophenolate Sodium peak is not more than 1.5.

Procedure

Inject test preparation (in single), after filtering through 0.45µm membrane filter (Millipore HVLP type) and record the chromatogram.

Calculations

Calculate the percentage of 5, 7-Dihydroxy-4-methylphthalide, any other impurity and total impurities by area normalization method.

The disregard limit for 5, 7-Dihydroxy-4- methylphthalide is 0.03%.

Disregard the peaks (if any) due to blank and placebo.

Note: *The RRT of 5, 7-Dihydroxy -4- methylphthalide is about 0.4 w.r.t. Mycophenolate Sodium peak.*

3.2.2.5 Analytical method development for dissolution samples

Standard Preparation

Working standard (32 mg) was transferred to 50 mL volumetric flask and dissolved in dissolution media. Five milliliter of this solution was transferred to 100 mL volumetric flask and the volume was made up to mark with dissolution media.

Test Preparation

The sample was filtered through 0.45µ filter. Two milliliter of the filtrate was transferred to 50 mL volumetric flask and made up-to the mark with dissolution media.

Placebo Preparation

Placebo solution was filtered as run under dissolution conditions mentioned above through 0.45 µ filter. Two millilitre of the filtrate was transferred in 50 mL volumetric flask and made up-to the mark with dissolution media.

Procedure

The absorbance of the Blank, Placebo, Standard (in five replicates) and test sample (dissolution samples) were measured, at 304 nm were measured. The amount of drug released from the formulation was calculated using the equation given below:

$$Mycophenolate\ Sodium = \frac{A_T}{A_S} \times \frac{Ws}{50} \times \frac{5}{100} \times \frac{900}{C} \times \frac{50}{2} \times \frac{P}{100} \times 100 \qquad \text{(vii)}$$

A_T = Area obtained for test

As = Area obtained for standard

W_S = Weight of standard

C = Claim value

P = Potency of standard

Analytical Method Validation for dissolution sample

Specificity

Blank Solution, Placebo Solution, Standard Solution and Test Solution were prepared and scanned for UV absorption in a range of 200-400 nm.

Linearity

Linearity of the method was studied using five different concentrations in the range of 50% to 150% of the actual working concentration as per the proposed method. The absorbance of the resulting solutions was taken in triplicates.

Precision

Precision of the method was studied by analyzing dissolution samples at 304 nm withdrawn after 24 h from the six dissolution jars.

Accuracy

Accuracy study was performed at three concentration levels (80%, 100% and 120% of test concentration) in triplicate. Drug was dissolved in dissolution media and spiked to the respective dissolution media-containing placebo. Samples were withdrawn at the end of the dissolution time. The samples were analyzed on UV at 304 nm. Drug was added to the dissolution media at three different levels.

3.2.3 Analytical Method Development and Validation for quantification of Mycophenolic acid in plasma samples

Summary of the Analytical Method

Mycophenolic acid and its internal stanadard Pioglitazone were extracted from plasma using protein precipitation method using 2% HCl in Acetonitrile as precipitant. Centrifuged and the supernatant was injected directly into the LC-MS/MS.

A simple, accurate, precise and sensitive Bio-analytical method was developed and evaluated with respect to the following validation parameters to ascertain its suitability for the determination of Mycophenolic Acid by LCMS/MS : Selectivity, matrix effect, accuracy and precision (Inter- day and intra-day accuracy and precision), Long term stability in matrix.

System suitability

System suitability was determined before start of each analytical run. Seven consecutive injections of un-extracted medium quality control (MQC's) were injected and evaluated for peak area ratio.

Selectivity and Matrix effect in human plasma samples

The selectivity of the method was determined by analyzing blank human plasma samples in duplicate. There was no interfering mass peak at the retention times of Mycophenolic acid and internal standard in all the human plasma blank samples. The matrix effect was determined by spiking MQC in selected human plasma sample.

Linearity

The linearity of the response for the method was determined by analyzing the calibration standards solutions of Mycophenolic acid (Cal-1 to Cal-7) in the concentration range of 0.25µg/mL to 32µg/mL.

Precision and Accuracy

To assess the precision and accuracy of the developed method, four different concentrations in the range of expected concentrations were evaluated using six determinations per concentration. Precision and accuracy was assessed at intra and inter-day basis.

Stability of extracted Sample Solutions

To evaluate the long term stability (at two different concentrations LQC, HQC), six aliquots of each concentration were maintained in the deep freezer (-20°C temperature) for 54 days and quantified against spiked calibration curve standards.

3.2.3.1 Bio-Analytical Method Description for the Determination of Mycophenolic acid In Human Plasma:

Preparation of solutions

***Buffer Solution (0.1% formic acid pH-5.2*)**: One millilitre of formic acid was added to a 1000 mL volumetric flask containing 500 mL of Milli-Q water and sonicated. Finally the volume was made up with Milli-Q water. The pH was adjusted to 5.2 with dilute ammonia solution and filtered through 0.45µm filter paper.

Rinsing Solution: 200 mL of methanol, 600 mL acetonitrile and 200 mL of Milli-Q water were transferred into a 1000 mL reagent bottle and mixed well. This solution was stored at room temperature.

Extraction solvent: 2% HCl in acetonitrile was used as an extraction solvent.

Sample diluent: Acetonitrile was used as sample diluent.

Preparation of Mycophenolic acid working solutions for the calibration standards (Table 9)

Calibration Standard VII (320 µg/mL): Accurately weighed amount (32 mg corrected to purity) of Mycophenolic acid was taken in a 100 mL volumetric flask. About 20 mL of acetonitrile was added and sonicated to dissolve. The volume was made up with acetonitrile and mixed well.

Calibration Standard VI (160 µg/mL): Twenty five millilitre of calibration standard VII was taken in a 50 ml volumetric flask. The volume was made up with acetonitrile and mixed well.

Calibration Standard V (80 µg/mL): Twenty five mililitre of Calibration Standard VII was taken in a 100 mL volumetric flask. The volume was made up with acetonitrile and mixed well.

Calibration Standard IV (40 µg/mL): Twenty five mililitre of Calibration Standard VI was taken in a 100 mL volumetric flask. The volume was made up with acetonitrile and mixed well.

Calibration Standard III (20 µg/mL): Twenty five millilitre of Calibration Standard V was taken in a 100 mL volumetric flask. The volume was made up with acetonitrile and mixed well.

Calibration Standard II (10 µg/mL): Twenty five mililitre of Calibration Standard IV was taken in a 100 mL volumetric flask. The volume was made up with acetonitrile and mixed well.

Calibration Standard I (2.5 µg/mL): Twenty five mililitre of Calibration Standard II was taken in a 100 mL volumetric flask. The volume was made up with acetonitrile and mixed well.

Quality Control Sample Solutions

Quality Control Stock Solution (1000 µg/mL): 100 mg (corrected to purity) of MPA was taken accurately in a 100 ml volumetric flask. 70 ml of acetonitrile was added and sonicated to dissolve. The volume was made up with acetonitrile and mixed well.

High Quality Control Sample Solution (HQC) (300 µg/mL): 3 ml of Stock solution was taken in a 10ml volumetric flask. The volume was made up with acetonitrile and mixed well.

Medium Quality Control Sample Solution (MQC) (200µg/mL): 2 ml of Stock solution was taken in a 10 ml volumetric flask. The volume was made up with acetonitrile and mixed well.

Quality Control Sample solution A (30µg/ml): Take 1ml of high quality control in 10 ml volumetric flask. Make up the volume with Acetonitrile and mix well.

Low Quality Control Sample Solution (LQC) (7.5µg/ml): Take 5 ml of quality control sample solution A in a 20ml volumetric flask. Make up the volume with Acetonitrile and mix well.

Table 9: Concentrations of Calibration Standard and Quality Control Sample Solutions

Solutions	Concentrations in µg/ml	
	Mycophenolate Sodium in solutions	Mycophenolate Sodium in plasma
Calibration Standard I	2.5	0.25
Calibration Standard II	10	1
Calibration Standard III	20	2
Calibration Standard IV	40	4
Calibration Standard V	80	8
Calibration Standard VI	160	16
Calibration Standard VII	320	32
Low Quality Control	7.5	0.75
Medium Quality Control	200	20
High Quality Control	300	30

Internal standard stock solution

Pioglitazone (50 mg) was taken in a 100 mL volumetric flask and 70 mL of Acetonitrile was added to it. It was sonicated to dissolve and volume was made up with methanol to get a concentration of 500µg/mL.

Preparation of Extracted Samples

One set of spiked human plasma calibration curve standards was withdrawn from deep freezer along with one or more sets of quality control samples to be analyzed and equilibrated to room temperature. The equilibrated samples were vortexed for complete mixing of contents. Ten microlitre of internal standard working solution (500 µg/mL) except in blank was added to it and 200 µL of spiked human plasma calibration curve and quality control samples were put into each micro centrifuge tubes and vortexed. 1 ml of extraction solvent was added and vortexed for 2 min. These micro centrifuge tubes centrifuged for 5 min at 8500 rpm. Supernatant organic solvent was decanted and transferred in to HPLC auto sampler vials for analysis.

Preparation of Un-Extracted Samples

Ten microlitre of internal standard working solution (except in blank) and 20µL of Quality control working Solution (LQC-W/MQC-W/HQC-W) were added into each micro centrifuge tubes. Tubes were vortexed for 2 min and 1.0 ml of extraction solvent was added to it. Tubes were vortexed for 2 min and loaded in to HPLC auto sampler vials for analysis.

Chromatographic Conditions

The chromatographic conditions are summarized in the following Table 10:

Table 10: Chromatographic Conditions and parameters

Column	Kromacil 4.6 x 125mm, 5µ
Mobile Phase	Solution A: Acetonitrile Solution B: 0.1% Formic Acid adjusted to pH-5.2 with dilute Ammonia Solution (85:15) v/v
Flow rate	0.6 mL/min
Injection volume	20 µL
Run time	3.0 min

Mass Spectrometric Conditions

The mass spectrometric conditions are summarized in the following Table 11 and 12:

Table 11: Mass Spectrometric Conditions for Pioglitazone (Internal Standard)

Product ion	DP	FP	IS voltage (V)	Collision energy (eV)	Temperature (°C)
357.2>134	70	110	5000	39	450.00

Table 12: Mass Spectrometric Conditions for MPA (Analyte)

Product ion	DP	FP	IS voltage (V)	Collision energy (eV)	Temperature (°C)
322>135.9	17	110	5000	30	450.00

Method Validation

The method was partially validated for the selectivity, linearity, precision, accuracy and stability of the samples. The partial validation was performed as per guidance for

industry on bio-analytical method validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for drug evaluation and research (CDER) and center for veterinary medicine (CVM), May 2001, page no-3 (change in species within matrix). Bio-analytical method was evaluated with respect to the following validation parameters to ascertain it's suitability for the determination of Mycophenolate Sodium by LCMS/MS.

Selectivity, matrix effect, accuracy and precision (Inter- day and intra-day accuracy and precision) and long term stability in matrix.

System suitability

The system suitability was determined before start of each analytical run. Seven consecutive injections of un-extracted MQC's were injected and evaluated for average peak ratio.

Selectivity and Matrix effect in human plasma samples

Selectivity of the method was determined by analyzing blank human plasma samples in duplicate. There was no interfering mass peak at the retention times of MPA and internal standard in all the human plasma blank samples. The matrix effect was determined by spiking medium quality control sample (MQC) in selected human plasma sample.

Linearity

The linearity of response for the method was determined by analyzing the calibration standards solutions of MPA (Cal-1 to Cal-7) in the concentration range of 0.25µg/mL to 32µg/mL.

Precision and Accuracy

To assess the precision and accuracy of the developed method, four different concentrations in the range of expected concentrations were evaluated using six determinations per concentration. Precision and accuracy was assessed at intra and inter-day basis. The intra-day and inter-day precision and accuracy study were carried out using a batch containing following samples:

- A calibration curve containing standards (Cal-1 to Cal-7).

- Six quality control (QC) samples of each of the QC types (LLOQ, LQC, MQC, HQC).
- The QC samples were prepared independently, i.e. from different master solutions, from the standards samples.

Following criteria were met in order to approve the intra- and inter- batch precision and accuracy:

Precision: For each concentration level, coefficient of variation (CV) should not be exceeded 15%.

Accuracy: Mean value of samples at each concentration level, within 85 - 115% of the nominal value.

Drug Stability in Plasma (Freeze Thaw):

Low and high control plasma samples were subjected to three cycles by storing them in freezer at -20 ± 10 °C and RT followed by analysis.

3.2.4 Formulation development of Mycophenolate Sodium MR Tablet

It was very challenging to develop a once daily sustained release formulation because of the high dose & high solubility of the drug. Hence, the formulation development activity was initiated with following two approaches to obtain desired drug release with good reproducibility. In both the approaches the strength of each tablet was 720 mg of Mycophenolic acid.

Approach I: Developing a prototype formulation using hydrophobic release rate controlling agent(s), optionally with functional coating as core-coat technology (Lipid Matrix Technology), and

Approach II: Developing a prototype formulation using hydrophilic release rate controlling agent(s), optionally with hydrophobic agent(s) as hydration inhibitor (Polymer Matrix Technology).

3.2.4.1 Lipid Matrix Technology

3.2.4.1.1 Dry Granulation Method (B. No. NAXLM-1)

Mycophenolate Sodium, lactose anhydrous, Eudragit RSPO, Aerosil 200 and Compritol 888 ATO were weighed and passed through #40 s.s. sieve, and mixed well. Half the quantity of magnesium stearate was passed through #60 s.s. sieve and mixed well

with the powder blend. The powder blend was compressed using slug punches and the slugs were de-slugged to obtain desired granules. The remaining quantity of #60 passed magnesium stearate was mixed with the granules and compressed into tablets using 18.67 X 8.5 mm standard concave caplet punch. Table 13 enlists the composition of the batch NAXLM -1 prepared by dry granulation method.

Table 13: Composition of NAXLM-1

S. No.	Ingredients	% w/w	mg/Tablet
1	Mycophenolate Sodium	79.9	776.5
2	Lactose anhydrous	0.5	4.85
3	Compritol 888 ATO	10.82	104.9
4	Aerosil 200	2.0	19.4
5	Eudragit RSPO	3.9	37.83
6	Magnesium stearate	1.0	9.7
Extra granular Addition			
7	Magnesium stearate	1.0	9.7
Total		**100**	**970**
Friability			**0.03%**
Hardness			**294 N**

3.2.4.1.2 Wet Granulation Method (Aqueous) (B No. NAXLM-2 to NAXLM-4)

Mycophenolate Sodium, and lactose anhydrous, were weighed and passed through #40 s.s. sieve, and mixed well. The powder blend was granulated with the aqueous dispersion of Eudragit RS 30D/ Eudragit NE 30D/ Kollicoat SR 30D and dried, and passed through s.s sieve no #24 to obtain granules. The granules were mixed well with Compritol 888 ATO, lubricated with #60 passed magnesium stearate, followed by compression to obtain tablets.

Table 14 shows the composition of the batch NAXLM-2, NAXLM-3, NAXLM-4 prepared by aqueous granulation method.

Table 14: Compositions of NAXLM-2, NAXLM-3 and NAXLM-4

S. No	Ingredients	NAXLM-2		NAXLM-3		NAXLM-4	
		% w/w	mg/ Tablet	% w/w	mg/ Tablet	% w/w	mg/ Tablet
Intra granulation							
1	Mycophenolate Sodium	81.46	776.5	81.46	776.5	81.46	776.5
2	lactose anhydrous	0.54	5.2	0.54	5.2	0.54	5.2
Granulating agent							
3	Eudragit RS 30D	6.00	17.28	-		-	
4	Eudragit NE 30D	-		6.00	17.28	-	
5	Kollicoat SR 30D	-		-		6.00	17.28
Extra granular addition							
6	Compritol 888 ATO	10.00	95.3	10.00	95.3	10.00	95.3
7	Magnesium stearate	2.00	19.0	2.00	19.0	2.00	19.0
Total		100	913.28	100	913.28	100	913.28
Friability		0.02%		0.02%		0.02%	
Hardness		246 N		249 N		269 N	

3.2.4.1.3 Wet Granulation Method (Non-Aqueous) (DCM: IPA Mixture) (B No. NAXLM-5)

Mycophenolate Sodium, lactose anhydrous, Aerosil 200 and Compritol 888 ATO were weighed, passed through #40 s.s. sieve, and mixed well. The powder blend was granulated with the non-aqueous solution of Eudragit RSPO in dichloromethane (DCM): Isopropyl alcohol (IPA) mixture and dried, and then passed through s.s sieve no #24 to obtain granules. The granules were lubricated with #40 passed magnesium stearate and compressed to obtain tablets.

Table 15 shows the composition of the batch NAXLM-5 prepared by non aqueous granulation method.

Table 15: Composition of NAXLM-5

S. No.	Ingredients	% w/w	mg/Tablet
Intra granulation			
1	Mycophenolate Sodium	79.9	776.5
2	Lactose anhydrous	10.1	98.17
3	Compritol 888 ATO	3.0	29.16
4	Aerosil 200	1.0	9.7
Granulating agent			
5	Eudragit RSPO	5.0	48.6
6	DCM:IPA (mL)	Lost in processing	
Extra granular Addition			
7	Magnesium stearate	1.0	9.7
Total		100	972.0
Friability		0.03%	
Hardness		276 N	

3.2.4.1.4 Wet Granulation Method (Non-Aqueous) (Ethanol: Water) (B No. NAXLM-6)

Mycophenolate Sodium, Aerosil 200, lactose anhydrous, Eudragit RSPO and Compritol 888 ATO were weighed and passed through # 40 s.s. sieve, and mixed well. Powder blend was granulated with the ethanol: water mixture and dried, and passed through #24 s.s. sieve to obtain granules. Granules were lubricated with # 40 passed magnesium stearate and compressed to obtain tablets.

Table 16 enlists the composition of the batch NAXLM-6 prepared by non aqueous granulation method.

Table 16: Composition of NAXLM-6

S. No.	Ingredients	% w/w	mg/Tablet
Intra granulation			
1	Mycophenolate Sodium	79.9	776.5
2	Lactose anhydrous	0.5	4.86
3	Compritol 888 ATO	13.70	133..2
4	Aerosil 200	1.0	9.7
5	Eudragit RSPO	3.9	37.9
6	Ethanol: Water (mL)	Lost in process	
Extra granular Addition			
7	Magnesium stearate	1.0	9.7
Total		100	972
Friability		**0.04 %**	
Hardness		**258 N**	

3.2.4.1.5 Wet Granulation followed by Rate Controlling Coating (B. No. NAXLM-7a, NAXLM-7b, NAXLM-7c, NAXLM-7d)

B. No. NAXLM-7a

Preparation of core Tablets

Mycophenolate Sodium, Aerosil 200, lactose anhydrous and Compritol 888 ATO were weighed and passed through # 40 s.s. sieve, and mixed well. Powder blend was granulated with Eudragit RSPO in DCM: (Ethanol: Water) [5:20 (19.2:0.8)] mixture and dried, and passed through #24 s.s. sieve to obtain granules. Granules were lubricated with #40 passed magnesium stearate, and compressed to obtain tablets.

Preparation of coating dispersion

Kollicoat IR was dissolved in de-mineralized water (DM water) under stirring. PEG 400 was added to the solution followed by the addition of talc. Kollicoat SR 30D was added to the dispersion and stirred to obtain a homogeneous dispersion. The prepared dispersion was filtered through #100 mesh and used to coat the tablets.

Table 17: Composition of NAXLM-7a

S. No.	Ingredients	% w/w	mg/Tablet
	Intra granulation		
1	Mycophenolate Sodium	79.9	776.5
2	Lactose anhydrous	0.5	4.86
3	Compritol 888 ATO	13.70	133.2
4	Aerosil 200	1.0	9.7
	Granulating agent		
5	Eudragit RSPO	3.9	37.90
6	DCM: (Ethanol: Water) [5:20 (19.2:0.8)]	Lost in process	
	Extra granular Addition		
7	Magnesium stearate	1.0	9.7
	Total	100	972
	Coating Composition		
8	Kollicoat SR 30D	35.0 gm	
9	Kollicoat IR	1.75 gm	
10	PEG 400	1.5 gm	
11	Talc	4.5 gm	
12	Water	q.s. to 200.0 ml	
% Build up		10%	
Friability		0.01%	
Hardness		268 N	

Table 17 enlists the composition of the batch NAXLM-7a prepared by non aqueous granulation method followed by rate controlling coat.

B. No. NAXLM -7b

Preparation of core Tablets

Mycophenolate Sodium, Aerosil 200, lactose anhydrous and Compritol 888 ATO were weighed and passed through #40 s.s. sieve, and mixed well. Powder blend was granulated with Eudragit RSPO in DCM: (Ethanol: Water) [5:20 (19.2:0.8)] mixture and dried, and passed through #24 s.s. sieve to obtain granules. Granules were lubricated with #40 passed magnesium stearate, and compressed to obtain tablets.

Preparation of coating dispersion

Kollicoat IR was dissolved in DM water under stirring. PEG 400 was added to the solution followed by the addition of talc. Kollicoat SR 30D was added to the dispersion and stirred to obtain a homogeneous dispersion. The prepared dispersion was filtered through #100 mesh and used to coat the tablets.

Table 18: Composition of NAXLM-7b

S. No.	Ingredients	% w/w	mg/Tablet
Intra granulation			
1	Mycophenolate Sodium	79.9	776.5
2	Lactose anhydrous	0.5	4.86
3	Compritol 888 ATO	13.70	133.2
4	Aerosil 200	1.0	9.7
Granulating agent			
5	Eudragit RSPO	3.9	37.90
6	DCM: (Ethanol: Water) [5:20 (19.2:0.8)]	Lost in process	
Extra granular Addition			
7	Magnesium stearate	1.0	9.7
Total		100	972
Coating Composition			
8	Kollicoat SR 30D	35.0gm	
9	Kollicoat IR	7.0 gm	
10	PEG 400	1.5 gm	
11	Talc	4.5 gm	
12	Water	Q.S. to 200.0 ml	
% Build up		5%	
Friability		0.01%	
Hardness		268 N	

Table 18 enlists the composition of the batch NAXLM-7b prepared by non aqueous granulation method followed by rate controlling coat.

B. No. NAXLM -7c

Preparation of core Tablets

Mycophenolate Sodium, Aerosil 200, lactose anhydrous and Compritol 888 ATO were weighed and passed through #40 s.s. sieve, and mixed well. Powder blend was granulated with Eudragit RSPO in DCM: (Ethanol: Water) [5:20 (19.2:0.8)] mixture and dried, and passed through #24 s.s. sieve to obtain granules. Granules were lubricated with #40 passed magnesium stearate, and compressed to obtain tablets.

Preparation of coating dispersion

Kollicoat IR was dissolved in DM water under stirring. PEG 400 was added to the solution followed by the addition of talc. Kollicoat SR 30D was added to the dispersion and stirred to obtain a homogeneous dispersion. The prepared dispersion was filtered through #100 mesh and used to coat the tablets.

Table 19: Composition of NAXLM-7c

S. No.	Ingredients	% w/w	Mg/Tablet
	Intra granulation		
1	Mycophenolate Sodium	79.9	776.5
2	Lactose anhydrous	0.5	4.86
3	Compritol 888 ATO	13.70	133.2
4	Aerosil 200	1.0	9.7
Granulating agent			
5	Eudragit RSPO	3.9	37.90
6	DCM: (Ethanol: Water) [5:20 (19.2:0.8)]	Lost in process	
Extra granular Addition			
7	Magnesium stearate	1.0	9.7
	Total	**100**	**972**
Coating Composition			
8	Kollicoat SR 30D	35.0gm	
9	Kollicoat IR	7.0 gm	
10	PEG 400	1.5 gm	
11	Talc	4.5 gm	
12	Water	q.s to 200.0 ml	
% Build up		7.5%	
Friability		0.01%	
Hardness		268 N	

Table 19 enlists the composition of the batch NAXLM-7c prepared by non aqueous granulation method followed by rate controlling coat.

B. No. NAXLM-7d

Preparation of core Tablets

Mycophenolate Sodium, Aerosil 200, lactose anhydrous and Compritol 888 ATO were weighed and passed through #40 s.s. sieve, and mixed well. Powder blend was granulated with Eudragit RSPO in DCM: (Ethanol: Water) [5:20 (19.2:0.8)] mixture and dried, and passed through #24 s.s. sieve to obtain granules. Granules were lubricated with #40 passed magnesium stearate, and compressed to obtain tablets.

Preparation of coating dispersion

Kollicoat IR was dissolved in DM water under stirring. PEG 400 was added to the solution followed by the addition of talc. Kollicoat SR 30D was added to the dispersion and stirred to obtain a homogeneous dispersion. The prepared dispersion was filtered through #100 mesh and used to coat the tablets.

Table 20: Composition of NAXLM-7d

S. No.	Ingredients	% w/w	mg/Tablet
Intra granulation			
1	Mycophenolate Sodium	79.9	776.5
2	Lactose Anhydrous	0.5	4.86
3	Compritol 888 ATO	13.70	133.2
4	Aerosil 200	1.0	9.7
Granulating agent			
5	Eudragit RSPO	3.9	37.90
6	DCM: (Ethanol: Water) [5:20 (19.2:0.8)]	Lost in process	
Extra granular Addition			
7	Magnesium stearate	1.0	9.7
Total		100	972
Coating Composition			
8	Kollicoat SR 30D	35.0 gm	
9	Kollicoat IR	7.0 gm	
10	PEG 400	1.5 gm	
11	Talc	4.5 gm	
12	Water	q.s. to 200.0 ml	
% Build up		10%	
Friability		**0.02%**	
Hardness		**270N**	

Table 20 enlists the composition of the batch NAXLM-7d prepared by non aqueous granulation method followed by rate controlling coat.

3.2.4.1.6 Wet Granulation followed by Rate Controlling Coating (B. No. NAXLM-8)

Preparation of core Tablets

Mycophenolate Sodium, Aerosil 200, lactose anhydrous and Compritol 888 ATO were weighed and passed through #40 s.s. sieve, and mixed well. Powder blend was granulated with Eudragit RSPO in ethanol: water mixture and dried, passed through #24 s.s. sieve to obtain granules. Granules were lubricated with #40 passed magnesium stearate, and compressed to obtain tablets.

Preparation of coating dispersion

Kollicoat IR was dissolved in DM water under stirring. PEG 400 was added to the solution followed by the addition of talc. Kollicoat SR 30D was added to the dispersion and stirred to obtain a homogeneous dispersion. The prepared dispersion was filtered through #100 mesh and used to coat the tablets.

Table 21: Composition of NAXLM-8

S. No.	Ingredients	% w/w	mg/Tablet
	Intra granular addition		
1	Mycophenolate Sodium	79.60	780.05
2	Lactose anhydrous	0.58	5.68
3	Compritol 888 ATO	13.32	130.54
4	Aerosil 200	1.0	9.8
Granulating agent			
5	Eudragit RSPO	4.0	39.20
6	Ethanol: Water (mL)	Lost in process	
Extra granular Addition			
7	Magnesium stearate	1.0	9.80
	Total	100	980.0
Coating composition			
8	Kollicoat SR 30D	76.09	37.28
9	Kollicoat IR	10.87	5.33
10	PEG 400	3.26	1.60
11	Talc	9.78	4.79
12	Water	q.s.	q.s.
% Weight gain		5.0	49.0
Tablet weight after coating (mg)		-	1029.0
Friability		**0.03 %**	
Hardness		**258 N**	

Table 21 contains the composition of the batch NAXLM-8 prepared by non aqueous granulation method followed by rate controlling coat.

3.2.4.2 Polymer Matrix Technology

3.2.4.2.1 Dry Granulation Method (B No. NAXPM-9)

Mycophenolate Sodium, Lactose anhydrous, HPMC K15M CR, Eudragit RSPO were weighed and passed through #40 s.s. sieve, and mixed well. Half the quantity of magnesium stearate was passed through #40 s.s. sieve and mixed well with the powder blend. The powder blends was compressed using slug punches and the slugs were de-slugged to obtain the desired granules. The remaining quantity of #40 passed magnesium stearate was mixed with granules and compressed to obtain tablets.

Table 22 enlists the composition of the batch NAXPM-9 prepared by dry granulation method.

Table 22: Composition of NAXPM-9

S. No	Ingredients	% w/w	mg/Tablet
1	Mycophenolate Sodium	64.94	779.32
2	Lactose anhydrous	14.06	168.68
3	HPMC K15 M CR	15.00	180.00
4	Eudragit RSPO	5.00	60.00
5	Magnesium stearate	1.00	12.00
Total		**100.00**	**1120.00**
Friability		**0.02%**	
Hardness		**268 N**	

3.2.4.2.2 Wet Granulation (Aqueous) followed by Slugging Method (B No. NAXPM- 10)

Mycophenolate Sodium and lactose anhydrous were weighed and passed through #40 s.s. sieve, and mixed well. Sufficient quantity of water was added in to aqueous dispersion of Eudragit RS 30D and the pH was adjusted to 2.0 using 0.2M HCl. The powder blend was granulated using granulating fluid and the wet mass was passed through s.s sieve no #24 and dried. After drying, the granules were mixed with HPMC K100M CR, HPMC K15 and Xanthan gum and lubricated with half the quantity of magnesium stearate. The lubricated granules were slugged using suitable slug punches and the slugs were deslugged to obtain the desired granules. The remaining

quantity of #40 passed magnesium stearate was mixed with granules and compressed to obtain tablets.

Table 23 enlists the composition of the batch NAXPM -10 prepared by aqueous granulation method followed by slugging.

Table 23: Composition of NAXPM-10

S. No	Ingredients	% w/w	mg/Tablet
1.	Mycophenolate Sodium	80.47	776.51
2.	Lactose anhydrous	0.53	5.14
3.	Eudragit RS 30D	4.00	38.60
4.	Xanthan Gum (Xantural 75)	2.00	19.30
5.	HPMC K100M CR	7.00	67.55
6.	HPMC K15	5.00	48.25
7.	Magnesium stearate	1.00	9.65
Total		**100.00**	**965.00**
Friability		**0.04 %**	
Hardness		**264 N**	

3.2.4.2.3 Wet Granulation (Aqueous) Method (B No. NAXPM-11)

Mycophenolate Sodium and lactose anhydrous were weighed and passed through #40 s.s. sieve, and mixed well. Sufficient quantity of water was added in to aqueous dispersion of Eudragit RS 30D and the pH was adjusted to 2.0 using 0.2M HCl. The powder blend was granulated using granulating fluid and the wet mass was passed through s.s sieve no #24 and dried. After drying, the granules were mixed with HPMC K100M CR, HPMC K15, Eudragit RSPO and Xanthan gum and lubricated with half the quantity of magnesium stearate. The lubricated granules were slugged using suitable slug punches and the slugs were deslugged to obtain the desired granules. The remaining quantity of #40 passed magnesium stearate was mixed with granules and compressed to obtain tablets.

Table 24 enlists the composition of the batch NAXPM-11 prepared by aqueous granulation method followed by slugging.

3.2.4.2.4 Wet Granulation (Non-aqueous) Method (B No. NAXPM-12)

Mycophenolate Sodium was weighed and granulated with ethanol: water mixture and the wet mass was passed through s.s sieve no #24 and dried. The granules were mixed with lactose anhydrous, HPMC K100M CR, and Eudragit RSPO and xanthan gum,

and lubricated with half the quantity of magnesium stearate. The lubricated granules were slugged using suitable slug punches and the slugs were deslugged to obtain the desired granules. The remaining quantity of #40 passed magnesium stearate was mixed with granules and compressed to obtain the tablets.

Table 24: Composition of NAXPM-11

S. No	Ingredients	% w/w	mg/Tablet
1	Mycophenolate Sodium	80.33	1164.76
2	Lactose anhydrous	0.17	2.49
3	Eudragit RS 30D	4.00	58.00
4	Eudragit RSPO	3.50	50.75
5	Xanthan Gum(Xantural75)	2.00	29.00
6	HPMC K15	2.00	29.00
7	HPMC K100M CR	7.00	101.50
8	Magnesium stearate	1.00	14.50
Total		**100**	**1450.00**
Friability		**0.02 %**	
Hardness		**276 N**	

Table 25 enlists the composition of the batch NAXPM-12 prepared by non-aqueous granulation method.

Table 25: Composition of NAXPM-12

S. No	Ingredients	% w/w	Mg/Tablet
1	Mycophenolate Sodium	78.24	782.37
2	Lactose anhydrous	2.76	27.63
3	Eudragit RSPO	4.00	40.00
4	Xanthan Gum (Xantural 75)	2.00	20.00
5	HPMC K100M CR	12.00	120.00
6	Magnesium stearate	1.00	10.00
7	Ethanol: Water	Lost in process	
Total		**100.00**	**1000.00**
Friability		**0.01%**	
Hardness		**241 N**	

3.2.4.2.5 Wet Granulation (Non-aqueous) Method (B No. NAXPM-13)

Mycophenolate Sodium was weighed, mixed and granulated with PVP K-30 dissolved in ethanol: water mixture and the wet mass was passed through s.s sieve no #24 and dried. The dried granules were mixed with lactose anhydrous, HPMC K100M CR, Eudragit RSPO and xanthan gum and lubricated with half the quantity of magnesium stearate. The granules were slugged using suitable slug punches and the

slugs were deslugged to obtain the desire granules. The remaining quantity of #40 passed magnesium stearate was mixed with granules and compressed to obtain tablets.

Table 26 enlists the composition of the batch NAXPM-13 prepared by non-aqueous granulation method.

Table 26: Composition of NAXPM-13

S. No	Ingredients	% w/w	mg/Tablet
1	Mycophenolate Sodium	78.24	782.37
2	Lactose anhydrous	1.94	19.62
3	Eudragit RSPO	4.00	40.00
4	PVP K-30	0.8	8.01
5	Xanthan Gum (Xantural 75)	2.00	20.00
6	HPMC K100M CR	12.00	120.00
7	Magnesium stearate	1.00	10.00
8	Ethanol: Water (19:1)	Lost in process	
Total		**100.00**	**1000.00**
Friability		0.02%	
Hardness		**279 N**	

3.2.4.2.6 Wet Granulation (Non-aqueous) Method (B No. NAXPM-14)

Mycophenolate Sodium, lactose anhydrous, Benecel MP 874, Eudragit RSPO and xanthan gum were weighed and granulated with PVP K-30 dissolved in ethanol: water mixture and the wet mass was passed through s.s sieve no #12 and dried. The dried granules were passed through s.s sieve no #24 and lubricated with magnesium stearate. The granules compressed to obtain tablets. Table 27 enlists the composition of the batch NAXPM-14 prepared by non-aqueous granulation method.

Table 27: Composition of NAXPM-14

S. No	Ingredients	% w/w	Mg/Tablet
1	Mycophenolate Sodium	78.01	780.05
2	Lactose anhydrous	1.99	19.95
3	PVP K-30	1.00	10.0
4	Eudragit RSPO	4.00	40.00
5	Xanthan Gum (Xentural 75)	2.00	20.00
6	Benecel MP 874 (HPMC)	12.00	120.00
7	Ethanol: water	Lost in process	
8	Magnesium stearate	1.00	10.00
Total		**100.00**	**1000.00**
Friability		**0.02%**	
Hardness		**238 N**	

3.2.4.2.7 Wet Granulation (Non-aqueous) Method (B No. NAXPM-15)

Mycophenolate Sodium was weighed and granulated with PVP K-30 in ethanol: water mixture and the wet mass was passed through s.s sieve no #24 and dried. The granules were mixed with lactose anhydrous, Benecel MP 874, Eudragit RSPO and xanthan gum and lubricated with half the quantity of magnesium stearate. The granules were slugged using suitable slug punches and the slugs were deslugged to obtain desire granules. The remaining quantity of #40 passed magnesium stearate was mixed with prepared granules and compressed to obtain tablets. Table 28 enlists the composition of the batch NAXPM-15 prepared by non-aqueous granulation method.

Table 28: Composition of NAXPM-15

S. No.	Ingredients (%)	% w/w	mg/Tablet
Intragranular addition			
1	Mycophenolate Sodium	78.05	780.05
Granulating agent			
2	PVP K-30	0.49	4.9
3	Ethanol : Water	Lost in process	
Extragranular addition			
4	Lactose anhydrous	2.49	24.9
5	Eudragit RSPO	4.0	40.0
6	Xanthan gum (Xentrual 75)	2.0	20.0
7	Benecel MP 874 (HPMC)	12.0	120.0
8	Magnesium stearate	1.0	10.0
Total		100	1000
Friability		0.02%	
Hardness		264 N	

The two formulations NAXLM-8 and NAXPM-15 were subjected to the biostudy.

3.2.5 Biostudy–I

Randomized, single dose, three way, crossover comparative bioavailability study under fasting condition (Polymer Matrix & Lipid matrix)

A randomized, open label, three treatment, three period, three sequence, single dose, crossover comparative bioavailability study, under fasting conditions comparing rate and extent of absorption of two test MR formulations of Mycophenolate Sodium of Panacea Biotec Ltd. with reference formulation in 9 + 3 (Standby) healthy adult human subjects. A wash out period of one week was kept between the administrations of the formulations. The study (Protocol No: 06-07/TDM-NEX/PANBIO/BE-202)

was performed at Therapeutic Drug Monitoring Laboratory, Nexus Bioceuticals Research Pvt. Ltd., 194, Scheme No. 6, Road No. 15, Sion- Koliwada, Sion (East) Mumbai – 400022, Maharashtra-India. The analysis of the plasma samples was carried out at Sampann Drug Delivery, R&D Center, Panacea Biotec Ltd., Lalru, Punjab (India).

Study Dose:

Test 1 (T1)	:	One MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose (Polymer Matrix)
Test 2 (T2)	:	One MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose (Lipid matrix)
Reference (R)	:	Two tablets of reference formulation (Cellcept® 500 mg) as single oral dose.

3.2.5.1 Subject Selection / Number of subjects

As per U.S. FDA guideline of bioavailability and bioequivalence studies for orally administered drug products, twelve healthy volunteers were enrolled in the study. The study required minimum 9 volunteers, with 3 volunteers as stand-by to replace dropouts.

3.2.5.2 Inclusion criteria

The subjects selected were healthy human male volunteers of the age group between 18 to 45 years and body mass index (BMI) within the range of 18.5 – 24.99 Kg/m^2. The subjects had signed the written informed consent form (ICF-research subject information and consent form) prior to recruitment in the study.

Medical history, physical examination (including vital signs and 12 lead ECG) and analysis of laboratory parameters (including complete blood count, differential cell count, liver and renal function tests and urine analysis) were performed within 21 days prior to commencement of the study. Subjects were also screened for HIV infection and hepatitis B surface antigen and were nonsmoker, with no history of alcohol abuse. Bodyweight was within 15% of ideal in relation to height, according to the Life Insurance Corporation of India weight chart for non-medical cases.

Following Laboratory tests were performed:

1. **Haematology**
   - Haemoglobin
   - Haematocrit
   - Total and differential count
   - Red blood cell count
   - Platelet count
   - Blood grouping and Rh typing
2. **Serum Biochemistry**
   - Cholesterol
   - Triglycerides
   - RBS
   - Blood Urea
   - Creatinine
   - Sodium
   - Potassium
   - Chloride
   - Uric Acid
3. **Hepatic profile**
   - Alkaline phosphatase
   - SGOT
   - SGPT
   - Total proteins
   - Albumin
   - Globulin
   - Total bilirubin
   - Direct bilirubin
4. **Urine Analysis**
   - pH
   - Specific gravity
   - Protein
   - Glucose
   - Ketone bodies
   - Bilirubin
   - RBC casts
   - Nitrite
   - Urobilinogen
   - Microscopic Examination
5. **Coagulation profile**

6. **HIV Test, VDRL, Hepatitis B and C.**
7. **Urine drug screening for opioids, amphetamine, benzodiazepines, barbiturates and cannabinoids**
8. **Tests such as ECG, Chest X-ray *etc.***

The standard biochemical, pathological and haematological laboratory tests list with reference values are given in the Table 29.

3.2.5.3 Exclusion Criteria

The subjects will be excluded based on the following criteria:

- Allergy or significant history of hypersensitivity or idiosyncratic reaction to active pharmaceutical ingredient (API) or any other excipients in the particular product.
- Cardiovascular, pulmonary, hepatic, renal, haematological, gastrointestinal, endocrinal, immunologic, dermatologic, neurological or psychiatric disease.
- Having systolic blood pressure <100 and >130 mmHg, diastolic blood pressure <60 and >80 mmHg and pulse rate <60 and >100
- Alcohol dependence, alcohol abuse or drug abuse within the past one year
- Smoking or consumption of tobacco products (no current history of smoking of 9 cigarettes or beedies per day and subject should not be a regular consumer of oral tobacco or tobacco containing products like pan masala or gutkha)
- History of difficulty in swallowing or coming for follow up.
- Clinically significant illness within 4 weeks before the start of the study.
- Subjects who have been on an unusual diet (for whatever reason, less than 200 kcal and more than 3000 kcal) during the four weeks preceding the study
- Positive results to HIV, HCV, RPR and HBsAg.
- Ulceration or history of gastric and / or duodenal ulcer Asthma, urticaria or other allergic type reactions after taking aspirin.
- Use of enzyme modifying drugs (like phenytoin, carbamazepine, barbiturates, griseofulvin *etc.*) in the previous 30 days before the study.
- Abnormal ECG and X-ray.

- Subjects who vomit at least once before two times of median T_{max} will be excluded from the statistical analysis, however the subject will be allowed to continue the study depends on the severity of emesis which will be decided by the clinical investigator.
- Subjects who would have donated blood in excess of 350 ml in the preceding 3 months including the blood loss anticipated in the present study
- Subjects who have participated in another clinical trial within 12 weeks of study start.
- Any waiver of these inclusion and exclusion criteria must be approved and documented by the clinical investigator and the sponsor on a case-by-case basis.

Table 29: Standard values of Biochemical, Pathological and Haematological Laboratory tests

INVESTIGATION	NORMAL RANGE		INVESTIGATION	NORMAL RANGE	
Haematology					
Haemoglobin	**Male**	**Female**	Serum chloride	98 – 106 mmol/L	
	13.2 – 17.3 gm/dl	11.7 – 15.5 gm/dl	Blood urea	15 – 40 mg/dl	
RBC count	4.3 – 5.7 mill/cu mm	3.8 – 5.1 mill/cu mm	Serum creatinine	**Male**	**Female**
WBC count	4500 – 11000 cells/cu mm			0.5 – 1.3 mg/dl	0.5 – 1.1 mg/dl
Haematocrit	39 – 49 %	35 – 45 %	Serum uric acid	4.4 – 7.6 mg/dl	2.3 – 6.6 mg/dl
Differential Count:			Total bilirubin	0.3 – 1.0 mg/dl	
			Direct bilirubin	0.1 – 0.3 mg/dl	
Neutrophils	45 – 74 %		SGOT (AST)	0 – 35 U/L	
Lymphocytes	16 – 45 %		SGPT (ALT)	0 – 35 U/L	
Eosinophils	1 – 5 %		Alkaline Phosphatase	100 – 400 U/L	110 – 380 U/L
Monocytes	2 – 7 %		Total protein	5.5 – 8.0 gm/dl	
Basophils	0 – 1 %		Albumin	3.5 – 5.5 gm/dl	
Blood grouping	NA		Globulin	2.0 – 3.5 gm/dl	
Rh typing	NA		A/G ratio	1.2 – 2.5:1	
Platelet count	1.3 – 4.0 lakh cells/cu mm		Serum FSH	Post Menopausal:35 – 151 mIU/mL	
RBC Morphology	Normochromic Normocytic		Serum Estradiol	Post Menopausal :<60 pg/mL	
ESR 1st hour	0 – 15 mm/hour	0 – 20 mm/hour	**Urine Analysis**		
Bleeding Time	1 – 3 minutes		pH	5.0 – 7.0	
Clotting Time	3 – 7 minutes		Specific Gravity	1.010 – 1.030	
Biochemistry			Protein	NA	
FBS/ RBS	60 – 110 / 80 – 120 mg/dl		Glucose	0.0 – 0.8 mmol/L	
Serum cholesterol	124 – 250 mg/dl	Ketones	NA		
Serum Triglycerides	40 – 160 mg/dl	35 – 150 mg/dl	Pus cells	1 – 2 cells/HPF	
Electrolytes			Epithelial cells	0 – 2 cells/HPF	
Serum sodium	136 – 145 mmol/L		RBC's	0 – 1 cells/HPF	
Serum potassium	3.5 – 5.0 mmol/L		Nitrite	NA	
			Urobilinogen	Less than 1 mg/dl	

Table 30: Drug administration schedule for various subjects

Subject Number	Period 1	Period 2	Period 3
4, 6, 7, 12	R	T1	T2
1, 2, 8, 11	T1	T2	T3
3, 5, 9, 10	T2	R	T1

Where:

Test 1 (T1): MR tablet of Mycophenolate Sodium (Code No. NAXPM-15)

Test 2 (T2): MR tablet of Mycophenolate Sodium (Code No.NAXLM-8)

R: IR tablet (Reference tablet) Cellcept 500mg (Batch No. U0399)

All subjects fasted overnight for 10 hr prior to administration of dose. Drinking water was not allowed from 1h pre dose until 2 hr post dose except during administration of study formulations. Single dose of study drug was administered as per schedule given in Table 30 with 240 mL of plain drinking water at room temperature. One glass (240 mL) of plain whole milk was provided 9 hr post-dose along with snacks.

3.2.5.4 Sample Collection

Blood samples were collected from an antecubical venous by an indwelling venous cannula in 5 mL EDTA Vacutainers®. On study day 1 of each study phase, the cannula was placed into the volunteer's forearm approximately half an hour before study drug administration and remained there for approximately 24 hrs after administration of the formulation. Blood samples subsequent to the removal of venous cannula were collected by direct vein puncture using a 10 mL hypodermic syringe (preferably) in sitting position. Blood samples for Test 1 and Test 2 formulations were collected at 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 11.0, 12.0, 13.0, 14.0, 15.0, 16.0, 18.0, 20.0, 22.0, 24.0, 30.0, 36.0, 42.0, 48.0, 60.0, 72.0 hr. Blood samples for Reference (R) formulation was collected at 0, 0.17, 0.33, 0.50, 0.67, 0.83, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 3.00, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00, 60.00, and 72.00th hr post administration.

3.2.5.5 Analysis of Plasma samples

A method for quantification of the drug in plasma was developed and validated as provided in Section 3.2.3. The analyst did not have access to the randomization schedule during the course of the study.

All concentration values below the limit of quantification were set to zero for all pharmacokinetic and statistical evaluations. Any missing samples or unrepeatable concentration values due to poor chromatography were reported as 'M' and not included for pharmacokinetic or statistical analysis. Samples from all subjects who completed the cross over were assayed for Mycophenolate Sodium using validated LC-MS/MS method at Panacea Biotec Ltd.

3.2.5.6 Pharmacokinetic Analysis

Pharmacokinetic analysis was performed at Panacea Biotec Ltd. The data from all subjects who had completed both the periods was analyzed by non-compartmental analysis using WinNonlin 5.2 (model 200) Pharsight, USA.

The following pharmacokinetic parameters were calculated for each subject and treatment (Table 31)

Table 31: Pharmacokinetic parameters

S. No.	Parameter	Description
1	AUC_{0-t}	The area under the plasma concentration versus time curve, from time zero to the last measurable concentration, as calculated by the linear trapezoidal method.
2	$AUC_{0-\infty}$	Area under the plasma concentration versus time curve, from time zero to infinity. $AUC_{0-\infty}$ was calculated as the sum of AUC_{0-t} plus the ratio of the last measurable plasma concentration to the elimination rate constant.
3	C_{max}	Maximum measured plasma concentration over the time span specified.
4	T_{max}	Time of the maximum measured plasma concentration. If the maximum value occurs at more than 1 time point, Tmax was defined as the first time point with this value.
5	λ_z	Apparent first-order terminal elimination rate constant calculated from a semi-log plot of the plasma concentration versus time curve. The parameter was calculated by linear least-square regression analysis using the maximum number of points in the terminal log-linear phase (e.g. three or more non-zero plasma concentrations).
6	$t_{1/2}$	The apparent first-order terminal elimination half-life was calculated as $0.693/\lambda_z$.

No value of λ_z, $AUC_{0-\infty}$ or $T_{1/2}$ was reported for cases that did not exhibit a terminal log-linear phase in the concentration versus time profile.

As per U.S. FDA guideline of bioavailability and bioequivalence studies for orally administered drug products, if pre-dose concentration (at 0.0^{th} Hr) was found to be less than or equal to 5% of mean C_{max}, the value considered as such for calculation. If

pre-dose concentration was found to be more than 5% of mean C_{max}, the respective subject was eliminated from the analysis.

3.2.5.7 Statistical Analysis

The following model was used as ANOVA model for testing sequence, formulation and period effect.

$$\mathbf{Y_{ijk}=\mu+S_{ik}+P_{jk}+F_j+e_{ijk}} \quad \text{------- (vii)}$$

Where:

$\mathbf{Y_{ijk}}$ = Pharmacokinetic response, e.g. C_{max} or Ln (C_{max})

i = Subject Number

j = Formulation Number

k = Sequence Number

μ = Overall Mean

$\mathbf{S_{ik}}$ = The random effect of i^{th} subject in the k^{th} sequence

$\mathbf{F_j}$ = Fixed effect of j^{th} formulation

$\mathbf{P_{jk}}$ = Fixed period effect at the j^{th} formulation in the k^{th} sequence

$\mathbf{e_{ijk}}$ = The random error in observing Y_{ijk}

The log-transformed pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were analyzed using ANOVA model (vii), with the main effects of sequence, period and formulations as fixed effects and subjects nested within sequence as random effect. The sequence effect was tested at the 0.10 level of significance using the subjects nested within sequence mean square as the error term. All other main effects were tested at the 0.05 level of significance against the residual error (mean square error) from the ANOVA model as the error term.

3.2.5.8 Bioavailability Criteria:

The natural logarithmic transformation of the pharmacokinetic exposure parameters Ln(Cmax), Ln(AUC0-t) and Ln(AUC0-∞) were used to assess the bioavailability. The classical 90% confidence interval for the ratio of the geometric least square mean of the test product and the reference product (Cellcept) were calculated for Ln(Cmax), Ln(AUC0-t) and Ln(AUC0-∞).

3.2.6 Optimized Formulation with Polymer Matrix Technology

3.2.6.1 Wet Granulation (Non-Aqueous) Method (B. No. NAXPM-16)

Mycophenolate Sodium, lactose anhydrous, Aerosil 200, PVP K-90**,** HPMC K15 M CR and Polyox WSR 301 were weighed and granulated with PVP K-30 dissolved in IPA and the wet mass was passed through s.s sieve no #24 and dried. The #40 passed magnesium stearate was mixed with the granules and compressed to obtain tablets. Table 32 enlists the composition of the batch NAXPM -16 prepared by non-aqueous granulation method.

Table 32: Composition of NAXPM-16

S. No.	Ingredients (%)	% w/w	mg/Tablet
Intra granular addition			
1	Mycophenolate Sodium	78.87	776.5
2	Lactose anhydrous	2.13	21
3	Aerosil 200	0.50	5
4	PVP K-90	5.00	49.25
5	HPMC K15 M CR	5.00	49.25
6	Polyox WSR 301	5.00	49.25
Granulating agent			
7	PVP K-30	2.50	24.62
8	IPA (mL)	Lost in processing	
Extra granular addition			
9	Magnesium stearate	1	9.85
Total		100	985
Friability		0.01%	
Hardness		320 N	

3.2.6.2 Wet Granulation (Non-Aqueous) Method (B.No. NAXPM-17, NAXPM-18).

Mycophenolate Sodium, Lactose anhydrous, Aerosil 200, PVP K-90**,** HPMC K15 M CR or HPMC K100 M CR were weighed and granulated with PVP K-30 dissolved in IPA and the wet mass was passed through s.s sieve no #24 and dried. The #40 passed magnesium stearate was mixed with granules and compressed to obtain tablets. Table 33 enlists the composition of the batch NAXPM -17 and 18 prepared by non-aqueous granulation method.

Table 33: Composition of NAXPM -17, NAXPM-18

S. No.	Ingredients (%)	NAXPM-17		NAXPM -18	
		% w/w	mg/Tablet	% w/w	mg/Tablet
Intra granular addition					
1	Mycophenolate Sodium	78.87	776..5	78.87	776.5
2	Lactose anhydrous	2.13	20.55	2.13	20.55
3	Aerosil 200	0.50	5	0.50	5
4	PVP K-90	5.00	50	5.00	50
5	HPMC K15 M CR	10.00	98.5	-	
6	HPMC K100 M CR	-		10.00	98.5
Extra granular Addition					
7	PVP K-30	2.50	24.6	2.50	24.6
8	IPA (mL)	Lost in process			
9	Magnesium Stearate	1.00	9.85	1.00	9.85
Total		**100**	**985**	**100**	**985**
Friability		**0.01%**		**0.01%**	
Hardness		**325 N**		**326 N**	

3.2.6.3 Wet Granulation (Non-Aqueous) Method (B. No. NAXPM-19, NAXPM-20)

Mycophenolate Sodium, lactose anhydrous, Aerosil 200, PVP K-90**,** HPMC K100 M CR and Polyox WSR 301 were weighed and granulated with PVP K-30 dissolved in IPA and the wet mass was passed through s.s sieve no #24 and dried. The #40 passed magnesium stearate was mixed with granules and compressed to form tablets. Table 34 enlists the composition of the batch NAXPM-19 and 20 prepared by non-aqueous granulation method.

Table 34: Composition of NAXPM-19, NAXPM-20

S. No.	Ingredients (%)	NAXPM-19		NAXPM-20	
		% w/w	mg/Tablet	% w/w	mg/Tablet
Intra granular addition					
1	Mycophenolate Sodium	77.35	776.5	73.67	776.5
2	Lactose DCL21	2.65	26.5	1.33	14
3	Aerosil 200	0.50	5.0	0.5	5.25
4	PVP K-90	2.5	25	2.5	26.25
5	HPMC K100 M CR	10.00	100	17.5	185
6	Polyox WSR 301	5.0	50	2.5	26.25
Granulating agent					
7	PVP K-30	1.0	10	1.0	10.5
8	IPA (mL)	Lost in process			
Extra granular addition					
9	Magnesium Stearate	1.0	10	1.0	10.5
Total		**100**	**1000**	**100**	**1050**
Friability		0.01%		0.01%	
Hardness		328 N		339 N	

The formulations NAXPM-20 and NAXPM- 19 were subjected to biostudy II and III respectively.

3.2.7 Biostudy II and III

3.2.7.1 Approval nos. of the studies from DCGI

a. F.No.12-3/99-DC (Pt-Panacea) 5172 dated 03/07/08
b. F.No.12-3/99-DC (Pt-Panacea) 5173 dated 03/07/08

3.2.7.2 Randomized, single dose, three way, crossover comparative bioavailability study under fasting and fed conditions (NAXPM-19 and NAXPM-20)

A randomized, open label, balanced, three treatment, three period, six sequence, single dose, three way crossover comparative bioavailability study of test MR formulations of Mycophenolate Sodium of Panacea Biotec Ltd. under fasting and fed conditions with reference formulation under fasting condition in 18 + 6 (Standby) healthy human adult male subjects. A wash out period of one week was kept between the administrations of the formulations. The study was performed at Quest Life Sciences Pvt., Ltd, SDF III, MEPZ, Tambaram, Chennai-600045, India. The analysis of the plasma samples were done at Sampann Drug Delivery, R&D Center, Panacea Biotec Ltd., Lalru, Punjab (India).

Study Dose for biostudy II (Slow release):

Test 1 (T1)	:	One MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose (NAXPM-20), Fasting condition
Test 2 (T2)	:	One MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose (NAXPM-20), Fed condition
Reference (R)	:	Two tablets of reference formulation (Cellcept 500® mg) as single oral dose, Fasting condition

Study Dose for biostudy III (Fast release):

Test 1 (T1)	:	One MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose (NAXPM-19), Fasting condition
Test 2 (T2)	:	One MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose (NAXPM-19), Fed condition
Reference (R)	:	Two tablets of reference formulation(Cellcept 500® mg) as single oral dose, Fasting condition

3.2.7.3 Subject Selection / Number of subjects for Biostudy II and III

Twenty four healthy human adult male volunteers were enrolled in the study. The study required minimum 18 volunteers, with 6 volunteers as stand-by to replace dropouts.

Same study design was used for biostudy II and III. Inclusion, exclusion criteria and statistical analysis method (ANOVA) for biostudy II and III were same as for biostudy I.

3.2.7.4 Sample Collection for Biostudy II and III

Blood samples including predose sample were collected from an antecubical vein by an indwelling catheter or a scalp vein upto 12 hr. After 12 hr, samples were collected by direct vein puncture. Heparin-lock technique was used to prevent clotting of the indwelling catheter. Blood samples for Test formulation T1(fasting), T2 (fed) and Reference (R) were collected at 0, 0.33, 0.50, 0.67, 0.83, 1.00, 1.25, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 7.50, 8.00, 9.00, 10.00, 11.00, 12.00, 14.00, 16.00, 18.00, 22.00, 24.00, 36.00 and 48.00 hr. Before each blood sample was drawn (0.00-12.00 hr), 0.5 mL of blood was discarded so as to prevent the heparin in the cannula from interfering with the analysis.

3.2.8 Characterization of the developed formulations

3.2.8.1 Hardness

The hardness of the tablet was measured using Erweka hardness tester (TBH-220 D).

3.2.8.2 Friability test

10 whole tablets were taken. The tablets were carefully dedusted prior to testing. The tablets were accurately weighed, and placed in the drum. The drum was attached to the horizontal axis of a device that rotates at 25±1 rpm. Thus, at each turn, the tablets roll or slide and fall onto the drum wall or onto each other. The drum was rotated for 100 times, and the tablets were removed. Any loose dust was removed from the tablets as before, and accurately weighed.

$$\% \text{ Friability} = (\text{Initial weight} - \text{final weight}) / \text{final weight} \times 100$$

3.2.8.3 Weight variation Test

The weight variation test of the tablets were performed by weighing 20 individual tablets.

3.2.8.4 Thickness

The thickness of the tablet was measured by using vernier calipers (Mitutoyo).

3.2.8.5 Drug release studies

The drug release studies were carried out in USP type II apparatus at 75 rpm using 900 ml pH 6.8 phosphate buffer. Sinkers were used in the drug release studies to present sticking of tablets to the bottom of dissolution vessel.

3.2.8.6 Water content

The water content was measured by using Karl Fisher titration method.

3.2.8.7 Stability studies

The optimized formulation was subjected to accelerated stability testing as per ICH guidelines and the protocol is mentioned in Table 35.

Table 35: Stability protocol for the developed tablet formulation

Period	Tests Required				
	Assay	RS	Dissolution	Water Content	Description
Initial	√	√	√	√	√
Storage Condition: 40ºC/75% RH					
1 M	√	√	√	√	√
2 M	√	√	√	√	√
3 M	√	√	√	√	√
6 M	√	√	√	√	√
Period	**Tests Required**				
	Assay	**RS**	**Dissolution**	**Water Content**	**Description**
Initial	√	√	√	√	√
Storage Condition: 30ºC/65% RH					
3 M	√	√	√	√	√
6 M	√	√	√	√	√
9 M	√	√	√	√	√
12 M	√	√	√	√	√
18 M	√	√	√	√	√
24 M	√	√	√	√	√

Period	Tests Required				
	Assay	RS	Dissolution	Water Content	Description
Period	Tests Required				
	Assay	RS	Dissolution	Water Content	Description
Initial	√	√	√	√	√
Storage Condition: 25ºC/60% RH					
3 M	√	√	√	√	√
6 M	√	√	√	√	√
9 M	√	√	√	√	√
12 M	√	√	√	√	√
18 M	√	√	√	√	√
24 M	√	√	√	√	√

3.2.9 Modeling of Drug Release Kinetics

The data obtained for dissolution profiles for different batches were fitted into various kinetic models, *viz.*, zero order, first order, Higuchi model, Hixon Crowell model and Korsmeyer Peppas. The regression coefficient and constant values were determined for each model and reported.

3.2.10 *In Vitro In Vivo* Correlation

The concept of correlation level is based upon the ability of the correlation to reflect the entire plasma drug concentration–time curve that will result from administration of the given dosage form. It is the relationship of the entire *in vitro* dissolution curve to the entire plasma level curve that defines the correlation.

Level A correlation - It represents a point-to-point relationship between *in vitro* dissolution and the *in vivo* input rate of the drug from the dosage form. This latter factor is sometimes referred to as *in vivo* dissolution. In such a correlation, the *in vitro* dissolution and *in vivo* input rate curves are either directly superimposable or may be made to be superimposable by the use of a constant offset value. Such a procedure is most applicable to modified-release systems that demonstrate an *in vitro* release rate that is essentially independent of the typical dissolution media usually employed in pharmaceutics. However, this is not a requirement for a Level A correlation. With this correlative procedure, a product's *in vitro* dissolution curve is compared to its *in vivo* input curve (*i.e.,* the curve produced by deconvolution of the plasma level data). This

may be done by use of mass balance model-dependent techniques, such as the Wagner–Nelson procedure or the Loo-Riegelman method, or by model-independent, mathematical deconvolution [121].

The advantages of a Level A correlation are as follows:

A point-to-point correlation is developed. This is not found with any other correlation level. It is developed using every plasma level and dissolution point that has been generated. Thus, it reflects the complete plasma level curve.

In-Vitro

The IVIVC tool kit of WinNonlin 5.2 was used for data analysis. The *in vitro* dissolution data for Test 1, Test 2 and reference was plotted as percent release vs. time. The fraction absorbed (F_{abs}) was calculated from percent release and plotted against time. Once all data input was provided then Hill dissolution model was fitted by using uniform weighting.

In-Vivo

The *in vivo* data was plotted for Test 1, Test 2 and reference formulation between plasma concentrations vs. time. A two exponential function was fitted on the basis of Akaike Information Criterion. The IVIVC tool kit of WinNonlin 5.2 was used to compute fraction absorbed over time by numerical deconvolution technique.

Correlation

In vitro – in vivo correlation was determined between Test 1, Test 2 and reference. Among the three correlation models F_{abs} = AbsScale*Diss (Tscale*T vivo) model was selected and used to fit fraction absorbed (F_{abs}). The software was run for IVIVC correlation. The plot between F_{abs} *vs.* $F_{dissolved}$ was generated. For validation of correlation, three parameters were selected i.e. AUC_{last}, linear trapezoidal rule and average as mean. The error was predicted by generating table of prediction errors for AUC and C_{max}.

4.0 Results and Discussion

4.0 RESULTS AND DISCUSSION

4.1 Preformulation Studies

4.1.1 Organoleptic properties

Mycophenolate Sodium was found to be off white crystalline powder and appeared as irregularly needle shaped crystals when observed under optical microscope.

4.1.2 pH dependent solubility studies

The solubility of Mycophenolate Sodium in various solvents was calculated and the results are given in Table 36. From the result it can be predicted that the solubility of Mycophenolate Sodium increases with increase in pH.

Table 36: Solubility Study for Mycophenolate Sodium at various pH

S. No.	Drug release medium (n=3)	Concentration (mg/mL)
1.	0.1 N HCl	0.05 ± 0.002
2.	pH 4.5 acetate buffer	0.09 ± 0.002
3.	Purified water	9.8 ± 0.012
4.	pH 6.8 phosphate buffer	10.0 ± 0.024

4.1.3 Physical characteristics

4.1.3.1 Following physical characteristics were recorded for Mycophenolate sodium.

Physical characteristics		Values
Melting point	:	183 to 186°C
Loss on drying (LOD)	:	1.64%
Loose Bulk density	:	0.297 gm/ml
Tapped Density	:	0.463 gm/ml
Hausner ratio	:	1.56
Compressibility index	:	35.85%
Angle of repose	:	46.39 °C

The values of angle of repose 30-40 are considered as good while values below 30 are excellent. Similarly, the values of compressibility index from 5-15% are excellent. It can be interpreted from above mentioned values that the Mycophenolate Sodium has poor flow properties and may require more than the standard amount of lubricant to increase the flow.

4.1.3.2 Particle size analysis

Particle size analysis of Mycophenolate Sodium was done using SYPMATEC particle size analyzer and the recorded results are given in Table 37, 38, 39 and Figure 4.

Table 37: Particle size analysis of Mycophenolate Sodium using SYMPATEC Particle size analyzer

S. No.	% Fraction	Particle size (µm)
1	X_{10}	0.99
2	X_{50}	3.68
3	X_{90}	14.31
4	X_{99}	36.08
5	SMD (Sauter mean diameter)	2.56 µm
6	VMD(Volume mean diameter)	6.18 µm

Particle size distribution graph for MPS

S_V = 2.34 m²/cm³ S_m= 5038.25 cm²/g

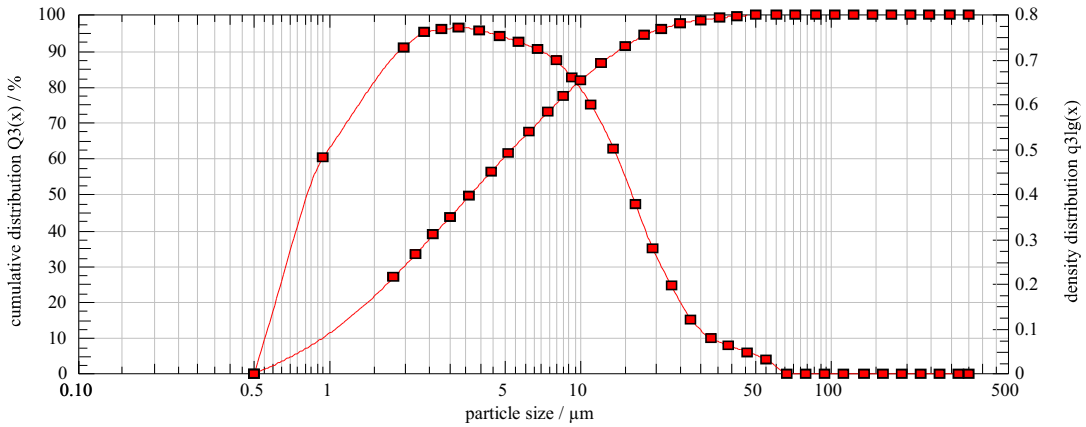


Figure 4: Particle size distribution graph for MPS

Table 38: Cumulative distribution for Mycophenolate Sodium

x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%	x_0/µm	Q_3/%
1.8	26.67	7.4	72.67	30	98.37	122	100
2.2	33	8.6	77.21	36	98.99	146	100
2.6	38.51	10	81.52	42	99.4	174	100
3	43.28	12	86.26	50	99.76	206	100
3.6	49.37	15	91.12	60	100	246	100
4.4	56.03	18	94.1	72	100	294	100
5.2	61.49	21	95.95	86	100	350	100
6.2	67.12	**25**	97.42	**102**	100		

Table 39: Density Distribution (log transformed data)

x_m/µm	$q_3$lg	x_m/µm	$q_3$lg	x_m/µm	$q_3$lg	x_m/µm	$q_3$lg
0.95	0.45	6.77	0.72	27.39	0.12	111.55	0.00
1.99	0.72	7.98	0.69	32.86	0.08	133.46	0.00
2.39	0.76	9.27	0.66	38.88	0.06	159.39	0.00
2.79	0.77	10.95	0.60	45.83	0.05	189.33	0.00
3.29	0.77	13.42	0.50	54.77	0.03	225.11	0.00
3.98	0.76	16.43	0.38	65.73	0.00	268.93	0.00
4.78	0.75	19.44	0.28	78.69	0.00	320.78	0.00
5.68	0.74	22.91	0.19	93.66	0.00		

It can be concluded from the above mentioned tables that 90% of the particles of Mycophenolate Sodium are below 14.31 µm

4.1.4 Compatibility study

Compatibility study for Mycophenolate Sodium formulation was carried out by XRD, DSC and detection of Related Substances. The relevant curves given as Figure 5 to 12 and results given in Table 40 and 41 are summarized below:

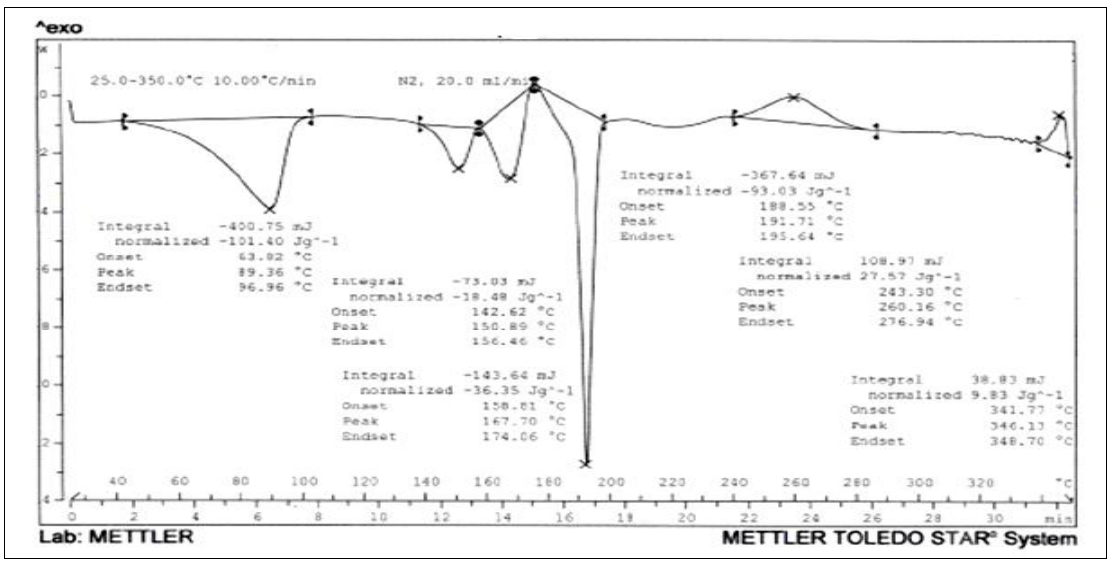


Figure 5: DSC study for MPS

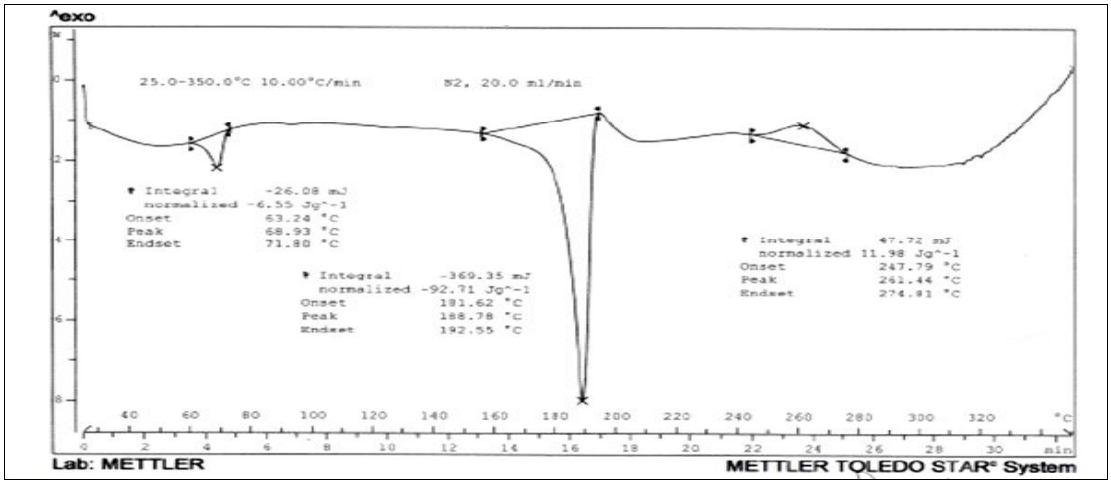


Figure 6: DSC study for Final proto type formulation

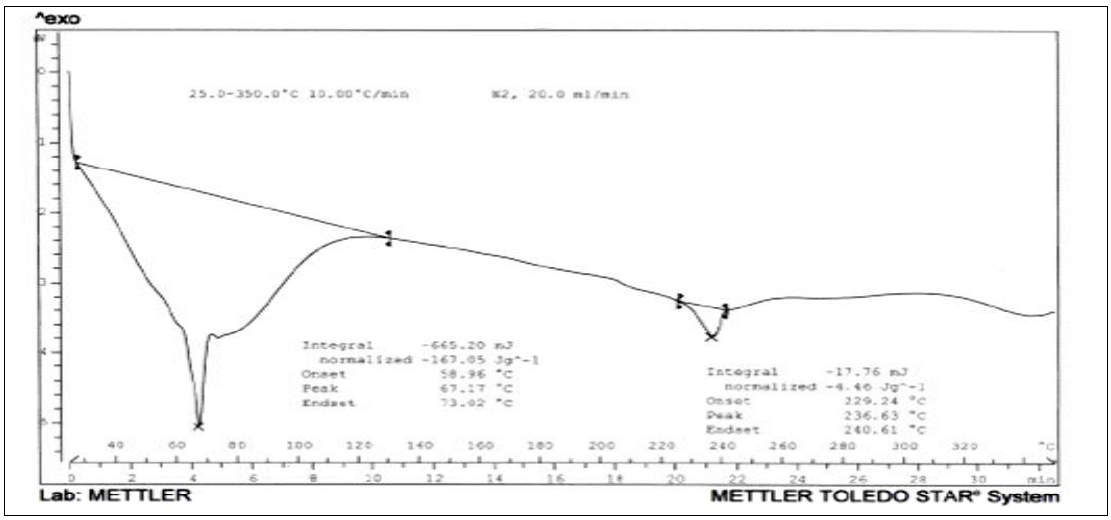


Figure 7: DSC study for Placebo Blend

Table 40: Compatibility study for Drug with Excipients

S. No.	Formulation	Onset	Peak	Endset
1	Mycophenolate Sodium	180.55°C	192.72°C	195.64°C
2	Final Prototype Formulation	181.62°C	188.78°C	192.55°C
3	Placebo Blend of final formulation	None	None	None

From the above observation, it can be seen that there was an insignificant change in the melting behavior of drug after its formulation in to the tablet.

4.1.4.1 XRD Data

The XRD evaluation of MPS and granules containing MPS revealed that the polymorphic form was same after granulation. The graphs of XRD for MPS, MPS

MR tablet blend (containing MPS) MR tablet blend (placebo), MPS MR tablet granules and MR tablet granules (placebo) are shown below in Figure 8-12.

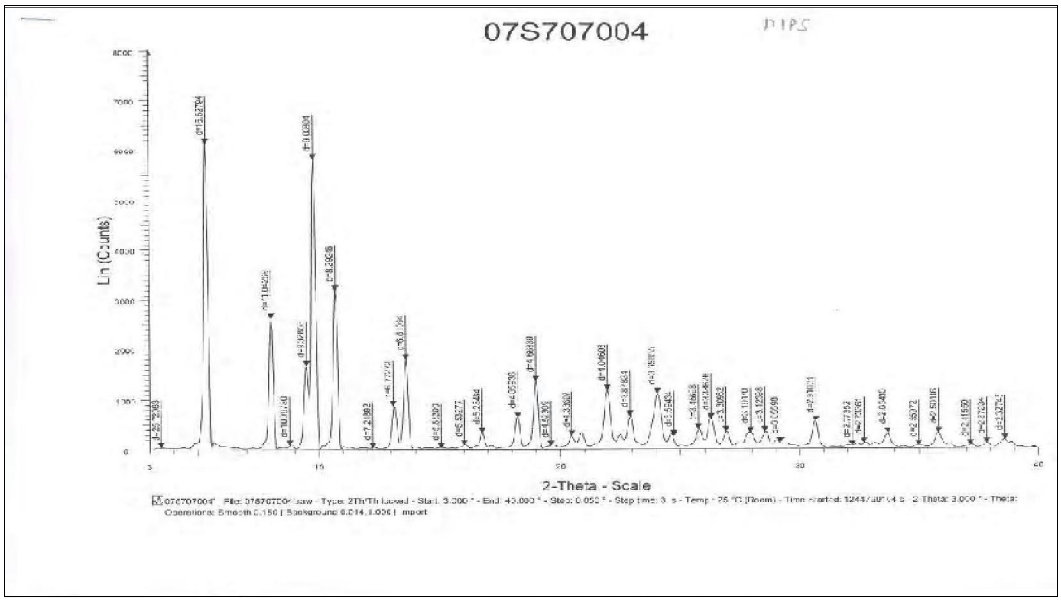


Figure 8: XRD study for MPS

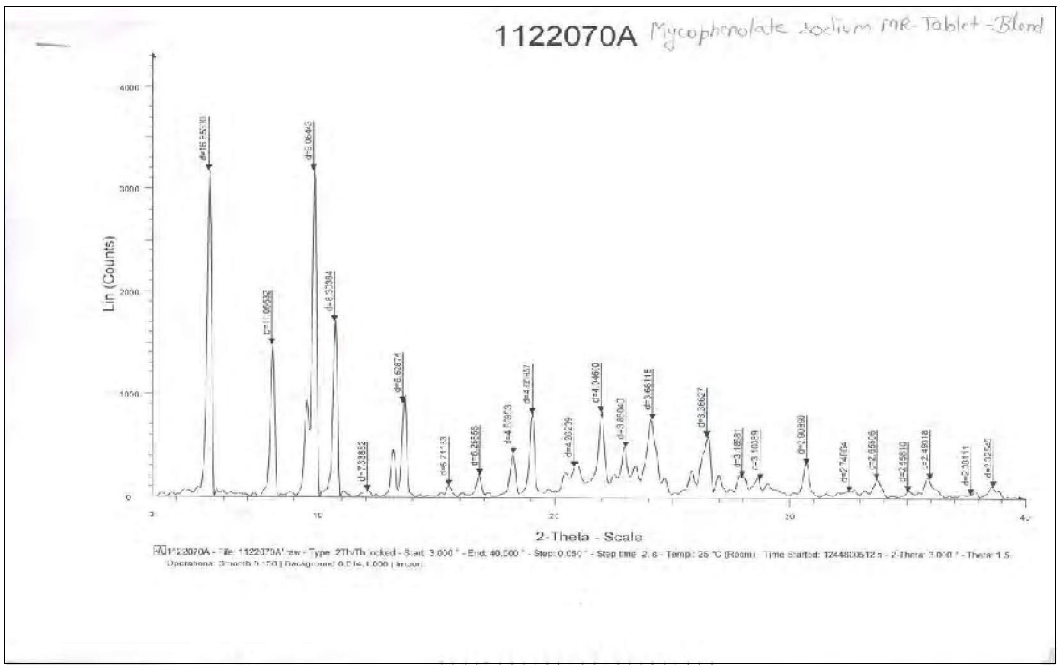


Figure 9: XRD study for Mycophenolate Sodium MR Tablets Blend

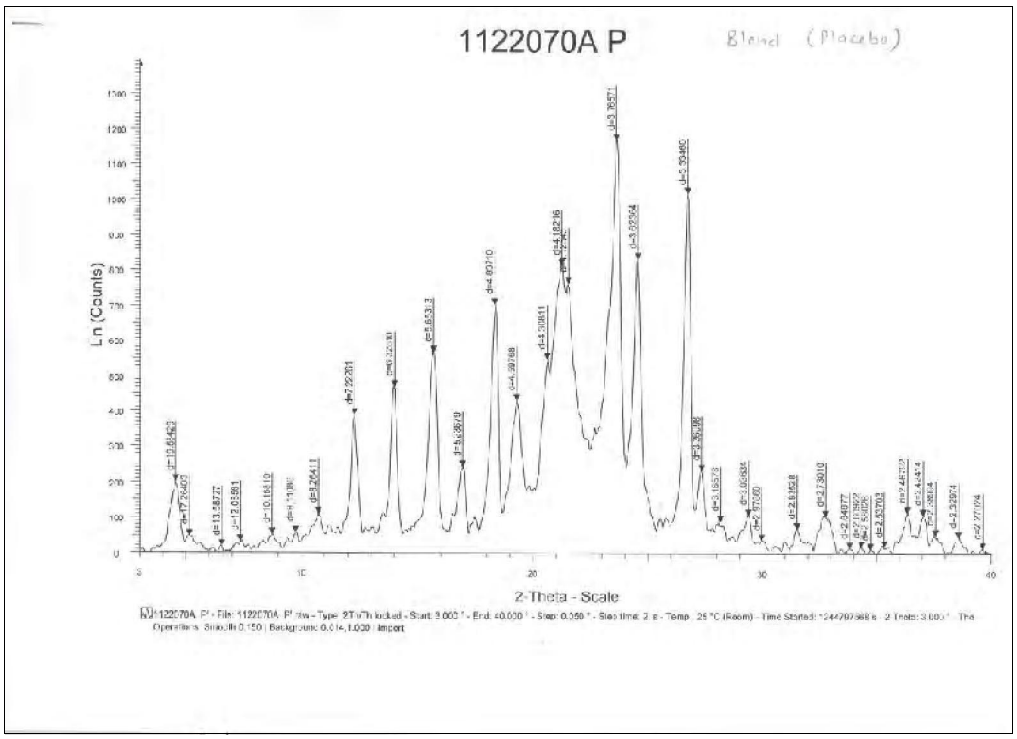


Figure 10: XRD study for Mycophenolate Sodium MR Tablets Blend (Placebo)

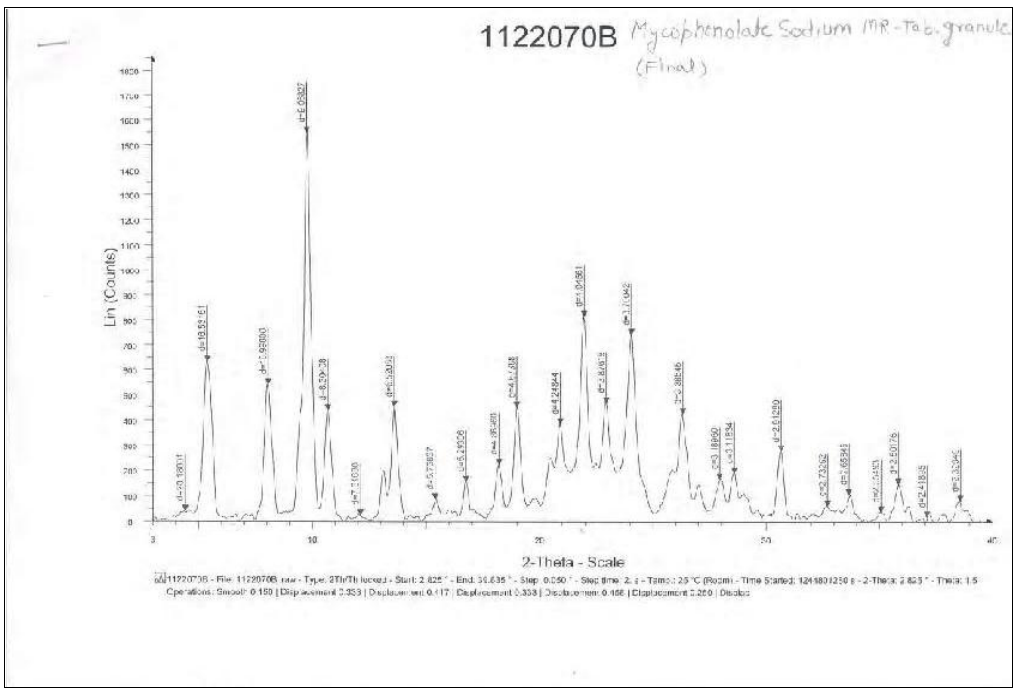


Figure 11: XRD study for Mycophenolate Sodium MR Tablet Granules (Final)

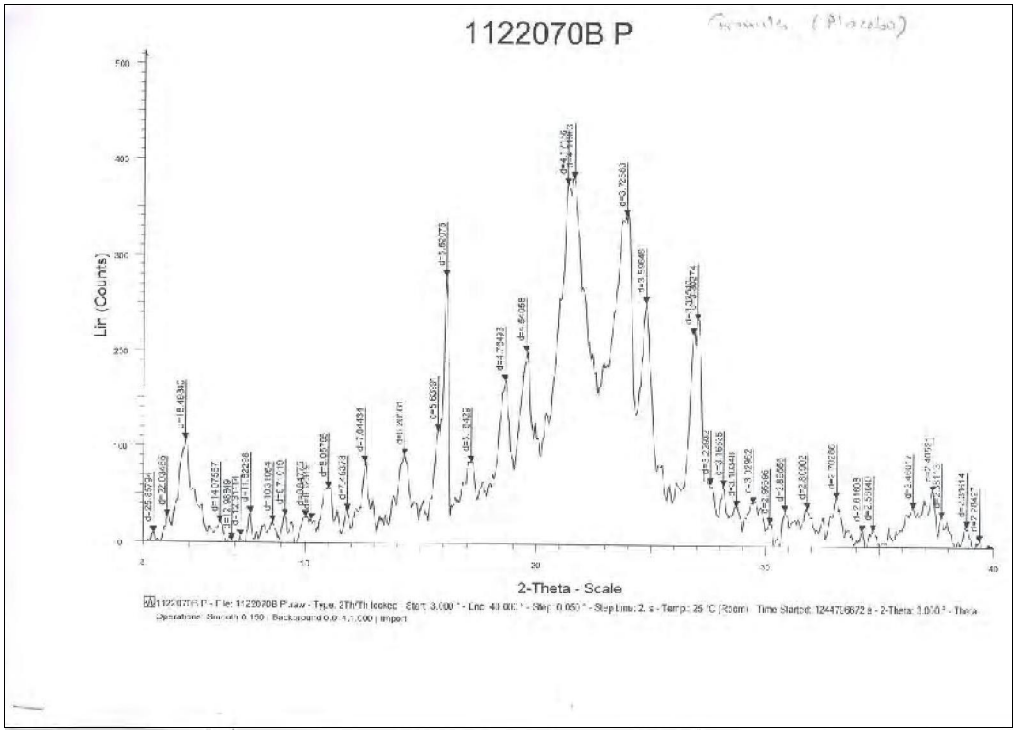


Figure 12: XRD study for Mycophenolate Sodium MR Tablet Granules (Placebo)

Table 41: Compatibility study for Drug with Individual Excipients

Sample Name	Initial			After 1M at 50°C			After 2M at 50°C		
	Impurity Profile			Impurity Profile			Impurity Profile		
	I	II	III	I	II	III	I	II	III
Mycophenolate Sodium	ND	0.07	0.07	ND	0.06	0.06	ND	0.06	0.06
Mycophenolate Sodium + Lactose anhydrous	ND	0.04	0.04	ND	0.04	0.04	ND	0.05	0.05
Mycophenolate Sodium + Polyvinylpyrrolidone (Plasdone K 30)	ND	0.05	0.05	ND	0.07	0.07	ND	0.05	0.05
Mycophenolate Sodium + Polyvinylpyrrolidone (Plasdone K 90)	ND	0.07	0.07	ND	0.07	0.07	ND	0.05	0.05
Mycophenolate Sodium + HPMC (Methocel K100 M CR)	ND	0.05	0.05	ND	0.06	0.06	ND	0.05	0.05
Mycophenolate Sodium + Polyethylene Oxide (Polyox WSR 301)	ND	0.05	0.06	ND	0.03	0.03	ND	0.05	0.05
Mycophenolate Sodium + Magnesium Stearate	ND	0.03	0.04	0.2	0.04	0.04	ND	0.08	0.08
Mycophenolate Sodium + Colloidal silicon dioxide	ND	0.03	0.04	ND	0.04	0.04	0.03	0.04	0.05
Mycophenolate Sodium+ Opadry II Yellow 85G82625	ND	0.07	0.07	ND	0.04	0.04	ND	0.05	0.09

I: 5,7-dihydroxy-4-methylphthalide
II: Any Other impurity
III: Total impurities

It can also be observed that there is not much increase in the related substances. It reveals that drug was stable within the composition.

4.1.5 Analytical Method Development and validation for Assay, Related substance and dissolution.

4.1.5.1 Analytical Method Development for Assay

λ_{max} determination

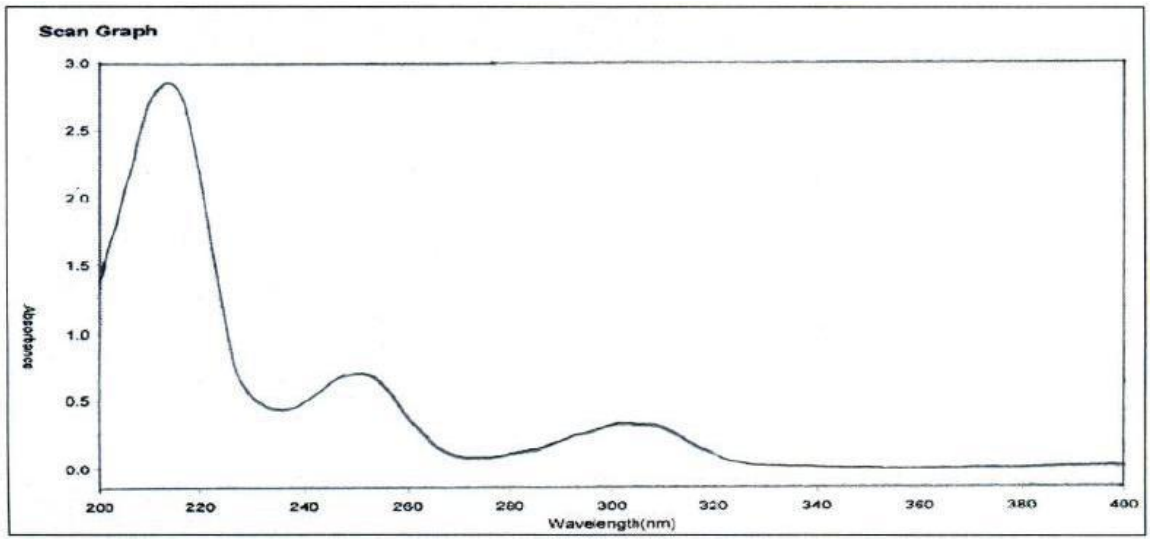


Figure 13: Scan of Mycophenolate Sodium in 0.1N HCl

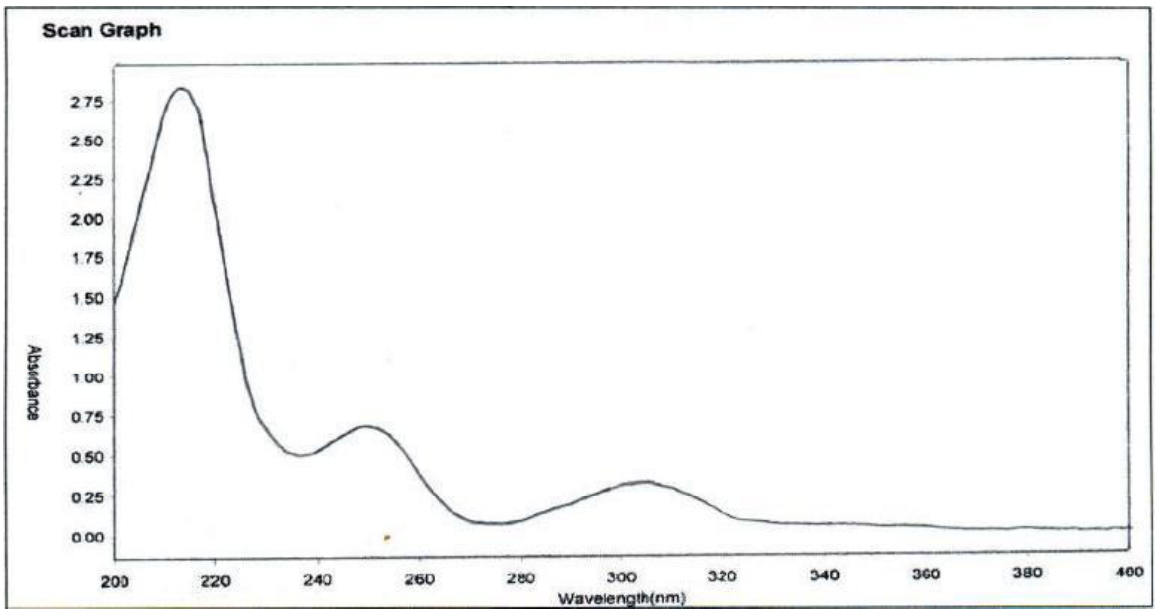


Figure 14: Scan of Mycophenolate Sodium in pH 6.8 Phosphate buffer

From Figure 13 and 14 it was found that drug showed maximum absorbance at 304 nm in both the medium.

HPLC Method Development

The method was developed using HPLC equipped with UV detector for the analysis of Mycophenolate Sodium. The chromatogram for the same is presented in Figure 15. Figure depicts the chromatogram of blank (top), Standard (middle) and test (bottom).

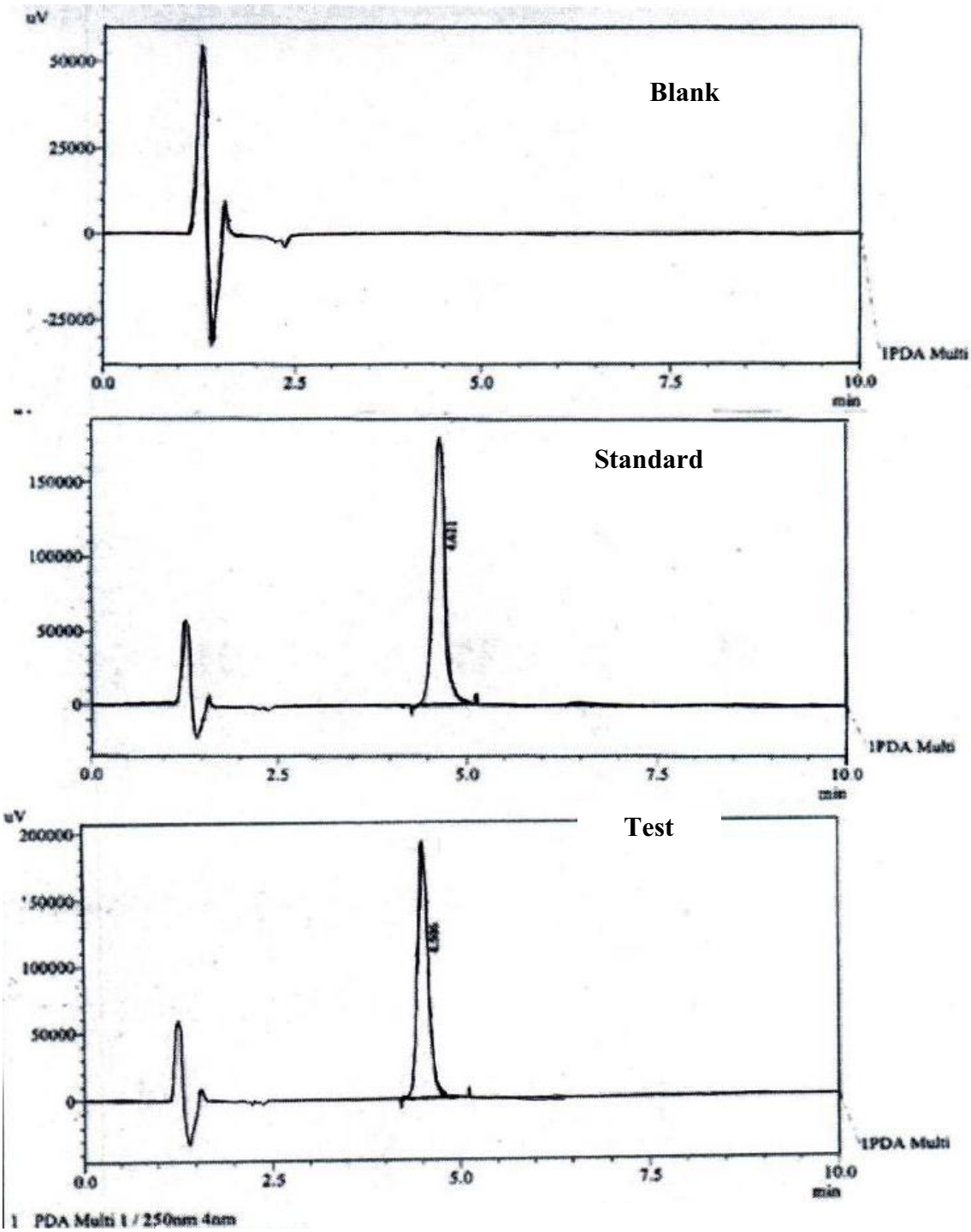


Figure 15: Chromatogram for blank, Standard and test samples respectively.

It can be observed from Figure 15 that the peak of the drug was obtained at a retention time of 4.56 min and detected at a wavelength of 250 nm.

4.1.5.2 Analytical Method Validation for Mycophenolate Sodium for Assay

Specificity

It was observed that there was no interference of mobile phase (diluent) and placebo at the retention time of Mycophenolate Sodium. It depicts that the developed method was specific for the analysis of Mycophenolate Sodium.

Forced Degradation Study

The conditions and results of forced degradation study are summarized in Table 42:

Table 42: Forced Degradation Study

Peak Purity test:			
Peak Stress conditions	**Assay of unstressed/ degraded samples w.r.t claim (%)**	**% Degradation w.r.t Assay**	**Peak Purity index**
Unstressed	100.05	NA	1.00
Acidic: 1 mL of 0.1 N Methanolic HCl /2hrs.	37.59	62.46	1.00
Alkaline: 1 mL of 0.01 N Methanolic NaOH/60°C 2 hr.	66.17	33.88	1.00
Oxidative; 1 mL of 50% H_2O_2 for 2 hrs	52.41	47.64	1.00
Humidity (Saturated Potassium nitrate solution) 24Hrs	100.22	-0.17	1.00
Thermal @ 50°C/24Hrs	94.40	5.65	1.00
UV cabinet for 12 hrs	100.50	-0.45	1.00
Acceptance criteria		Peak purity index should be more than 0.99	

During forced degradation, the Mycophenolate Sodium peak was found to be separated from placebo peaks and the peak purity index for each sample was within acceptance limits. The chromatogram for forced degradation in one of the conditions (acidic) is depicted in Figure 16.

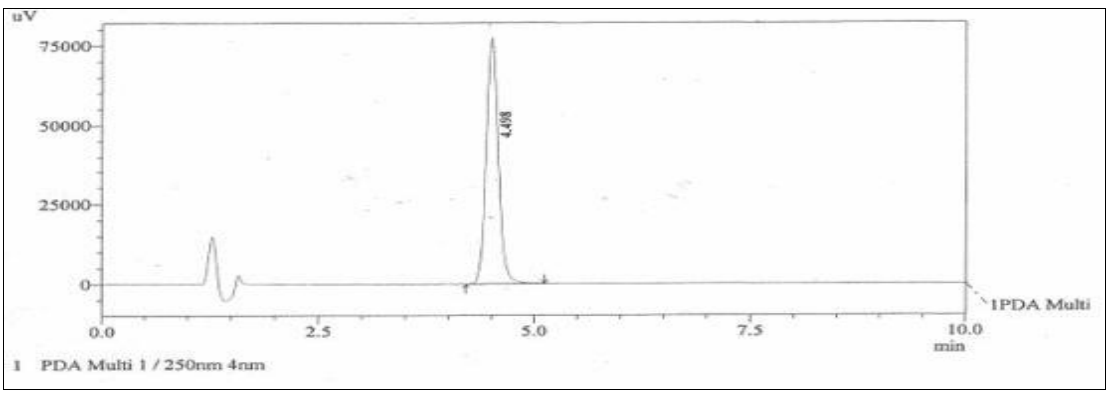


Figure 16: Representative chromatogram for sample subjected to Forced degradation study

Linearity

Linearity of the method was studied from 50%, 60%, 80%, 100%, 120% and 140% of Mycophenolate Sodium of the test concentration in the test as per the proposed method. The injections were made in duplicate. The results obtained are enlisted in the Table 43. Linearity plot for analytical method validation of Mycophenolate Sodium by HPLC is given in Figure 17.

Table 43: Observation for linearity of the analytical method

	50%	**60%**	**80%**	**100%**	**120%**	**140%**
Conc.(ppm)	195	234	312	390	468	585
Mean Area	943052	1133030	1509035	1906952	2376106	2873172
Regression Equation	y = 5037.2x-43320					
Coefficient of Determination	R^2 = 0.9981					
Acceptance Criteria	Coefficient of Determination R^2 should be more than 0.99					

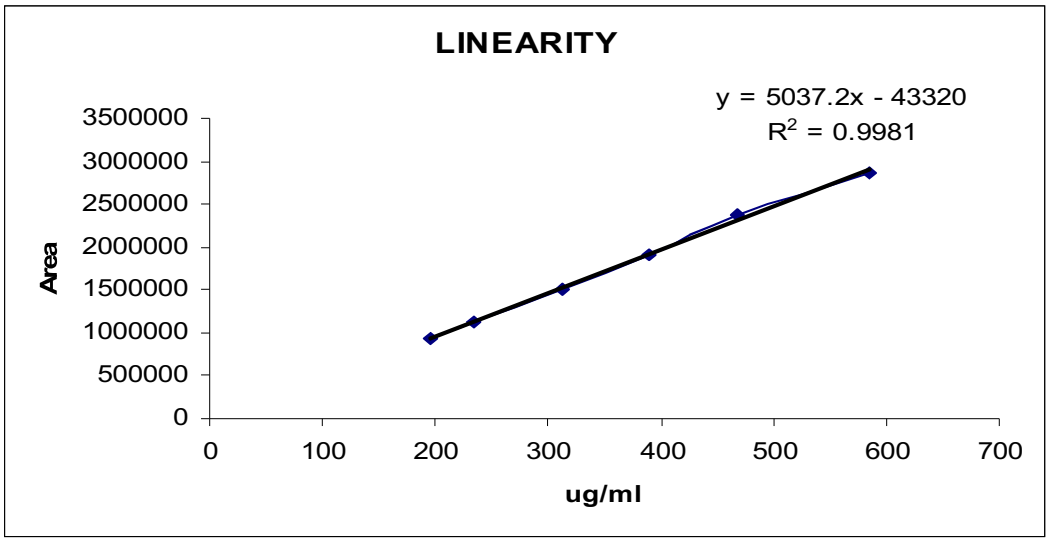


Figure 17: Linearity plot for analytical method validation of Mycophenolate Sodium by HPLC.

The linearity of a method is a measure of how well a calibration plot of response vs. concentration approximates a straight line, or how well the data fits to the linear equation:

$$y = mx + c$$

where y is the response, x is the concentration, m is the slop and c is the intercept of a

line fitting to the data. Ideally a linear relationship is preferred because it is more precise, easier for calculation, and can be confined with few standards. Also, UV detector response for a dilute sample is expected to follow Beer's law, and should be linear. Therefore, a linear calibration gives evidence that the system is performing properly in the concentration range of interest.

Precision

Precision can be defined as "the degree of agreement among test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample". [122]

A more comprehensive definition proposed by the International Conference on Harmonization devides precision into these types: (1) repeatability (2) intermediate precision, and (3) reproducibility. Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of time is instrumental precision. This is measured by the sequential, repetitive injection of the same homogeneous sample (typically 10 or times), followed by the averaging of the peak areas or height value and determination of relative standard deviation of all injections. A second aspect is sometimes termed intra-assay precision and involves multiple measurement of the same sample (different parameters) under the same conditions (Table 44). Instrumental precision is the agreement of complete measurement when the same method is applied many times within the same laboratories.

Table 44: Precision (repeatability) data for method validation of Mycophenolate Sodium

	Test 1	**Test 2**	**Test 3**	**Test 4**	**Test 5**	**Test 6**
Mean Area	1914559	1916478	1915115	1905759	1905575	1923649
Assay (in %)	98.46	98.56	98.49	98.00	98.00	98.92
Mean Area for Standard	1978764					
Mean Assay	**98.40**					
%RSD	**0.36**					
Acceptance criteria	**% RSD should not be more than 5.0%**					

Intermediate Precision

Intermediate Precision of the proposed assay method was assessed by making six determinations at 100% of test concentration by different analysts on different

instrument. Six aliquot of the test samples were analyzed as per the proposed method. The data is compiled in the Table 45.

Table 45: Intermediate precision observation for method validation

	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Mean Area	1899944	1898664	1884368	1881324	1867685	1894652
Assay (in %)	99.83	99.76	99.01	98.85	98.13	99.55
Mean Area for Standard	1916082					
Mean Assay	**99.19**					
%RSD	**0.66**					
Acceptance criteria	**% RSD should not be more than 5.0%**					

There was no significant variation in the six values obtained, the precision and intermediate precision (repeatability) method hence validated.

Determination of variation between the results of precision and intermediate precision

Table 46: Variation between the results of precision and intermediate precision

S. No	Precision	Intermediate precision	%Variation
1	98.40%	99.19%	0.79

Acceptance Criteria: The percent variation should not be more than 5.0. The value of percentage variation obtained was 0.79% indicating the precision of method.

From the results in the Table 46, it was clear that the analytical method was "Precise".

Accuracy

Accuracy of the method was studied at three levels (80%, 100% and 120% of assay concentration) in triplicate. The amount of analyte recovered was calculated and compared with the amount of Mycophenolate Sodium added. Accuracy is defined as the closeness of the measured value to be the true value. The true value can be obtained by direct comparision to a standard or by analyte recovery or by the method of standard addition. The data given in the Table 47 represents the percentage recovery at three different levels.

Table 47: Accuracy data for the method validation of Mycophenolate Sodium

Mean area of peak due to Mycophenolate Sodium peak = 1891375									
Concentration	**80%**			**100%**			**120%**		
	I	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
Mean Area of Mycophenolate Sodium	1558574	1518484	1545043	194793	1921940	1919971	227727	2332171	2310533
Mycophenolate Sodium Found (mg)	625.15	609.07	619.72	781.32	770.90	770.11	913.42	935.44	926.76
% Recovery	101.16	98.63	100.35	101.31	100.00	99.89	98.49	101.06	100.01
Range	98.63-101.31								
Acceptance Criteria	% Recovery should be between 95.0 and 105.0								

Acceptance criteria for accuracy for all the concentration range fall within the range i.e. ±5%, which confirmed the accuracy of developed method.

Robustness

Change in flow rate

Robustness was studied by altering the flow rate of the mobile phase from 1.5 mL to 1.5 ± 0.1 mL/min. The results are compiled in Table 48.

Table 48: Robustness (change in flow rate) data for the method validation of Mycophenolate Sodium

	Initial Condition (Taken from Repeatability)	**Change in Flow rate of Mobile Phase**	
		1.4 mL/min.	**1.6 mL/min.**
Assay (%)	98.40	97.53	97.36
% variation	--	-0.88	-1.05
Acceptance Criteria	% variation in assay should not be more than 5.0% by changing the flow rate 1.5±0.2 mL/min. w.r.t. initial assay (Repeatability).		

Since the percent variation in terms of assay (%) by changing the flow rate by ±0.1 mL/min was within the acceptable range for Mycophenolate Sodium, and so the change in flow rate by ±0.1 mL/min. will not affect the assay.

Change in column oven temperature

Robustness was studied by changing the column oven temperature from 45°C±5°C. The results are displayed in Table 49.

Table 49: Robustness (change in column oven temperature) data for the method validation of Mycophenolate Sodium

	Initial Condition	Change in column oven temperature	
		40°C	50°C
Assay (%)	98.40	98.44	97.34
% variation	--	0.04	-1.07
Acceptance Criteria	% Variation in assay should not be more than 5.0% by changing the column oven temperature. w.r.t. initial assay (Repeatability)		

The value of percentage variation was found to be below 5 % on changing the column oven temperature from 45°C to 45±5°C will not affect the assay. It could be inferred that the method was robust.

4.1.5.3 Analytical method validation for Mycophenolate Sodium for related substance

Specificity

It was observed that there is no interference of sample diluent and placebo, at the retention time of Mycophenolate Sodium peak. Due to higher concentration, the samples did not pass the peak purity as the absorbance was more than 1. So the samples were further diluted 10 times. After dilution the samples passed the peak purity test.

Forced degradation study: During forced degradation, Mycophenolate Sodium peak was well separated from degraded peaks with no interference, of placebo and degradant peaks. Peak Purity for Mycophenolate peak was within acceptance criteria. The results of forced degradation study are given in the Tables 50 and 51.

Table 50: Forced degradation study data of B. No. NAXMP20

Stress conditions	% of Impurities in degraded/unstressed samples (%)			Peak Purity Test for Mycophenolate Sodium peak in dilute test sample	
	5,7-Dihydroxy-4-methyl phthalide	Single Highest impurity	Total impurities	Peak Purity Angle	Peak Purity Threshold
Unstressed	ND	0.03	0.1	0.080	0.279
Acidic: 5 mL of 0.5 N HCl/@ 100°C for 4 hrs	2.29	2.29	10.28	0.070	0.272

Stress conditions	% of Impurities in degraded/unstressed samples (%)			Peak Purity Test for Mycophenolate Sodium peak in dilute test sample	
	5,7-Dihydroxy-4-methyl phthalide	Single Highest impurity	Total impurities	Peak Purity Angle	Peak Purity Threshold
Alkaline: 5 mL of 0.5 N NaOH/@ 100°C for 4 hrs	13.28	13.28	14.5	0.057	0.264
Oxidative; 5 mL of 5% H_2O_2@RT for 4 hrs	0.1	0.1	0.44	0.069	0.276
Light: exposed in UV light for 24Hrs.	0.03	0.03	0.13	0.079	0.280
Humidity : exposed in Humidity condition (Saturated Potassium nitrate solution %RH-92%) for 24Hrs.	0.02	0.03	0.13	0.075	0.280
Heat: exposed @ 100°C for 24Hrs.	ND	0.04	0.27	0.083	0.279
Acceptance criteria	Purity angle should be less than Purity threshold.				

Table 51: Forced degradation study data of B. No. NAXPM19

Stress conditions	% of Impurities in degraded/unstressed samples (%)			Peak Purity Test for Mycophenolate Sodium peak in dilute test sample	
	5,7-Dihydroxy-4-methyl phthalide	Single Highest impurity	Total impurities	Peak Purity Angle	Peak Purity Threshold
Unstressed	ND	0.03	0.06	0.082	0.280
Acidic: 5 mL of 0.5 N HCl/@ 100°C for 4 hrs	0.88	5.52	8.2	0.069	0.272
Alkaline: 5 mL of 0.5 N NaOH/@ 100°C for 4 hrs	10.75	10.75	12.36	0.060	0.263
Oxidative; 5 mL of 5% H_2O_2@RT for 4 hrs	0.37	0.37	0.65	0.075	0.279
Light: exposed in UV light for 24Hrs.	0.02	0.03	0.11	0.071	0.287
Humidity : exposed in Humidity condition (Saturated Potassium nitrate solution %RH-92%) for 24Hrs.	0.02	0.03	0.1	0.089	0.286
Heat: exposed @ 100°C for 24Hrs.	0.01	0.03	0.13	0.087	0.282
Acceptance criteria	Purity angle should be less than Purity threshold.				

Precision

The data compiled in the Table 52 and 53 show that method is precise.

Table 52: Data representing precision of single highest impurity and total impurity for B. No. NAXPM20

Parameters	% of Single highest impurity	% of Total Impurities
Replicate-1	0.04	0.08
Replicate-2	0.04	0.07
Replicate-3	0.04	0.06
Replicate-4	0.04	0.07
Replicate-5	0.04	0.07
Replicate-6	0.04	0.07
Mean	0.04	0.07
%RSD	0.0	9.0
Acceptance criteria	**RSD should not be more than 10.0%**	

Table 53: Data representing precision of single highest impurity and total impurity for B. No. NAXPM19

Parameters	% of Single highest impurity	% of Total Impurities
Replicate-1	0.04	0.06
Replicate-2	0.04	0.06
Replicate-3	0.04	0.06
Replicate-4	0.04	0.05
Replicate-5	0.04	0.06
Replicate-6	0.04	0.06
Mean	0.04	0.06
%RSD	0.0	7.0
Acceptance criteria	**RSD should not be more than 10.0%**	

Accuracy: The data compiled in the Table 54 shows that method is "Accurate"

Table 54 represents the accuracy data for known impurities *i.e.*, 5,7-Dihydroxy-4-methyl phthalide at different levels defined in Materials and Method section.

Table 54: Data representing accuracy of known impurity for B. No. NAXPM20

Concentration	80%			100%			120%			150%			200%		
Drug Added (mg)	0.1471	0.1471	0.1471	0.1883	0.1883	0.1883	0.2354	0.2354	0.2354	0.2942	0.2942	0.2942	0.3825	0.3825	0.3825
Drug Found (mg)	0.1501	0.1552	0.1485	0.185	0.1917	0.185	0.2318	0.233	0.2310	0.2937	0.2865	0.2899	0.3741	0.3747	0.3747
% Recovery	102.0	105.5	101.0	98.2	101.8	98.3	98.5	99.0	98.2	100.2	102.7	101.5	102.2	102.1	102.1
Mean % Recovery	102.8			99.4			98.6			101.5			102.1		
Range (%)	98.2 – 105.5														
Mean	100.9%														
Acceptance Criteria	% Recovery should be between 90.0 and 110.0														

Determination of LOQ & LOD Limits

Table 55 represents the LOQ and LOD limits

Table 55: LOQ & LOD Limits:

Parameters	LOQ (0.6 µg/mL)	LOD (0.2 µg/mL)
Replicate-1	12656	3960
Replicate-2	12110	3780
Replicate-3	12293	3920
Replicate-4	12198	4121
Replicate-5	12072	3864
Replicate-6	12340	4111
Mean	12278	3959
%RSD	1.7	3.4
Acceptance criteria	**RSD should not be more than 10.0%**	**RSD should not be more than 33.0%**

Stability of solution of LOQ & LOD Limits

It was observed in Table 56 that the test solution was stable for 12 hrs during the analysis and the percent variance in release of Mycophenolic Acid obtained with test solution were within the acceptable range**.**

Table 56: Stability of solution

Time	5, 7-Dihydroxy-4-methyl Phthalide	% Variation	Single highest impurity	% Variation	Total impurities	% Variation	Myco-phenolate Sodium	% Variation
Initial 0 hr.	ND	-	0.04	-	0.07	-	99.93	
After 3 hr.	ND	-	0.04	0.0	0.07	0.0	99.92	- 0.01
After 6 hr.	ND	-	0.04	0.0	0.07	0.0	99.93	0.0
After 9 hr.	ND	-	0.04	0.0	0.07	0.0	99.93	0.0
After 12 hr.	ND	-	0.04	0.0	0.07	0.0	99.93	0.0
Acceptance criteria	**RSD should not be more than 10.0%**							

Filter Standardization

Data compiled in Table 57 shows that HVLP and Whatman-42 both the filters are suitable.

Table 57: Filter standardization

Sample type	5, 7-Dihydroxy-4-methyl Phthalide	% Variation	Single highest impurity	% Variation	Total impurities	% Variation	Myco phenolate Sodium	% Variation
centrifuged Sample	ND	-	0.03	-	0.05	-	99.95	-
Filtered with HVLP Filter	ND	-	0.03	0.0	0.05	0.0	99.96	0.01
Filtered with Whatman-42 Filter	ND	-	0.03	0.0	0.05	0.0	99.95	0.0
Acceptance Criteria	The % Variation of test preparation filtered HVLP and Whatman-42 filter should not be more than 5.0%, calculated w.r.t unfiltered standard.							

Table 58 summarizes the results obtained during partial validation of analytical method for Related Substances.

Table 58: Results of partial validation of analytical method for Related Substances

Analytical Performance Characteristics	Acceptance Criteria	Results
Specificity	**Interference of sample matrix, and sample diluent:** Should have no interference at the retention time of Mycophenolate and known impurities peak	Complies
	Peak Purity: Purity angle should be less than purity threshold	Complies
	Degradation of samples: Method should be capable to measure the degradation of the drug	Complies
Precision	% RSD for repetitive determination for single highest impurity should not be more than 10.0%	NAXPM19 – 9.8%
	% RSD for repetitive determination for total impurities should not be more than 10.0%	NAXPM20-8.4%
% Recovery	% Recovery should be between 90%-110%	98.2 %– 105.5%
Determination of LOQ	% RSD for repetitive determination for LOQ should not be more than 10.0%	1.72%
Determination of LOD	% RSD for repetitive determination for LOD should not be more than 33.0%	3.42%
Stability of the solutions	The % variation should not be more than 10.0% w.r.t. the results obtained at initial (0 hours).	The test solution is stable up to 12 hrs.
Filter standardization	The % variation of test preparation filtered through HVLP and Whatman-42 filters should not be more than ±5.0%, calculated w.r.t. unfiltered standard	**NAXPM20** Single highest impurities HVLP = 0.0% Whatman -42 = 0.0% Total impurities

Analytical Performance Characteristics	Acceptance Criteria	Results
		HVLP =0.0% Whatman -42 = 0.0% Mycophenolate HVLP = 0.01% Whatman -42 = 0.0%

4.1.5.4 Analytical method validation for dissolution samples

Specificity

Table 59 summarizes the data of system suitability performed on UV spectrophotometer.

Table 59: λmax determination for Mycophenolate Sodium

S.No.	Name	λmax
1	Standard Preparation	304.06
2	Test preparation	304.58
Acceptance Criteria	The λ_{max} should be 304±2	

There is no interference of blank and placebo at 304 nm, the λ_{max} was within 304±2. Hence the specificity of the method was validated.

Linearity

Linearity of the method was studied using five different concentrations in the range of 50% to 150% of the actual working concentration as per the proposed method. The absorbance of the resulting solutions was taken in triplicates.

It can be interpreted from Table 60 and Figure 18 that the method was linear in the desired range of concentration of Mycophenolate Sodium.

Table 60: Linearity observations for method validation for dissolution samples

Conc. (ppm)	Abs 1	Abs 2	Abs 3	Mean Abs
17	0.2104	0.211	0.2102	0.2105
20.4	0.248	0.2483	0.2481	0.2481
27.2	0.3186	0.3185	0.3192	0.3188
34	0.4106	0.4103	0.4098	0.4102
40.8	0.5019	0.5026	0.5025	0.5023
51	0.6421	0.6416	0.6421	0.6419

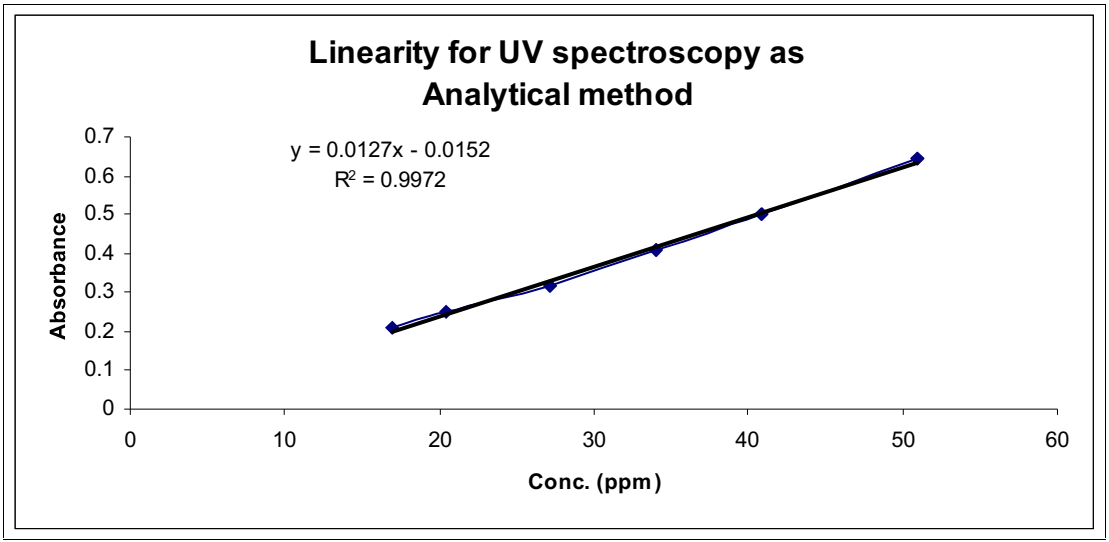


Figure 18: Linearity plot for method validation of dissolution samples

Precision

Precision of the method was studied by analyzing dissolution samples at 304 nm withdrawn after 24 h from the six Dissolution Jars. The intervessel and intravessel precision results are given in Table 61 and 62 respectively.

Table 61: Intervessel precision for the developed method

Weight of Mycophenolate Sodium WS taken: 29.5 mg Mean Absorbance: 0.3912						
	Tablet 1	**Tablet 2**	**Tablet 3**	**Tablet 4**	**Tablet 5**	**Tablet 6**
Absorbance	0.4612	0.4613	0.4618	0.4699	0.4706	0.4663
% Release w.r.t claim	100.8	100.8	100.9	102.7	102.8	101.9
Mean (%)	**101.7**					
%RSD	**0.9**					
Acceptance criteria	**% RSD should not be more than 5.0%**					

Table 62: Intravessel precision

Weight of Mycophenolate Sodium WS taken: 29.5 mg Mean Absorbance: 0.3912						
	Test 1	**Test 2**	**Test 3**	**Test 4**	**Test 5**	**Test 6**
Absorbance	0.4616	0.461	0.4612	0.4612	0.4615	0.4612
% Release w.r.t claim	100.9	100.8	100.9	102.7	102.8	101.9
Mean (%)	**100.9**					
%RSD	**0.9**					
Acceptance criteria	**% RSD should not be more than 2.0%**					

From above results it is clear that the % RSD for both intervessel and intravessel precision was within the acceptance limit, therefore the method was "Precise" (Table 62).

Accuracy

Accuracy study was performed at three concentration levels (80%, 100% and 120% of test concentration) in triplicate. The drug was dissolved in dissolution media and spiked to the respective dissolution media-containing placebo. Samples were withdrawn at the end of the dissolution time. The samples were analyzed on UV at 304 nm. Drug added to the dissolution media is displayed in Table 63 at three different levels.

Table 63: Accuracy data for method development for dissolution samples

Concentration	80%			100%			120%		
Placebo Taken	**1 Tablet**	**1 Tablet**	**1 Tablet**	**1 Tablet**	**1 Tablet**	**1 Tablet**	**1 Tablet**	**1 Tablet**	**1 Tablet**
Drug Added (mg)	617.34	617.02	616.80	771.68	771.25	770.58	926.46	925.87	909.54
Absorbance	0.3683	0.3685	0.3702	0.4595	0.4592	0.4588	0.5365	0.5368	0.5366
Found (mg)	624.27	624.61	627.49	771.68	771.25	770.58	926.46	925.87	926.18
% Recovery	101.1	101.2	101.7	100.9	100.9	100.9	98.2	98.3	98.2
Mean (%)	**101.33**			**100.9**			**98.23**		
Overall Mean (%)	**100.15**								
Range	**98.2-101.7**								
Acceptance Criteria	**% Recovery should be between 95.0 and 105.0**								

Results showed that % recovery was within the acceptable range, depicting the validation of accuracy.

4.1.6 Analytical method development and validation for quantification of Mycophenolic Acid in plasma samples

4.1.6.1 Analytical method development of Mycophenolic Acid in plasma samples

Bio-analytical method developed for the determination of Mycophenolic acid by LCMS/MS and the chromatogram are as given in Figure 19.

It can be observed from the above mentioned figures that no peak was observed in blank plasma (A), while in case of test sample of volunteers a sharp peak of

Mycophenolic Acid was observed (C). The value of LOD was found to be 0.1 µg/mL (B).

4.1.6.2 Analytical method validation of Mycophenolic Acid in plasma samples

System suitability

System suitability results for bioanalytical method are summarized in Table 64.

Table 64: System suitability for bioanalytical method

S. No.		Mycophenolic acid (area)	Internal Standard (IS) (area)	Peak Area ratio
1	Solv.MQC-1	710000	3000000	0.236
2	Solv.MQC-2	648000	2870000	0.225
3	Solv.MQC-3	685000	2860000	0.240
4	Solv.MQC-4	659000	2730000	0.242
5	Solv.MQC-5	637000	2680000	0.237
6	Solv.MQC-6	629000	2480000	0.254
7	Solv.MQC-7	646000	2350000	0.275
Mean		659142.86	2710000	0.244
SD		28725.30	229492.19	0.02
%CV		4.36	8.47	6.59

Criteria: %CV of the system suitability should be less than 15

For a system suitability % CV should be less than 15 which was 6.59 % in the above case, hence system was validated for system suitability.

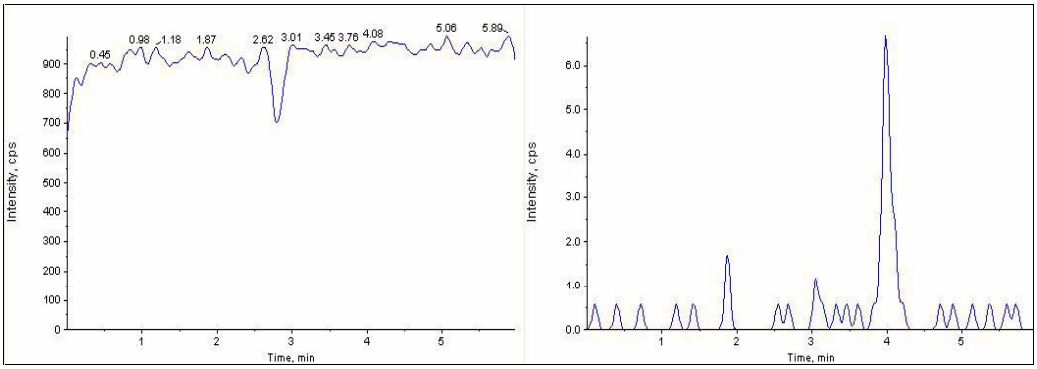
Selectivity and Matrix effect in human plasma samples

The results for selectivity and matrix effect are summarized in the Table 65 below:

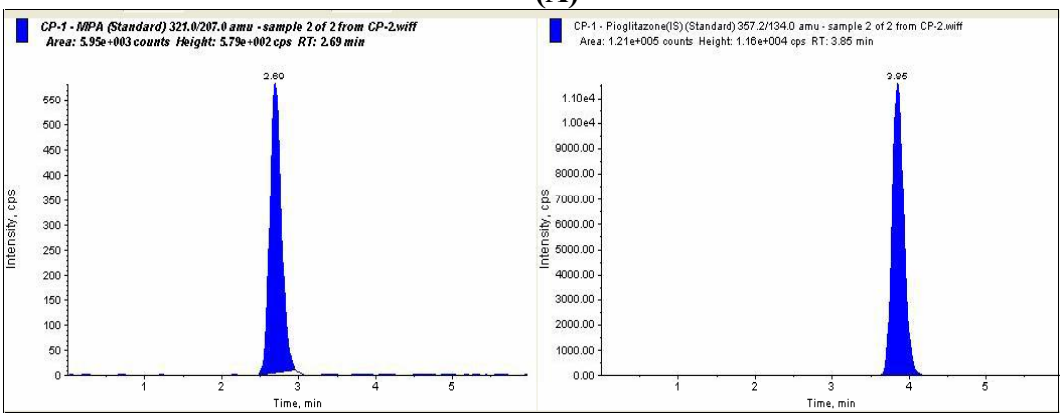
Table 65: Selectivity and Matrix effect in human plasma

MQC	% Accuracy
MQC-1	93.4
MQC-2	102
MQC-3	105
MQC-4	102
MQC-5	105
MQC-6	115

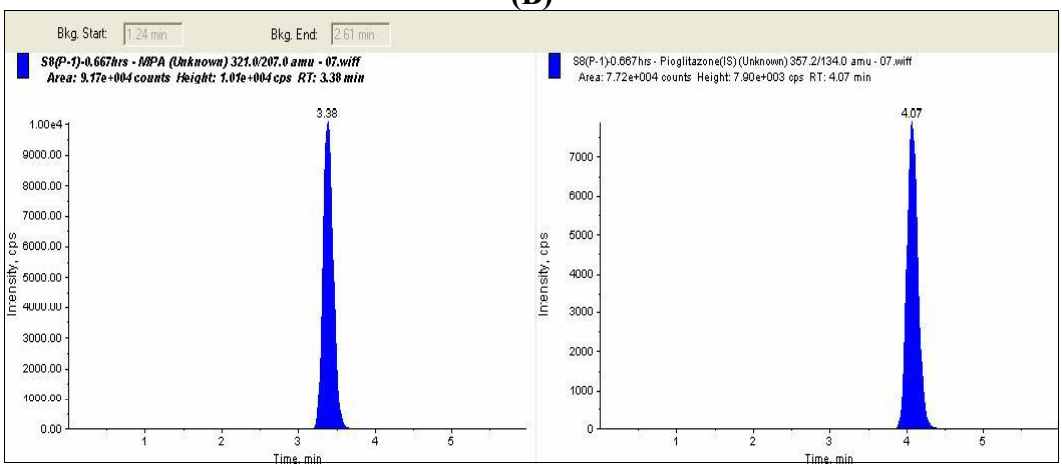
For validating selectivity and matrix effect % recovery should be between 85%-115%. Results demonstrate that % recovery fall within the range which reveals that the method is validated for both selectivity and matrix effects.



(A)



(B)



(C)

Figure 19: LCMS/MS Chromatograms for Mycophenolic Acid (Bio-analytical Method)

[(A) Blank, (B) LLOQ calibration curve plasma (0.1mcg) with internal standard, (C) Test sample]. The left hand chromatogram is for analyte (MPA) and right hand chromatogram is for internal standard.

Linearity

The linearity of response for the method was determined by analyzing the calibration standards solutions of Mycophenolic Acid (Cal-1 to Cal-7) in the concentration range of 0.25µg/mL to 32µg/mL. The results show that the ratio of Mycophenolic Acid to internal standard peak area is linear with in the concentration range of the analysis. The correlation coefficient was > 0.98.

Table 66: Linearity data for bioanalytical method validation

	Nominal Concentration	SET-1		SET-2		SET-3	
	ng/ml	Actual Conc (µg/ml).	Peak Area Ratio	Actual Conc. (µg/ml)	Peak Area Ratio	Actual Conc. (µg/ml)	Peak Area Ratio
1.	0.25	0.237	0.015	0.251	0.015	0.259	0.014
2.	1	0.943	0.055	1.03	0.067	1.03	0.056
3.	2	2.19	0.125	1.94	0.126	1.98	0.108
4.	4	4.34	0.241	3.9	0.250	3.75	0.201
5.	8	7.45	0.400	8.2	0.503	7.68	0.397
6.	16	15.9	0.778	15.9	0.894	17.1	0.808
7.	32	32.2	1.290	32	1.440	31.3	1.270
	SLOPE	0.0401		0.0453		0.0408	
	INTERCEPT	0.0524		0.0612		0.040	
	CORRELATION COEFFICENT	0.987		0.983		0.991	

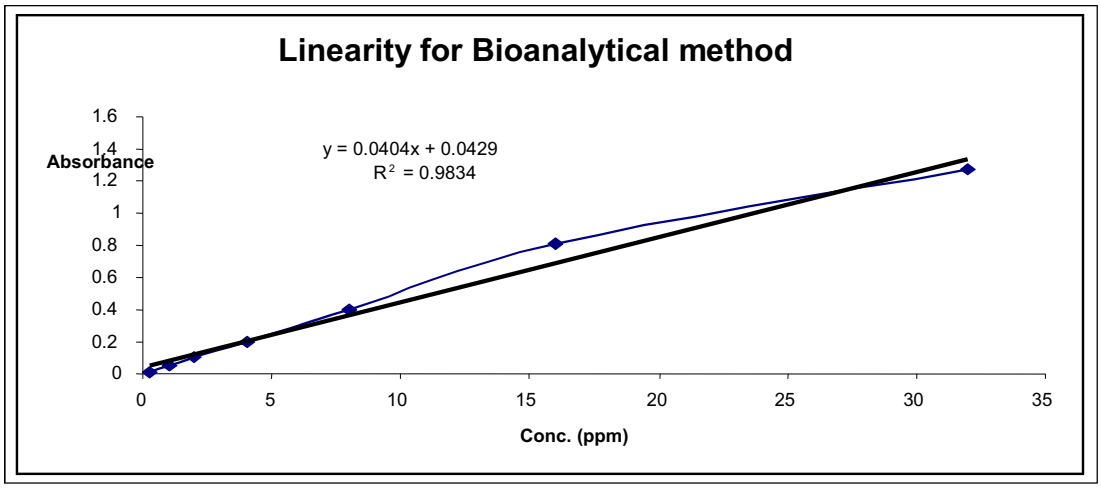


Figure 20: Linearity plot for bioanalytical method

From the linearity Table 66 and Figure 20 it was found that the method is linear in the range of concentration 0.25 µ/mL to 32 µ/mL.

Precision and accuracy

To assess the precision and accuracy of the developed method, four different concentrations in the range of expected concentrations were evaluated using six determinations per concentration. Precision and accuracy was assessed at within-day basis and between day basis. The results are summarized below:

Inter day Accuracy was found to be at:

Lower limit of quantitation (LLOQ) : 87.5-112% with %CV of 8.07

Limit of quantitation (LQC) : 88-115% with % CV of 7.37

Middle-quality control (MQC) : 89.8-115% with %CV of 5.22

High-quality control (HQC) : 86-115% with %CV of 9.44

Intra day Accuracy was found to be at:

LLOQ: 87.5-110% with %CV of 7.49

LQC: 88-111% with %CV of 7.79

MQC: 89.8-106% with %CV of 7.05

HQC: 86.8-115% with %CV of 9.53

For a system to be validated for precision CV should be less than 15% and for accuracy mean value of samples at each concentration level should be within 85 - 115% of the nominal value. From the result above it was found that the system was validated for precision and for accuracy with % CV less than 15% at LQC, MQC, and HQC levels.

Stability of extracted sample solutions

Long-term stability in human plasma samples

To evaluate the long term stability (at two different concentrations LQC, HQC), six aliquots of each concentration were maintained in the deep freezer (-20°C temperature) for 54 days and quantified against spiked calibration curve standards. The results demonstrate that the samples are stable for 54 days when maintained in the deep freezer (-20°C temperature). The mean % stability after 54 days was 85% for LQC and 103% HQC respectively.

4.1.6.3 Freeze thaw stability:

The percentage change in LQC and HQC samples was 0.8 and -1.3 % after three cycles which was within the acceptance limit.

The percentage of mean ratio LQC and HQC sample was 97.3 and 101.2% respectively, which was within the acceptance range of 90-110%, when compared with the freshly processed quality control samples.

4.2 Formulation development of Mycophenolate Sodium

The objective of the present work was to prepare once a day tablet formulation of the Mycophenolate Sodium. The drug is presently available in the market in the form of conventional tablet and prescribed twice daily. The half life of the drug is 12±5 hr. The drug follows linear pharmacokinetics. Thus, it was planned to design once in a day tablet formulation of the Mycophenolate Sodium which is has following in-vitro drug release profile.

Target Dissolution Profile for the proposed product:

Percentage of Drug Dissolved	Time (Hours)
~20%	2±1
~50%	6±2
~85%	12±2

4.2.1 Lipid Matrix Technology

The lipid matrix technology is generally used for the formulation of modified release formulations. Medium chain triglycerides (Compritol) were selected as release rate retarding material as it is hydrophobic and approved by the regulatory authorities for oral use [123-126]. Dry granulation and wet granulation (aqueous and non aqueous) method were selected for the development of modified release tablet formulation.

4.2.1.1 Dry Granulation Method (B. No. NAXLM-1)

In the tablets prepared by dry granulation technique, sticking was observed during slugging. Almost 100 % of the drug was released in 4 hr. (Table 67 and Figure 21).

Table 67: Dissolution Profile of NAXLM-1

Time (h)	% Drug Release	% RSD
1	63.6	0.6
2	92.0	1.3
4	100.7	0.6

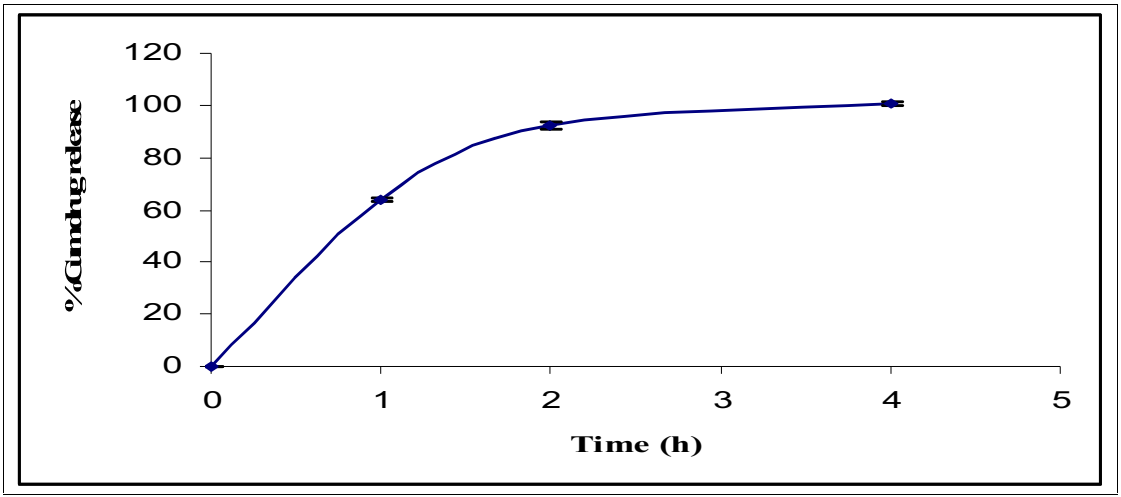


Figure 21: Drug release profile of NAXLM-1

The stickiness observed during slugging can be due to poor flow property of the drug. Since the drug comprises major portion of the composition, even concentration greater than 2% of Magnesium Stearate and Aerosil 200 could not prevent sticking. The particle size of the drug powder was about 15 micron and the flow properties were also poor (angle of repose 48°) which leads to the stickiness during slugging operation. Thus, it was planned to use wet granulation technique for the preparation of the tablet formulation. The drug release was very fast and not meeting with the target release profile so it was also decided to granulate the blend with polymer solution to achieve desired sustained drug release profile.

4.2.1.2 Wet Granulation (Aqueous) Method (B No. NAXLM - 2 to NAXLM - 4)

When tablets were prepared by wet granulation (aqueous) technique, no sticking was observed during compression of tablet as the flow properties of the prepared granules were good (angle of repose 30°). Three batches were prepared by using Eudragit RS 30D, Eudragit NE 30D and Kollicoat SR 30D as granulating agent and using Compritol as release modifying agent. The drug release was faster (Table 68 and Figure 22) and released completely in about 6-8 hr.

Table 68: Dissolution Profile of NAXLM -2, NAXLM -3 and NAXLM -4

Time (h)	NAXLM-2		NAXLM-3		NAXLM-4	
	% Drug release	% RSD	% Drug release	% RSD	% Drug release	% RSD
1	49.5	7.8	50.7	5.3	88.1	1.8
2	65.1	1.6	69.8	4.8	93.4	0.6
3	76.8	1.2	81.1	3.7	94.8	0.7
4	85.5	0.7	89.8	1.8	96.9	1.2
6	95.4	1.3	97.6	2.6	98.0	0.5
8	99.0	1.8	101.8	1.1	-	-
10	99.2	0.6	101.8	0.9	-	-
12	100.0	1.3	-	-	-	-

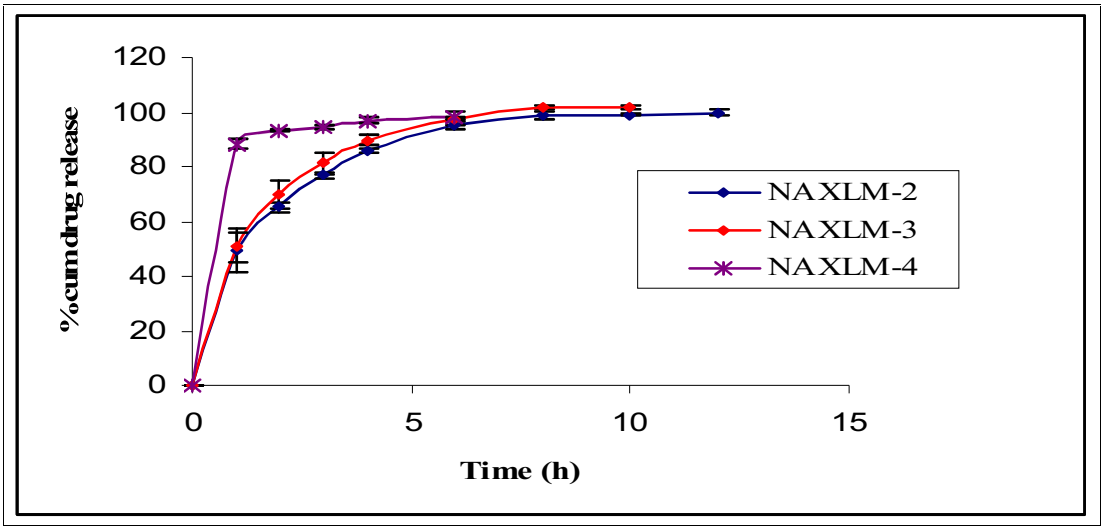


Figure 22: Drug release profile of NAXLM-2, NAXLM-3, NAXLM-4

The amount of polymer during granulation with Eudragit RS 30 D, Eudragit NE 30 D and Kollicoat SR 30 was not sufficient to sustain the drug release for desired time interval. Out of three polymers used Eudragit RS 30 D sustained the drug release for longer time period as compared to other used polymers. Thus, it was decided to use more quantity of Eudragit RS polymer for achieving desired sustained drug release profile. Further, it was noted that the drying time for 100 gm batch size was about 2 hr (LOD < 2%) so it was planned to use non-aqueous granulation technique to reduce the process time.

4.2.1.3 Wet Granulation (Non-Aqueous) Method (DCM: IPA Mixture) (B No. NAXLM-5)

The tablets were prepared with non aqueous granulation technique using DCM: IPA (1:1) mixture. The drying time for granulation was reduced to 30 min from 2 hr as compared to the aqueous granulation but the strength of the prepared granules was not good. The Eudragit RSPO content was increased to 5% as compared to 1.8 % from NAXLM-2,3,4 (in all the three batches 6% of aqueous polymer dispersion was used which contains 30% of the polymer) but still the release of the drug was faster and the drug released completely in 6 hr (Table 69 and Figure 23).

Table 69: Dissolution Profile of NAXLM-5

Time (h)	% Drug release	% RSD
1	45.1	5.0
2	71.9	1.6
3	86.9	5.0
4	92.6	1.4
6	99.6	2.0
8	102.0	1.6

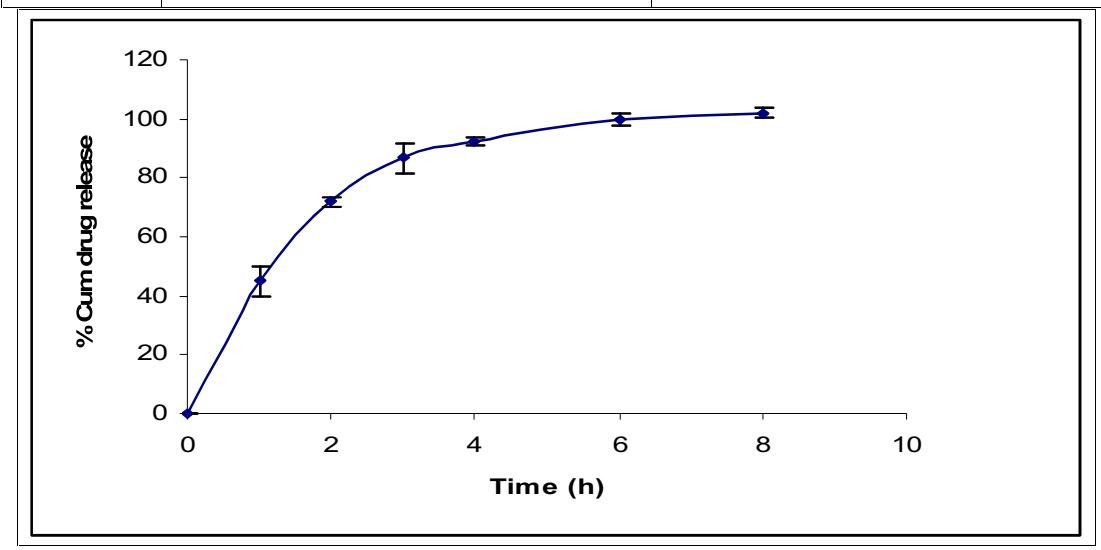


Figure 23: Drug release profile of NAXLM-5

Based on drug release profile it was planned to use more quantity of Compritol to control the drug release and also decided to explore ethanol: water mixture for the granulation purpose.

4.2.1.4 Wet Granulation (Non-Aqueous) method (Ethanol: Water) (B No. NAXLM-6)

The tablets were prepared with non aqueous granulation technique using ethanol: water (19.2: 0.8) mixture. The strength of the granules was good and the drying time was about 45 minutes. The drug release was faster (Table 70 and Figure 24) and released completely in 8 hr.

Table 70: Dissolution Profile of NAXLM-6

Time (h)	% Drug Release	% RSD
1	47.6	3.7
2	64.4	2.4
3	75.4	1.4
4	85.3	0.2
6	94.0	1.2
8	99.6	0.4

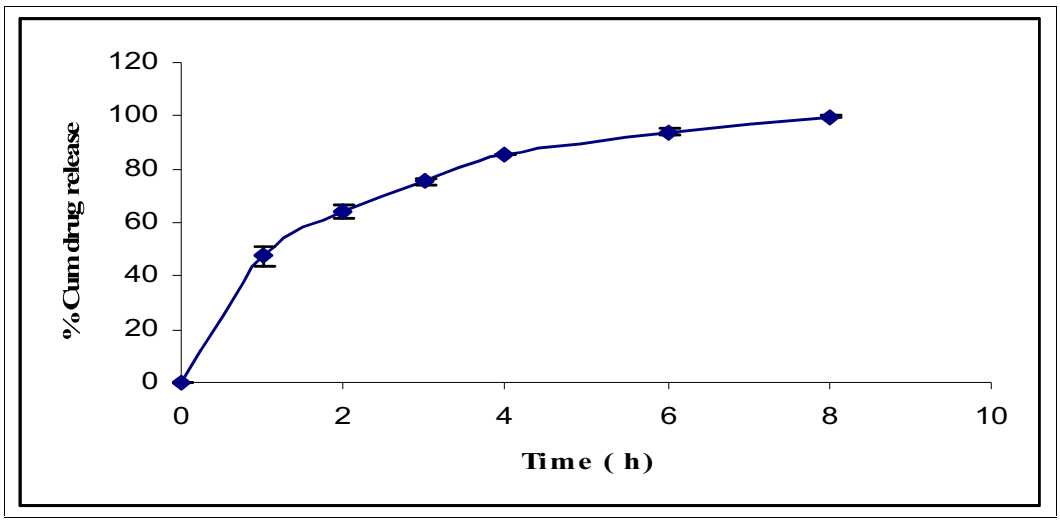


Figure 24: Drug release profile of NAXLM-6

Based on drug release profile it was concluded that single lipid can not control the release of the hydrophilic drug so it was planned to use functional coating for controlling the drug release for desired time interval).

4.2.1.5 Wet Granulation followed by Rate Controlling Coating (B. No. NAXLM - 7a, NAXLM-7b, NAXLM-7c, NAXLM-7d)

The tablets were prepared with non aqueous granulation technique using DCM: (Ethanol: Water) [5:20 (19.2:0.8)] mixture and tablets were coated with aqueous dispersion of Kollicoat SR 30 D and Kollicoat IR. It can be interpreted from Table 71

and Figure 25, that for batch No. NAXLM-7a, initially there was no release of drug upto 4 hr and only 35 % of drug was released in the 12 hr duration due to less amount of Kollicoat IR in the coating dispersion. In the batch no. NAXLM-7 b, the amount of Kollicoat IR was increased and it was observed that initially drug release was faster and the release was sustained for 14 hrs duration. Based on the drug release profile of batch no. NAXLM-7b, it was planned to increase the coating thickness from 5% to 10% to sustain the drug release for longer duration. On increasing the coating thickness, batch no. NAXLM-7c (7.5%) and batch no. NAXLM-7d (10%) initial burst release was controlled but within 12-14 hr drug was completely released.

Table 71: Dissolution Profile of NAXLM-7a, NAXLM-7b, NAXLM-7c, NAXLM-7d

Time (h)	NAXLM-7 a		NAXLM-7 b		NAXLM-7 c		NAXLM-7 d	
	% Drug Release	%RSD	% Drug Release	%RSD	% Drug Release	%RSD	% Drug Release	%RSD
1	0.0	0	23.4	2.8	10.7	8.3	9.7	7.4
2	0.0	0	43.5	1.3	34.9	16.8	31.0	1.5
3	0.0	0	57.0	0	50.5	2.4	47.3	0.1
4	0.0	0	67.1	1.1	61.2	1.4	57.7	0.3
6	2.5	11.8	79.1	0.2	77.4	1.7	74.8	2.2
8	7.9	4.6	87.2	0.8	86.0	0.2	84.4	0
10	18.9	0.7	92.8	0.4	91.7	0.1	90.4	0.5
12	35.6	1.6	95.2	0.7	95.4	1.1	93.8	0.8
14	--		98.2	0.4	95.7	0.8	94.4	0.8
16	--		--		98.6	1.3	97.8	0.1

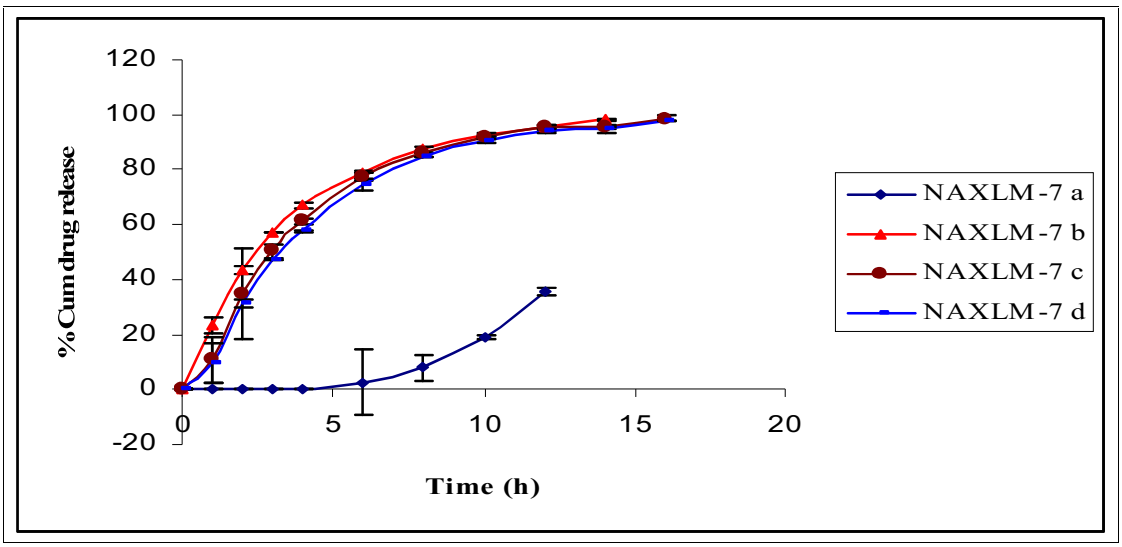


Figure 25: Drug release profile of NAXLM-7 a, NAXLM-7b, NAXLM-7c, NAXLM-7d

Based on the above experiments it was concluded that the drug release was mainly dependent on the amount of Kollicoat IR. For getting better sustained release it was decided to optimize the amount of Kollicoat IR to control the drug release.

4.2.1.6 Wet Granulation followed by Rate Controlling Coating (B No. NAXLM-8)

The tablets were prepared with non aqueous granulation technique using ethanol: water (19.2: 0.8) mixture and aqueous coated with Kollicoat SR 30 D and Kollicoat IR. The amount of Kollicoat IR was optimized to a ratio of 7.5: 1 (Kollicoat SR 30D: Kollicoat IR) and found that initial burst release was controlled and drug release was sustained for 20 hr duration (Table 72 and Figure 26). Since the formulation met the criteria for dissolution profile, it was selected for first investigational bioavailability study.

Table 72: Dissolution Profile of NAXLM-8

Time (h)	% Drug released	% RSD
1	5.3	4.5
2	13.8	5.9
4	28.0	4.5
6	48.5	5.1
8	63.4	4.1
10	73.7	4.7
12	80.3	4.8
14	84.3	6.4
20	90.3	1.9
24	96.3	3.7

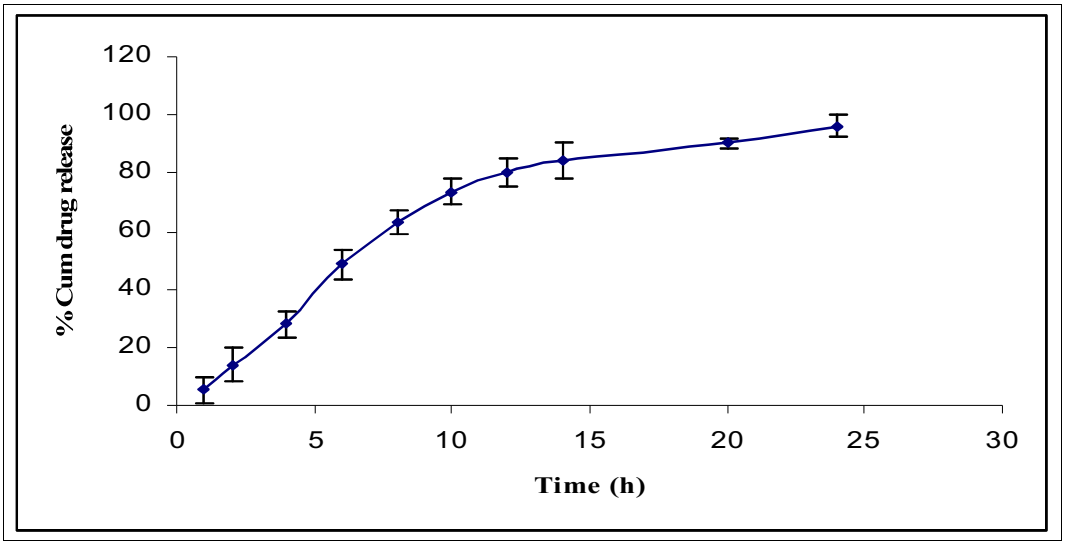


Figure 26: Drug release profile of NAXLM-8

4.2.1.7 Stability studies of the developed formulation:

The accelerated stability studies of the developed tablet formulation (NAXLM-8) were performed as per ICH guidelines. The tablets were packaged in HDPE bottle, PVDF blister and Alu-Alu blister pack and charged for stability. The result of the accelerated stability study showed (Table 73) that the tablet is stable upto 3 month in all the packaging material and there is no degradation or change in the drug release behaviour. After observing the three months stability results it was decided to carry out *in vivo* bioavailability study of the developed formulation. (NAXLM-8)

Table 73: Stability data of the developed tablet formulation in different packaging material (B. No. NAXLM-8)

	% Cumulative Drug Release						
	Initial	Pack Type					
		HDPE Bottle		PVDC Blister		Alu-Alu Blister	
Time (Hrs) / Month	Condition	40°C/75%RH					
	Initial	1	3	1	3	1	3
1	5.3	9.5	4.6	7.1	6.2	6.3	6.6
2	13.8	12.6	14.8	13.4	15.7	11.4	18.2
4	45.5	41.3	43.5	40.1	44.8	40.1	44.9
6	63.4	67.1	62.6	63.9	64.8	63.7	68.1
8	75.7	78.1	76.8	79.5	75.7	75.6	78.3
10	85.3	87.7	84.4	89.2	87.2	84.9	87.8
12	84.3	93.2	92.1	95.6	92.9	87.4	93.5
14	96.3	97.2	95.6	98.0	95.6	90.6	96.1
20	100.3	101.1	98.5	101.7	99.1	98.1	99.4

Chemical stability										
Pack Type	-	HDPE			PVDC			Alu-Alu		
Condition	-	40°C/75%RH			40°C/75%RH			40°C/75%RH		
Month	Initial	1	2	3	1	2	3	1	2	3
Assay (%)	97.4	98.6	97.3	94.9	98.7	98.1	95.3	98.0	97.4	97.0
SHI (%)	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.08
Total Impurity (%)	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.08
Water (%)	1.03	0.92	-	1.02	1.11	-	1.30	0.96	-	0.99
Hardness (N)	210	212	215	209	213	210	208	209	213	210
Friability	0.02%	0.01%	0.02%	0.02%	0.01%	0.02%	0.01%	0.02%	0.02%	0.01%
Thickness (mm)	6.51	6.51	6.51	6.51	6.51	6.51	6.51	6.51	6.51	6.51

4.2.2 Polymer Matrix Technology

The polymer matrix technology is commonly used for controlling the drug release for hydrophilic drugs. Generally hydrophilic polymers *viz.,* HPMC [127-132], PEO [123,133], xanthan gum [134-137], guar gum, sodium CMC [138] are commonly used for designing of modified release formulations of hydrophilic drugs.

4.2.2.1 Dry Granulation Method (B No. NAXPM -9)

Even with polymer matrix technology sticking was observed during slugging. The drug release was faster (Table 74 and Figure 27) and released completely in 10 hr.

Table 74: Dissolution Profile of NAXPM-9

Time (h)	% Drug released	% RSD
1	24.6	7.1
2	40.3	5.1
3	51.6	7.2
4	64.0	5.8
6	73.1	4.6
8	86.0	4.0
10	93.9	2.9

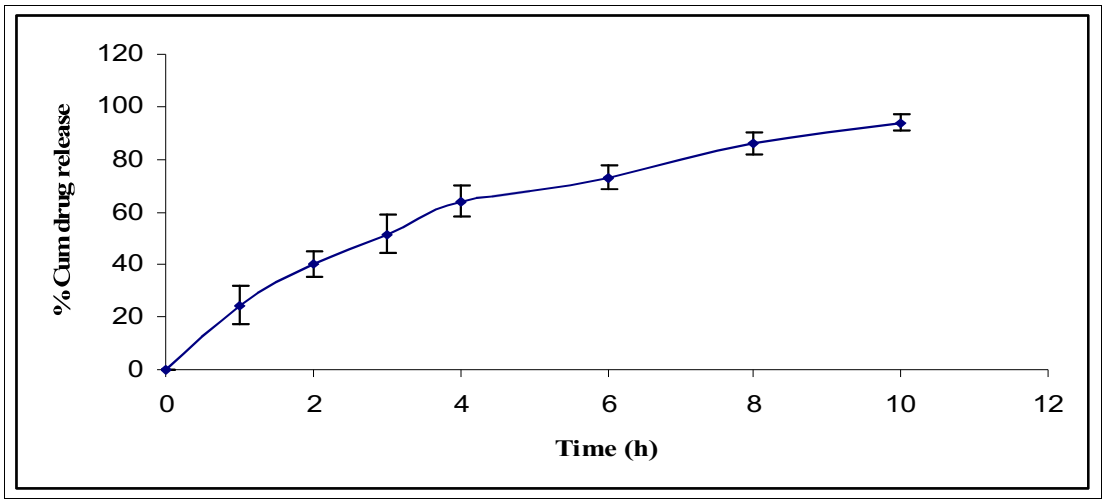


Figure 27: Drug release profile of NAXPM-9

As already mentioned, that stickiness was due to small particle size of the drug powder (15 micron) and poor flow properties (angle of repose 48°). Thus, it was planned to use wet granulation technique for the preparation of the tablet formulation. The drug release was very fast and did not meet the target release profile, so it was

decided to granulate the blend with polymeric solution to achieve the desired sustained drug release profile.

4.2.2.2 Wet (Aqueous) Granulation Followed By Slugging Method (B No. NAXPM-10)

The tablets were prepared by wet granulation followed by slugging method, no sticking was observed during compression of tablet as the flow properties of the prepared granules were good (angle of repose 30°). Tablets were prepared by using Eudragit RS 30D as granulating agent and using xanthan gum and HPMC as release modifying agent. The drug release was initially faster (Table 75 and Figure 28) and released completely in about 10 hr.

Table 75: Dissolution Profile of NAXPM-10

Time (h)	% Drug released	% RSD
1	30.8	0.6
2	38.5	12.9
3	49.8	11.2
4	59.9	9.1
6	78.2	4.8
8	90.9	4.8
10	96.4	2.9
12	97.3	1.8
14	96.1	2.0

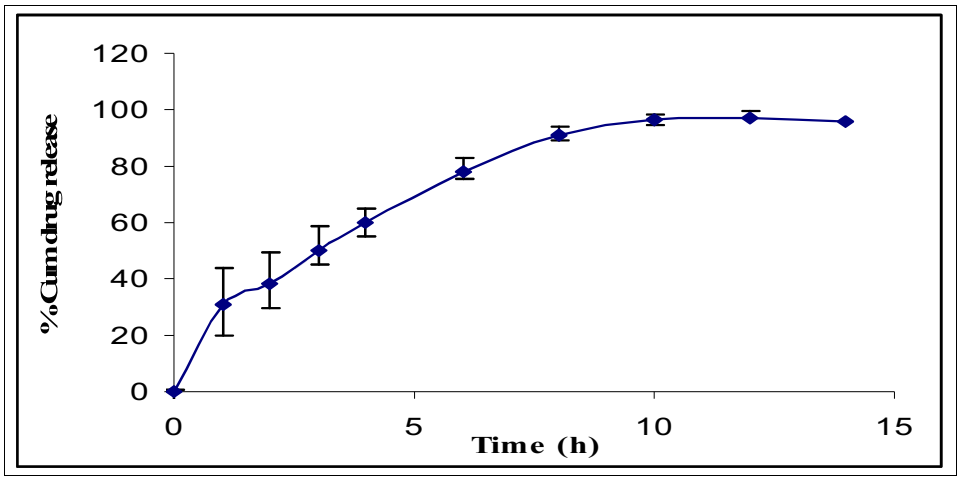


Figure 28: Drug release profile of NAXPM-10

Based on the drug release profile it was decided to add another rate controlling polymer to sustain the drug release.

4.2.2.3 Wet (Aqueous) Granulation Followed By Slugging Method (B No. NAXPM-11)

The tablets were prepared by wet granulation followed by slugging method. Tablets were prepared by using Eudragit RS 30 D as granulating agent and using Xanthan gum, HPMC and Eudragit RSPO as release modifying agent. Initial burst release was reduced and approx. 90% of the drug was released in 10 hr (Table 76 and Figure 29).

Table 76: Dissolution Profile of NAXPM-11

Time (h)	% Drug released	% RSD
1	11.7	7.1
2	22.4	1.1
4	47.1	1.9
6	64.9	1.8
8	79.8	1.2
10	86.2	0.9
12	89.0	0.6
14	89.5	0.9

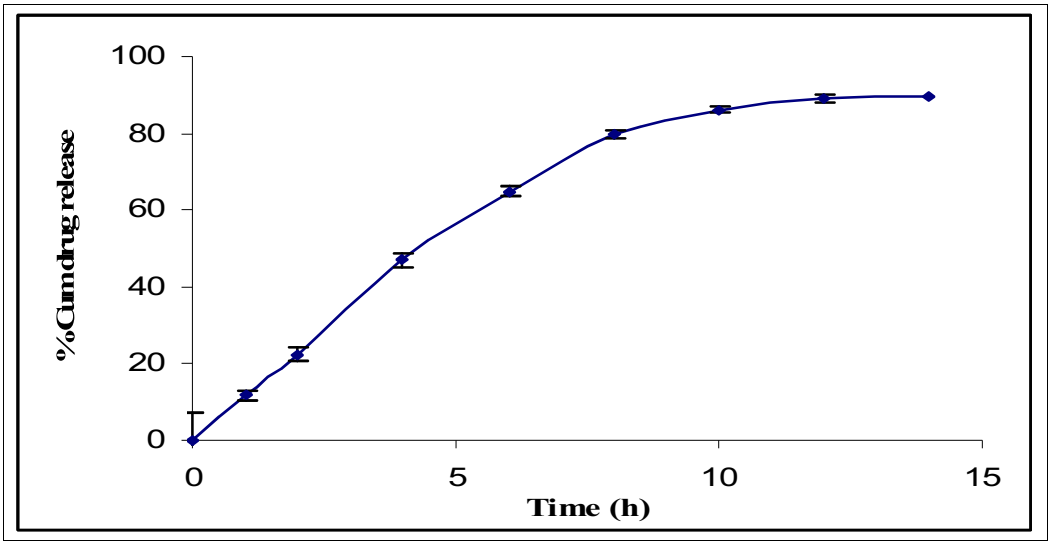


Figure 29: Drug release profile of NAXPM-11

It was concluded that granulation with Eudragit RS 30D was not capable of sustaining the drug release for desired time interval. Thus, it was planned to granulate with ethanol: water mixture and use more quantity of high viscous HPMC to sustain the drug release for more time.

4.2.2.4 Wet (Aqueous) Granulation Followed By Slugging Method (B No. NAXPM -12)

The tablets were prepared by wet granulation followed by slugging method. Tablets were prepared by using ethanol: water mixture as granulating agent and using xanthan gum, high viscous HPMC and Eudragit RSPO as release modifying agent. Complete drug was released in 10 hr (Table 77 and Figure 30).

Table 77: Dissolution Profile of NAXPM-12

Time (h)	% Drug released	%RSD
1	17.4	5.4
2	32.6	2.0
4	52.9	0.5
6	73.9	6.3
8	87.7	5.8
10	101.4	0.4
12	104.4	0.7

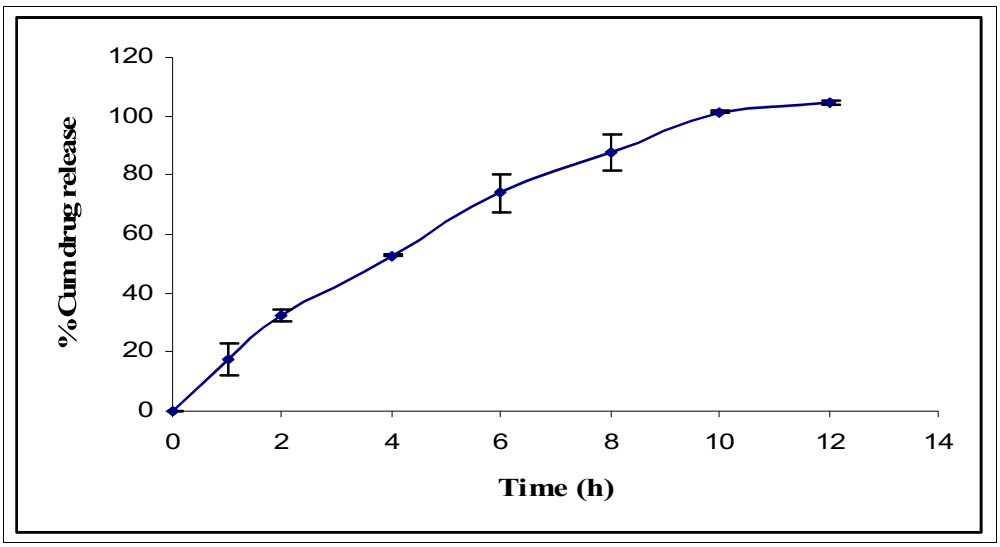


Figure 30: Drug release profile of NAXPM-12

Based on drug release profile it was decided to do granulation with binding agent to form the hard matrix tablet to achieve desired sustained drug release profile.

4.2.2.5 Wet (Aqueous) Granulation Followed By Slugging Method (B No. NAXPM-13)

The tablets were prepared by wet granulation followed by slugging method. Tablets were prepared by using PVP K 30 dissolved in ethanol: water mixture as granulating

agent and using xanthan gum, high viscous HPMC (100 K cps) and Eudragit RSPO as release modifying agent. Complete drug was released in 20 hr (Table 78 and Figure 31).

Table 78: Dissolution Profile of NAXPM-13

Time (h)	% Drug released	% RSD
1	23.6	1.8
2	25.3	7.4
3	30.8	6.7
4	38.1	6.1
6	49.7	5.3
8	59.7	5.9
10	69.3	6.2
12	77.3	5.5
14	83.4	6.0
16	95.4	4.1
20	102.6	3.2

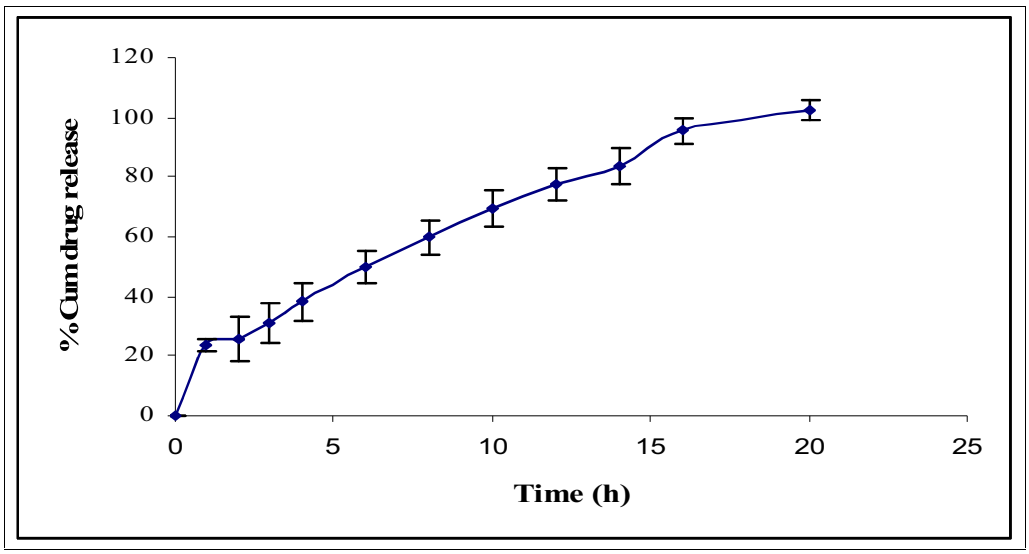


Figure 31: Drug release profile of NAXPM-13

The drug release profile showed that drug releases in sustained manner upto 20 hr but there was 23 % initial burst drug release which needs to be controlled. It was planned to use higher viscous Benecel MP 874 to control the initial burst release.

4.2.2.6 Wet (Aqueous) Granulation Followed By Slugging Method (B No. NAXPM-14)

The tablets were prepared by wet granulation followed by slugging method. Tablets were prepared by using PVP K 30 dissolved in ethanol: water mixture as granulating

agent and using xanthan gum, high viscous Benecel MP 874 and Eudragit RSPO as release modifying agent. Complete drug was released in 20 hr (Table 79 and Figure 32).

Table 79: Dissolution Profile of NAXPM-14

Time (h)	% Drug released	% RSD
1	14.0	3.0
2	24.8	2.4
4	40.8	0.7
6	53.5	0.5
8	65.1	0.4
10	74.9	0.5
12	82.2	1.1
14	87.8	0.4
16	92.9	1.2
20	98.0	1.2

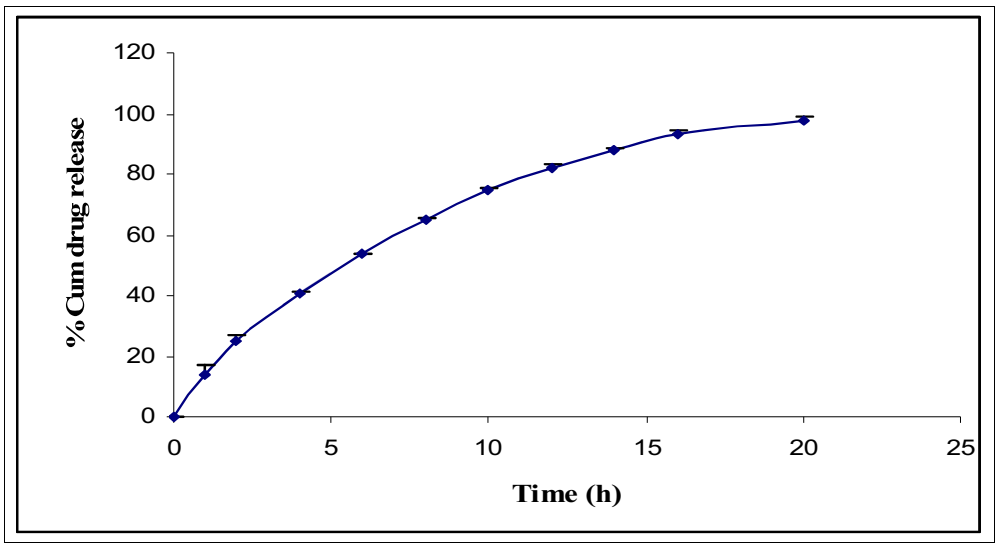


Figure 32: Drug release profile of NAXPM-14

The drug release profile showed that there is no initial burst drug release followed by sustained drug release upto desired time interval. Thus, it was planned to take stability batch with the same formula and carry out stability studies as per ICH guidelines.

4.2.2.7 Wet (Aqueous) Granulation Followed By Slugging Method (B No. NAXPM -15)

The tablets were prepared with the optimized composition (NXPM-14) Complete drug was released in 20 hr (Table 80 and Figure 33).

Table 80: Dissolution Profile of NAXPM-15

Time (h)	% Drug Released	% RSD
1	15.6	8.7
2	24.6	9.6
3	41.7	18.6
4	54.7	16.7
6	67.4	7.6
8	78.1	6.4
10	88.1	5.3
12	95.0	3.0
14	98.5	1.1
16	100.3	1.2
20	104.0	1.4

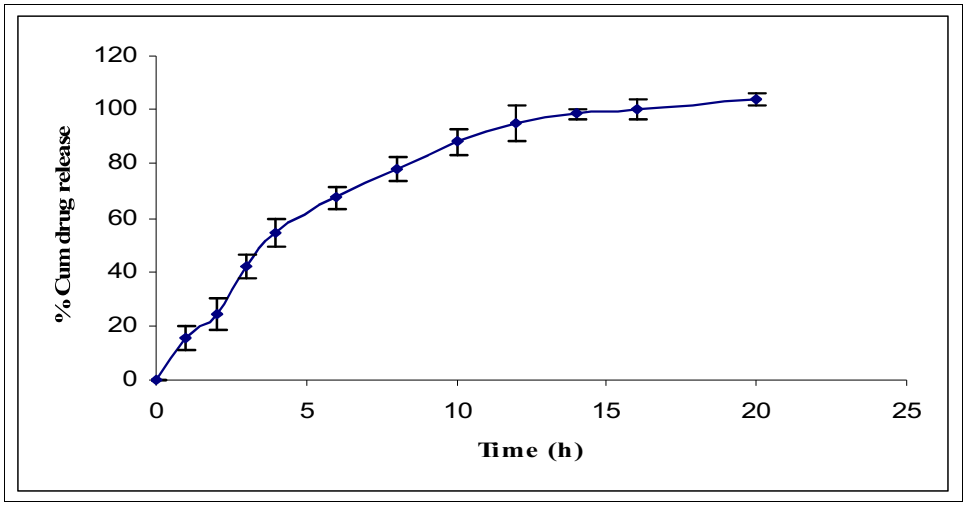


Figure 33: Drug release profile of NAXPM-15

Based on the results of drug release the NAXPM-15 formulation was short listed for first investigational Bioavailability study and also subjected stability study.

4.2.2.8 Stability studies of the developed formulation

The accelerated stability studies of the developed tablet formulation (NAXPM-15) were performed as per ICH guidelines. The tablets were packaged in HDPE bottle, PVDF blister and alu-alu blister pack and charged for stability. The result of the accelerated stability study showed (Table 81 and Figure 34) that the tablet is stable in all the packaging material and there is no degradation or change in the drug release behaviour. On the basis of stability results it was decided to carry out *in vivo* bioavailability study of the developed formulation.

Table 81: Stability data of the developed tablet formulation in different packaging material (B. No. NAXPM-15)

Chemical stability													
Pack Type	-	**HDPE**				**PVDC**				**Alu-Alu**			
Condition	-	**40°C/75%RH**				**40°C/75%RH**				**40°C/75%RH**			
Month	Initial	1	2	3	6	1	2	3	6	1	2	3	6
Assay (%)	96.38	99.7	99.20	98.3	98.30	100.20	100.30	98.50	97.80	100.60	99.50	99.30	100.30
SHI (%)	0.05	0.07	0.07	0.06	0.07	0.05	0.06	0.06	0.06	0.06	0.06	0.05	0.07
Total Impurity (%)	0.06	0.07	0.07	0.06	0.11	0.06	0.06	0.06	0.08	0.06	0.06	0.05	0.1
Water (%)	2.12	1.28	-	2.11	2.29	2.03	-	2.58	3.21	1.65	-	2.0	1.78
Hardness	198 N	205 N	195 N	198 N	202 N	199 N	196 N	198 N	204 N	199 N	198 N	198 N	196 N
Friability	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
Thickness	6.5 mm	6.5 mm	6.5 mm	6.5 mm	6.5 mm	6.5 mm	6.5 mm	6.5 mm	6.5 mm	6.5 mm	6.5 mm	6.5 mm	6.5 mm

	% Cumulative Drug Release									
	Initial	**Pack Type**								
		HDPE Bottle			**PVDC Blister**			**Alu-Alu Blister**		
	Condition	**40°C/75%RH**								
Time (Hrs) ↓ / Month →	**Initial**	**1**	**3**	**6**	**1**	**3**	**6**	**1**	**3**	**6**
1	15.6	16.4	15.6	18.3	15.2	14.6	19.9	15.2	15.0	17.1
2	24.6	27.6	25.2	29.5	25.1	24.3	27.8	24.5	24.7	26.4
4	41.7	43.2	40.4	44.2	40.5	39.5	41.9	40.6	39.2	40.6
6	54.7	54.7	53.6	56.4	53.9	53.4	54.1	53.4	52.7	52.3
8	67.4	67.1	65.2	67.6	67.1	66.2	66.3	65.2	64.9	65.9
10	78.1	74.5	75.5	77.2	77.3	76.3	74.6	76.4	75.3	73.6
12	88.1	87.0	85.1	85.9	87.6	85.4	84.3	86.8	84.3	82.2
14	95.0	95.3	92.5	91.7	93.1	90.7	89.9	93.5	91.6	90.6
16	98.5	98.9	97.0	94.9	96.1	95.6	96.4	97.9	96.6	95.1
20	100.3	101.6	99.4	99.7	99.5	99.0	100.6	99.1	98.0	100.1
24	104.0	102.8	99.3	99.3	-	-	101.0	99.6	99.8	101.4

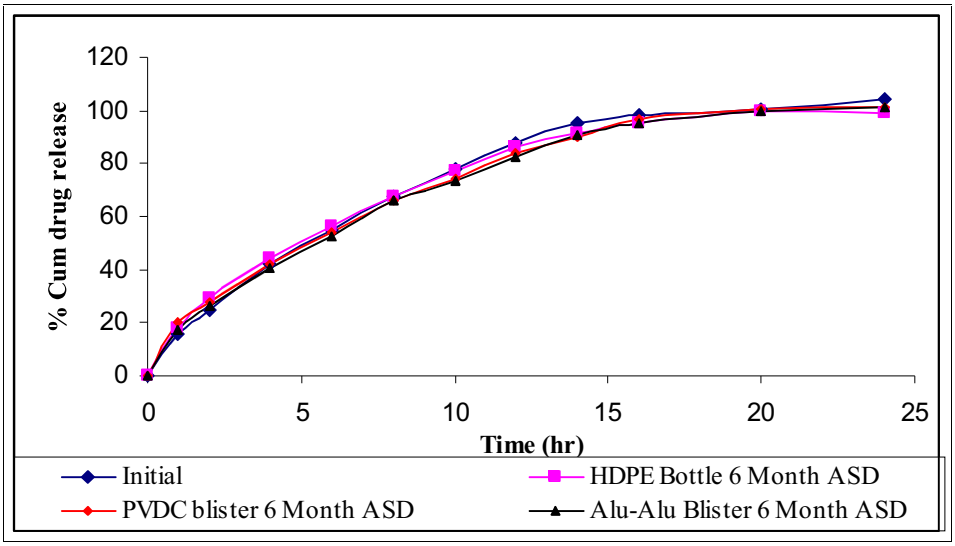


Figure 34: Results of the accelerated stability studies of the developed tablet formulation in different packaging material (B. No. NAXPM-15)

4.3 Bioavailability Studies

To get an optimal therapeutic activity, an active moiety should be delivered at the site of its action in an effective concentration during the desired period. To allow prediction of the therapeutic effect, the performance of the pharmaceutical formulation containing the active substance should be reproducible and thus the bioavailability too. A randomized, open label, three treatment, three period, three sequence, single dose, crossover comparative bioavailability study, under fasting conditions was performed for comparing rate and extent of absorption of two test MR formulations of Mycophenolate Sodium of Panacea Biotec Ltd. with reference formulation in 9+3 (Standby) healthy adult male human subjects. The pharmacokinetic parameters were obtained by subjecting the data to non-compartmental analysis using WinNonlin 5.2 software. Table 82 enlists the values of the various pharmacokinetic parameters *viz.*, C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, λ_z and $T_{1/2}$ for reference (R), Test 1 (T1) (NAXPM-15) and Test (T2) (NAXLM-8). The plasma concentration time data has been summarized in Table 87, 88 and 89.

The objective of Biostudy I was to compare the pharmacokinetic parameters obtained for Lipid Matrix (NAXLM-8) and Polymer Matrix (NAXPM-15) MR formulations having similar drug release profiles with that of commercial, marketed product (Cellcept tablets). It was intended to select the technology which provided pharmacokinetic parameters closer to that of reference formulation and acceptance criteria followed by further optimization.

4.3.1 Randomized, single dose, crossover comparative bioavailability study under fasting conditions (Study-I)

Table 82: PK Parameters Table

Sub.	Sequence	C_{max} (ug/mL)			$T_{1/2}$ (hr)			T_{max} (hr)			AUC_{0-t} (hr*ug/mL)			$AUC_{0-\infty}$ (hr*ug/mL)			λ_z (1/hr)		
		R	T1	T2	R	T1	R	T1	T2	T2	R	T1	T2	R	T1	T2	R	T1	T2
1	T1T2R	8.42	5.52	2.73	8.70	2.41	0.76	1.00	3.00	4.00	10.66	23.36	6.34	15.12	24.38	6.83	0.080	0.288	0.907
2	T1T2R	10.50	2.82	4.15	21.15	4.98	5.61	0.50	1.00	5.00	36.39	21.95	21.43	43.35	23.37	23.77	0.033	0.139	0.124
3	T2RT1	13.95	4.09	2.12	6.49	5.32	9.39	0.50	1.00	10.00	19.43	17.07	20.75	20.73	19.82	22.96	0.107	0.130	0.074
4	RT1T2	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
5	T2RT1	25.50	6.19	10.20	7.82	5.32	4.05	1.00	4.00	3.00	55.89	41.95	52.02	57.99	43.74	54.45	0.089	0.130	0.171
6	RT1T2	18.90	6.47	3.73	5.98	22.63	16.95	0.50	1.00	5.00	57.07	55.60	38.67	64.97	81.04	46.54	0.116	0.031	0.041
7	RT1T2	15.40	4.95	4.93	8.28	6.32	3.90	0.83	4.00	3.00	54.03	24.40	21.17	64.16	26.62	21.31	0.084	0.110	0.178
8	T1T2R	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
9	T2RT1	10.00	2.41	4.23	5.52	6.67	4.91	0.50	5.00	3.00	27.41	19.71	23.22	30.65	22.32	25.22	0.126	0.104	0.141
10	T2RT1	SB	SB	SB	SB	SB	SB	SB	SB	SB	SB	SB	SB	SB	SB	SB	SB	SB	SB
11	T1T2R	13.10	2.81	2.07	5.07	3.83	6.87	0.67	7.50	3.00	21.54	24.71	23.18	23.93	26.11	25.27	0.137	0.181	0.101
12	RT1T2	12.80	3.30	5.34	13.65	6.29	3.17	0.33	5.00	5.00	25.91	21.34	29.00	35.44	24.65	31.73	0.051	0.110	0.218
N		9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Mean		14.29	4.28	4.39	9.18	7.09	6.18	0.65	3.50	4.56	34.26	27.79	26.20	39.59	32.45	28.68	0.091	0.136	0.217
SD		5.23	1.55	2.47	5.17	5.98	4.70	0.24	2.24	2.24	17.46	12.60	12.85	19.03	19.47	14.16	0.034	0.069	0.264
Min		8.42	2.41	2.07	5.07	2.41	0.76	0.33	1.00	3.00	10.66	17.07	6.34	15.12	19.82	6.83	0.033	0.031	0.041
Median		13.10	4.09	4.15	7.82	5.32	4.91	0.50	4.00	4.00	27.41	23.36	23.18	35.44	24.65	25.22	0.089	0.130	0.141
Max		25.50	6.47	10.20	21.15	22.63	16.95	1.00	7.50	10.00	57.07	55.60	52.02	64.97	81.04	54.45	0.137	0.288	0.907
CV%		36.64	36.17	56.25	56.31	84.43	76.06	37.42	63.89	49.22	50.96	45.36	49.04	48.07	60.01	49.39	37.500	51.100	121.770

Note:

- M denotes Missing values.
- SB denotes standby
- Dropout 4 and 8 were replaced by standby 12 and 11 respectively. Standby 10 was not included in the analysis.

Table 83: ANOVA Table

Parameter	Unit	Effects	P-value
$Ln(C_{max})$	ug/mL	Sequence	0.337
	ug/mL	Formulation	0.000
	ug/mL	Period	0.854
$Ln(AUC_{0-t})$	hr*ug/mL	Sequence	0.331
	hr*ug/mL	Formulation	0.185
	hr*ug/mL	Period	0.144
$Ln(AUC_{0-\infty})$	hr*ug/mL	Sequence	0.294
	hr*ug/mL	Formulation	0.104
	hr*ug/mL	Period	0.225

On applying analysis of variance (Table 83), it was observed that no statistically significant effect (P>0.05) was observed, due to periods for $Ln(C_{max})$, $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$. Formulation effect was statistically significant for $Ln(C_{max})$ (P<0.05) but for $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$ it was found to be non significant (P>0.05). No significant difference (p>0.10) was observed due to sequence for $Ln(C_{max})$, $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$. The results of the statistical comparisons performed on the single-dose pharmacokinetic parameter estimates are summarized in Table 84, (Test-1) (NAXPM-15) and Table 85, (Test-2) (NAXLM-8).

Table 84: Comparison of Pharmacokinetic parameters of Test 1 and Reference

Test 1 (T1) Formulation – Non Transformed Data								
	Units	Least Sq. Mean[1]		Ratio%[2]	CV%[3]	90% Confidence Interval[4]		Power
		R	T1			Lower	Upper	
C_{max}	ug/mL	14.2856	4.2844	29.99	-	15.44	44.54	0.7404
AUC_{0-t}	hr*ug/mL	34.2603	27.7877	81.11	-	63.38	98.84	0.5887
$AUC_{0-\infty}$	hr*ug/mL	38.3772	32.1211	83.70	-	62.77	104.63	0.4713
T_{lag}	hr	0.5830	0.1040	5.58	-	-	-	-
Test 1 (T1) Formulation - Ln Transformed Data								
$Ln(C_{max})$	ug/mL	13.5452	4.0327	29.77	33.48	22.71	39.03	0.3838
$Ln(AUC_{0-t})$	hr*ug/mL	30.0333	25.8622	86.11	29.20	67.90	109.20	0.4602
$Ln(AUC_{0-\infty})$	hr*ug/mL	34.4737	28.7103	83.28	30.46	65.03	106.66	0.4351

1. Least-squares geometric means for Ln-transformed data.
2. Ratio calculated as Test 1 least-squares mean divided by the Reference least-squares mean.
3. Estimated intra-subject coefficient of variation, CV%=100*SQRT (e^{MSE}-1), where MSE is the mean square error term from the ANOVA.
4. Confidence interval on the ratio.

Table 85: Comparison of Pharmacokinetic parameters of Test 2 and Reference

Test 2 (T2) Formulation - Non Transformed Data								
	Units	Least Sq. Mean[1]		Ratio%[2]	CV%[3]	90 % Confidence Interval[4]		Power
		R	T2			Lower	Upper	
C_{max}	ug/mL	14.2856	4.3889	30.72	-	16.17	45.27	0.7404
AUC_{0-t}	hr*ug/mL	34.2603	26.1966	76.46	-	58.73	94.19	0.5887
$AUC_{0-\infty}$	hr*ug/mL	38.3772	28.4666	74.18	-	53.24	95.11	0.4713
T_{lag}	hr	0.7870	0.1040	7.53	-	-	-	-
Test 2 (T2) Formulation - Ln Transformed Data								
$Ln(C_{max})$	ug/mL	13.5452	3.9040	28.82	33.48	21.99	37.78	0.3838
$Ln(AUC_{0-t})$	hr*ug/mL	30.0333	23.0996	76.91	29.20	60.65	97.54	0.4602
$Ln(AUC_{0-\infty})$	hr*ug/mL	34.4737	24.9468	72.36	30.46	56.51	92.68	0.4351

1. Least-square geometric means for Ln-transformed data.
2. Ratio calculated as Test 1 least-squares mean divided by the Reference least-squares mean.
3. Estimated intra-subject coefficient of variation, CV%=100*SQRT (e^{MSE}-1), where MSE is the mean square error term from the ANOVA.
4. Confidence interval on the ratio.

The values of AUC_{0-t} and $AUC_{0-\infty}$ for test1 (NAXPM-15) were found to be 13.88 % and 16.71 % lower than that of reference respectively (Table 84). While in case of test 2 (NAXLM-8), the values were more than 20% lower than that of reference (Table 85). As expected for modified-release products, both Test formulations had lower peak concentrations and longer times to peak concentration compared to the reference (Table 85). The mean concentration *vs.* time graph on linear scale is shown in the Figure 35.

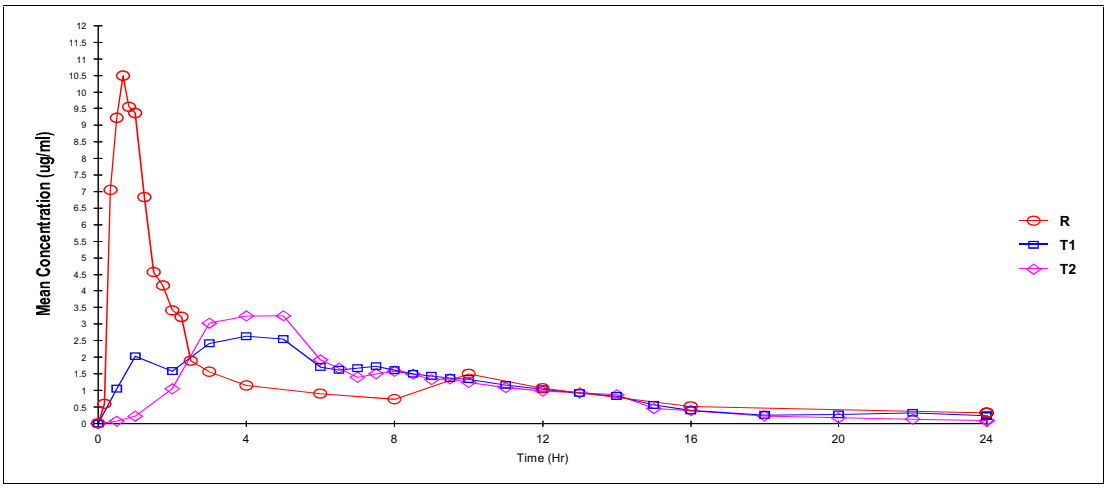


Figure 35: Mean Concentration *vs.* Time Graph (Linear Plot)

A second peak obtained in the profile for T1 was due to the high variability in the mean plasma concentration for T1.

Steady State Simulations

Steady-state simulations for the Test formulations and Reference were performed using the method of nonparametric super positioning as implemented in WinNonlin 5.2. Steady-state concentrations were calculated only for those subject-treatment cases where an elimination rate constant was estimable. Subject 4 of Test 2 and subject 8 of reference were omitted from the study because their elimination rate constant could not be estimated. Based on the 0-24 hr simulated steady-state concentrations, area under the curve (AUC_{ss}), peak concentration (C_{maxss}), minimum concentration (C_{minss}), time of peak (T_{max}) concentration and percent fluctuation (100% x $[C_{max}-C_{min}]/C_{ave}$; C_{ave} = AUC/24) were calculated. AUC_{ss} and C_{maxss} were transformed by taking the natural logarithms of their values prior to statistical evaluation. Comparisons between Test and Reference with regard to their steady-state pharmacokinetic parameters are summarized in Table 86.

Table 86: Simulated MPA Pharmacokinetics (0-24 hr at steady-state) for Test 1 and Test 2 and Reference.

Parameters	Acceptance Criteria	Results	
		T1 (NAXPM-15)	T2 (NAXLM-8)
AUC: T/R	>0.8	0.816	0.709
C_{max}: T/R	Preferably ≤ 1 but not more than 1.25	0.280	0.105
Fluctuation: T/R	Preferably ≤ 1 but not more than 1.25	0.783	0.887
Inter individual variation for test	Less than that of reference	Less than reference	Less than reference
C_{min}:	0.8µg/mL or more	0.170	0.064

The mean steady-state concentrations chart for 10 subjects who had data for all treatments was shown in the Figure 36. Neither test formulations appeared to provide coverage comparable to that of Reference in the 12-24 hour interval at steady-state. Test 1 (NAXPM-15) and Test 2 (NAXLM-8) formulations had lower average C_{max}, as desired. However both the formulations also had lower C_{min} concentrations than that was observed with the reference formulation (Table 86). The mean percent steady-state fluctuation for the Test 1 and Test 2 formulations was slightly less than reference.

It could be interpreted from Biostudy I that both the test formulations had lower values of AUC than the reference. However, the value of T/R for AUC in case of polymer matrix product was more than 0.8 thus meeting the acceptance criteria. Additionally, the values of C_{min} (predicted from steady state data) for test formulations were lower than that of reference. The above mentioned results clearly indicate that polymer matrix technology product performed better than that of lipid matrix technology, thus it was selected for futher optimization. It was also decided to simplify the existing manufacturing procedure of polymer matrix technology so that there are fewer complications during scale up. It was further envisaged to modify the existing formulation in order to improve the value of C_{min} for MR formulation. It was conceived to prolong the dissolution profile of the formulation in order to achieve the desired C_{min}. As per the original strategy, further biostudies were planned to be conducted on two formulations having different release profiles.

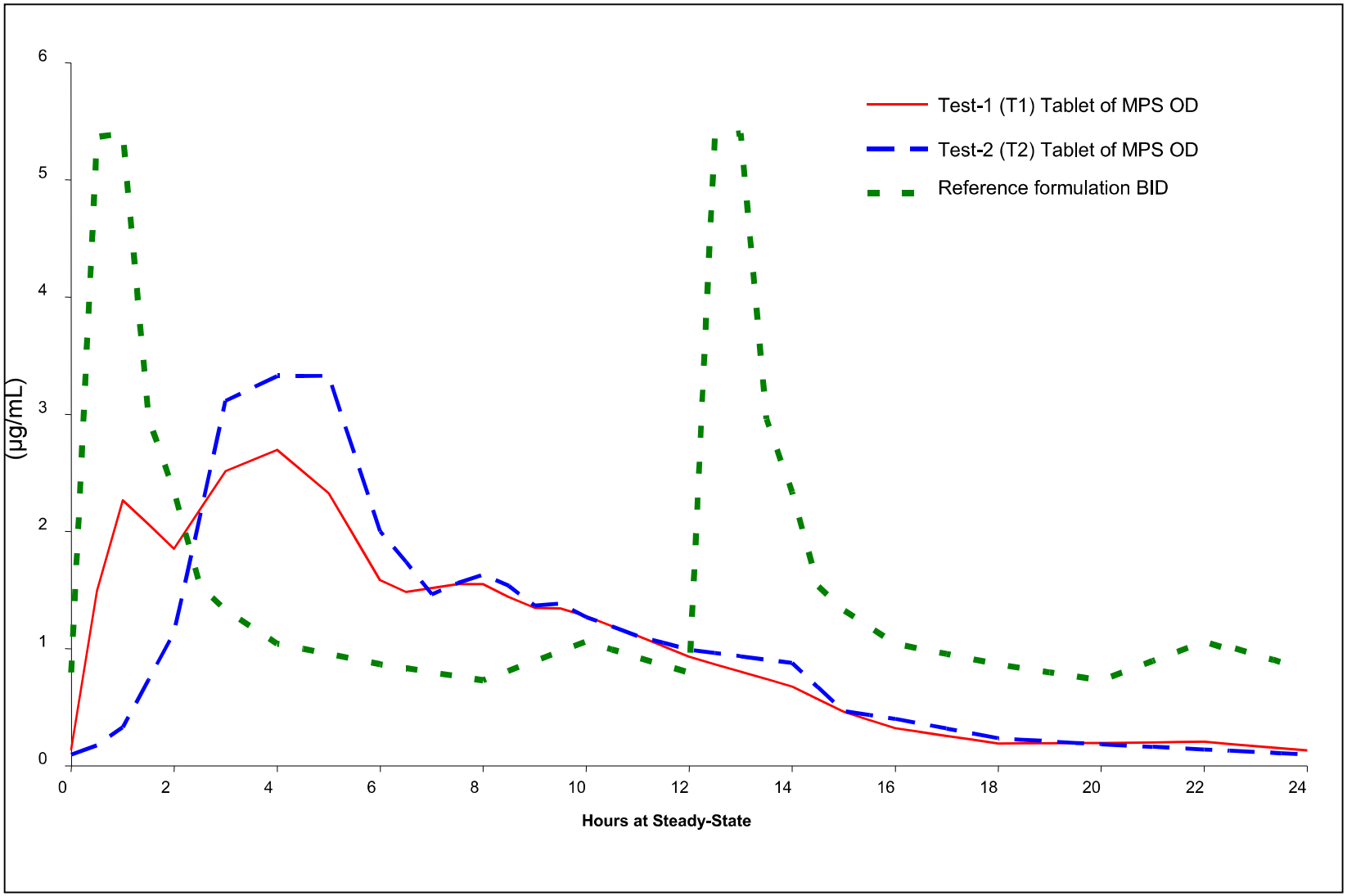


Figure 36: Simulated Mycophenolate Sodium mean concentration at steady state in fasted subjects with complete data.

Table 87: Concentration *vs.* Time Data for Reference (R), (Study-I, Batch No. U0399)

Subject	Sequence	Reference (R) - Concentration (µg/mL)																							
		Time (hr)																							
		0	0.17	0.33	0.5	0.67	0.83	1	1.25	1.5	1.75	2	2.25	2.5	3	4	6	8	10	12	16	24	36	48	60	72
1	T1T2R	0.00	0.40	1.88	2.65	3.76	5.18	8.42	3.62	2.66	2.17	2.16	1.64	1.09	0.63	0.50	0.42	0.36	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	T1T2R	0.00	0.88	7.29	10.50	8.89	6.57	5.38	4.27	3.98	3.99	3.94	3.87	2.59	2.46	2.30	0.45	0.82	0.88	0.77	0.52	0.29	0.36	0.23	0.00	0.00
3	T2RT1	0.08	1.84	8.53	13.95	8.61	8.40	5.38	3.68	3.06	2.89	1.86	2.48	1.85	0.91	0.30	0.58	0.29	0.61	0.47	0.19	0.14	0.00	0.00	0.00	0.00
5	T2RT1	0.00	0.00	0.00	0.72	17.70	16.60	25.50	20.40	10.20	8.92	7.94	7.19	2.97	2.57	2.97	1.68	1.42	2.09	1.49	0.84	0.26	0.19	0.00	0.00	0.00
6	RT1T2	0.00	1.14	13.50	18.90	15.30	12.30	7.75	7.70	7.40	6.74	3.24	4.40	2.25	2.20	1.74	1.07	0.90	4.88	3.04	1.05	0.92	0.00	0.00	0.00	0.00
7	RT1T2	0.00	0.00	8.34	11.70	12.70	15.40	13.80	11.90	7.00	6.16	6.20	6.07	3.22	3.84	1.42	1.92	1.03	1.60	0.76	0.61	0.75	0.85	0.00	0.00	0.00
9	T2RT1	0.00	0.87	7.91	10.00	7.83	4.44	3.85	2.18	1.52	1.87	1.02	0.86	0.97	0.52	0.36	0.56	0.27	1.81	1.92	0.93	0.41	0.00	0.00	0.00	0.00
11	T1T2R	0.00	0.63	10.20	10.40	13.10	11.00	9.30	7.29	3.13	2.33	1.41	0.97	0.83	0.36	0.33	0.77	0.67	0.69	0.77	0.33	0.00	0.00	0.00	0.00	0.00
12	RT1T2	0.00	0.20	12.80	10.20	7.32	5.34	4.14	3.11	2.60	1.77	1.62	1.47	1.09	1.34	0.78	0.83	0.99	1.34	0.89	0.49	0.48	0.00	0.00	0.00	0.00
N		9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Mean		0.01	0.66	7.83	9.89	10.58	9.47	9.28	7.13	4.62	4.09	3.27	3.22	1.87	1.65	1.19	0.92	0.75	1.58	1.12	0.55	0.36	0.16	0.03	0.00	0.00
SD		0.03	0.60	4.48	5.46	4.41	4.56	6.83	5.83	2.90	2.58	2.38	2.30	0.92	1.18	0.98	0.54	0.39	1.37	0.91	0.35	0.32	0.29	0.08	0.00	0.00
Min		0.00	0.00	0.00	0.72	3.76	4.44	3.85	2.18	1.52	1.77	1.02	0.86	0.83	0.36	0.30	0.42	0.27	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Median		0.00	0.63	8.34	10.40	8.89	8.40	7.75	4.27	3.13	2.89	2.16	2.48	1.85	1.34	0.78	0.77	0.82	1.34	0.77	0.52	0.29	0.00	0.00	0.00	0.00
Max		0.08	1.84	13.50	18.90	17.70	16.60	25.50	20.40	10.20	8.92	7.94	7.19	3.22	3.84	2.97	1.92	1.42	4.88	3.04	1.05	0.92	0.85	0.23	0.00	0.00
CV%		300.00	90.32	57.17	55.15	41.64	48.20	73.57	81.85	62.74	62.99	72.88	71.66	49.23	71.88	81.94	58.99	51.87	86.51	80.77	63.10	87.85	186.40	300.00	-	-

Table 88: Concentration *vs.* Time Data for Test (T1), (Study-I, Batch No. NAXPM-15)

Sub.	Sequence	Test 1 Formulation - Concentration (µg/mL)																															
		Time (hr)																															
		0	0.5	1	2	3	4	5	6	6.5	7	7.5	8	8.5	9	9.5	10	11	12	13	14	15	16	18	20	22	24	30	36	42	48	60	72
1	T1T2R	0.00	0.77	0.53	0.46	5.52	2.35	1.97	1.90	1.99	2.04	2.16	1.83	1.88	1.97	1.84	1.06	1.00	1.10	0.50	0.46	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	T1T2R	0.00	2.48	2.82	2.23	1.66	1.68	1.64	0.94	0.94	0.89	1.30	1.40	1.53	1.42	1.27	1.02	0.69	0.80	0.75	0.56	0.76	0.30	0.21	0.18	0.28	0.20	0.00	0.00	0.00	0.00	0.00	0.00
3	T2RT1	0.00	2.14	4.09	1.32	1.09	1.35	1.30	1.31	0.91	1.07	1.16	1.27	0.81	1.00	0.77	1.07	0.72	0.44	0.41	0.56	0.28	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	T2RT1	0.00	1.72	3.45	4.69	5.82	6.19	6.04	1.82	1.30	1.12	0.76	0.86	0.73	0.67	0.79	1.03	1.11	1.54	1.57	1.29	0.71	0.54	0.43	0.56	0.64	0.23	0.00	0.00	0.00	0.00	0.00	0.00
6	RT1T2	0.00	1.25	6.47	4.31	3.66	4.02	3.50	3.31	3.53	3.42	3.30	2.85	2.58	2.10	2.22	2.68	2.86	1.47	1.27	1.06	0.83	0.82	0.70	0.92	0.98	0.97	0.78	0.00	0.00	0.00	0.00	0.00
7	RT1T2	0.00	0.00	0.00	0.00	1.65	4.95	1.75	1.03	1.21	1.45	1.20	1.11	1.52	1.53	1.60	1.34	1.39	1.21	1.22	0.89	0.60	0.53	0.44	0.47	0.64	0.24	0.00	0.00	0.00	0.00	0.00	0.00
9	T2RT1	0.00	1.37	1.65	1.57	1.63	1.53	2.41	1.33	1.32	1.21	1.33	1.00	0.81	0.74	0.81	0.86	0.55	0.68	0.60	0.71	0.45	0.42	0.30	0.17	0.28	0.27	0.00	0.00	0.00	0.00	0.00	0.00
11	T1T2R	0.00	0.76	1.27	1.19	1.23	1.21	1.34	1.71	2.07	2.79	2.81	2.56	2.11	2.07	1.64	1.43	1.24	1.36	1.50	1.01	0.62	0.62	0.21	0.20	0.14	0.25	0.00	0.00	0.00	0.00	0.00	0.00
12	RT1T2	0.00	0.00	0.00	0.00	0.52	1.76	3.30	1.45	1.51	1.64	2.21	2.03	1.93	1.73	1.66	1.63	0.69	0.54	0.54	0.64	0.35	0.39	0.31	0.30	0.29	0.22	0.36	0.00	0.00	0.00	0.00	0.00
N		9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Mean		0.00	1.17	2.25	1.75	2.53	2.78	2.60	1.65	1.64	1.74	1.80	1.66	1.54	1.47	1.40	1.35	1.14	1.02	0.93	0.80	0.54	0.44	0.30	0.31	0.36	0.27	0.13	0.00	0.00	0.00	0.00	0.00
SD		0.00	0.87	2.16	1.72	1.97	1.82	1.50	0.71	0.82	0.86	0.86	0.71	0.65	0.56	0.52	0.56	0.71	0.41	0.46	0.28	0.21	0.23	0.20	0.30	0.33	0.29	0.27	0.00	0.00	0.00	0.00	0.00
Min		0.00	0.00	0.00	0.00	0.52	1.21	1.30	0.94	0.91	0.89	0.76	0.86	0.73	0.67	0.77	0.86	0.55	0.44	0.41	0.46	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Median		0.00	1.25	1.65	1.32	1.65	1.76	2.00	1.45	1.32	1.45	1.33	1.40	1.53	1.53	1.60	1.07	1.00	1.10	0.75	0.71	0.60	0.42	0.30	0.20	0.28	0.23	0.00	0.00	0.00	0.00	0.00	0.00
Max		0.00	2.48	6.47	4.69	5.82	6.19	6.00	3.31	3.53	3.42	3.30	2.85	2.58	2.10	2.22	2.68	2.86	1.54	1.57	1.29	0.83	0.82	0.70	0.92	0.98	0.97	0.78	0.00	0.00	0.00	0.00	0.00
CV%		-	74.80	95.95	98.40	78.00	65.30	59.00	43.00	49.60	49.60	47.54	42.70	42.19	37.80	37.10	41.20	61.80	40.60	49.40	35.00	38.39	51.90	76.00	95.10	91.20	107.00	215.00	-	-	-	-	-

Table 89: Concentration *vs.* Time Data for Test (T2), (Study-I, Batch No. NAXPM-8)

Sub.	Sequence	Test 2 Formulation - Concentration (µg/mL)																															
		Time (hr)																															
		0	0.5	1	2	3	4	5	6	6.5	7	7.5	8	8.5	9	9.5	10	11	12	13	14	15	16	18	20	22	24	30	36	42	48	60	72
1	T1T2R	0.00	0.23	0.50	1.31	1.05	2.73	0.53	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	T1T2R	0.00	0.00	0.00	1.23	2.54	3.07	4.15	0.98	1.13	1.04	1.03	1.07	1.02	1.03	1.39	1.16	0.92	0.65	0.76	0.72	0.31	0.26	0.25	0.23	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	T2RT1	0.00	0.00	0.33	0.90	1.13	1.24	1.34	1.33	1.28	1.18	1.38	1.73	2.01	1.93	2.05	2.12	1.65	0.99	0.66	0.63	0.25	0.13	0.31	0.23	0.30	0.25	0.16	0.00	0.00	0.00	0.00	0.00
5	T2RT1	0.00	0.00	0.00	0.55	10.20	8.42	7.62	4.15	2.61	2.64	3.08	3.29	2.19	1.21	1.23	1.60	1.64	2.30	2.92	2.87	0.56	0.94	0.34	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	RT1T2	0.00	0.52	1.09	2.36	3.03	2.90	3.73	2.78	2.69	2.38	2.34	2.30	2.50	2.42	2.43	2.02	1.59	1.55	1.22	1.04	1.16	0.64	0.69	0.53	0.45	0.41	0.32	0.00	0.00	0.00	0.00	0.00
7	RT1T2	0.00	0.00	0.35	2.02	4.93	4.43	3.44	1.33	0.82	0.47	0.34	0.49	0.74	0.91	0.80	0.50	0.45	0.32	0.27	0.26	0.15	0.10	0.15	0.10	0.08	0.02	0.03	0.00	0.00	0.00	0.00	0.00
9	T2RT1	0.00	0.00	0.00	0.61	4.23	2.39	1.78	1.76	1.79	1.55	1.55	1.63	1.38	1.20	1.00	1.12	1.77	1.72	1.24	0.97	0.43	0.39	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	T1T2R	0.00	0.00	0.00	0.83	2.07	2.07	2.04	1.72	1.67	1.29	1.32	1.36	1.19	0.95	1.05	1.09	1.34	1.13	1.07	1.01	0.84	0.64	0.28	0.29	0.24	0.26	0.21	0.00	0.00	0.00	0.00	0.00
12	RT1T2	0.00	0.00	0.00	0.00	0.49	4.35	5.34	2.22	2.07	2.66	3.14	3.07	3.21	3.06	3.08	2.50	1.21	0.91	0.95	0.94	0.84	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N		9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Mean		0.00	0.08	0.25	1.09	3.30	3.51	3.30	1.86	1.56	1.47	1.58	1.66	1.58	1.41	1.45	1.35	1.17	1.06	1.01	0.94	0.50	0.41	0.30	0.20	0.15	0.10	0.08	0.00	0.00	0.00	0.00	0.00
SD		0.00	0.18	0.37	0.74	2.98	2.10	2.20	1.10	0.86	0.94	1.10	1.10	0.99	0.91	0.93	0.80	0.61	0.72	0.83	0.81	0.38	0.31	0.20	0.19	0.17	0.16	0.12	0.00	0.00	0.00	0.00	0.00
Min		0.00	0.00	0.00	0.00	0.49	1.24	0.50	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Median		0.00	0.00	0.00	0.90	2.54	2.90	3.40	1.72	1.67	1.29	1.38	1.63	1.38	1.20	1.23	1.16	1.34	0.99	0.95	0.94	0.43	0.39	0.30	0.23	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max		0.00	0.52	1.09	2.36	10.20	8.42	7.60	4.15	2.69	2.66	3.14	3.29	3.21	3.06	3.08	2.50	1.77	2.30	2.92	2.87	1.16	0.94	0.70	0.53	0.45	0.41	0.32	0.00	0.00	0.00	0.00	0.00
CV%		-	216.00	146.60	67.90	90.30	59.80	66.00	59.00	55.20	64.10	69.91	66.00	62.31	64.70	64.20	59.60	52.00	67.30	82.10	86.20	75.15	76.30	81.00	96.20	113.00	151.00	151.00	-	-	-	-	-

4.3.2 Optimized Formulations with Polymer Matrix Technology

a) It was decided to further optimize the Polymer Matrix based formulation.

b) It was decided to develop two formulations, one with faster release profile and other with slow release profile for further PK studies.

4.3.2.1 Wet (Non-Aqueous) Granulation Method (B. No. NAXPM-16)

The tablets were prepared by wet granulation technique as single pot method. Tablets were prepared by using PVP K 30 dissolved in IPA as granulating agent and using, low viscous HPMC (15 K cps), PVP K90 and polyethylene oxide (PEO) as release modifying agent. Complete drug was released in 6 hr (Table 90 and Figure 37).

Table 90: Dissolution Profile of NAXPM-16

Time (h)	% Drug Release	% RSD
1	26.3	10.2
2	42.3	4.4
3	60.2	5.8
4	72.2	0.0
6	103.8	1.9

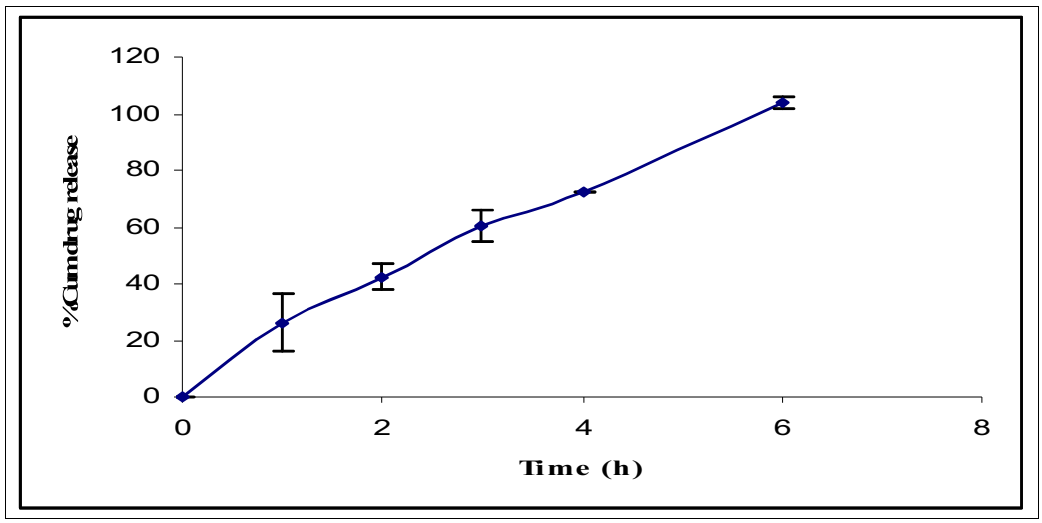


Figure 37: Drug release profile of NAXPM-16

The drug release profile showed that drug releases in sustained manner only for 6 hrs so it was planned to increase the amount of polymers to form strong matrix.

4.3.2.2 Wet (Non-Aqueous) Granulation Method (B. No. NAXPM -17, NAXPM -18)

The tablets were prepared by wet granulation technique as single pot method. Tablets were prepared by using PVP K 30 dissolved in IPA as granulating agent and using, low viscous HPMC (15 K cps) in batch no. NAXPM-17 and HPMC (100 K cps) in batch no. NAXPM-18) as release modifying agent. Complete drug was released in 8-10 hr (Table 91 and Figure 38).

Table 91: Dissolution Profile of NAXPM-17, NAXPM-18

Time (h)	NAXPM-17		NAXPM -18	
	% Drug Release	% RSD	% Drug Release	% RSD
1.0	23.0	2.7	19.7	4.5
2.0	42.1	6.1	31.1	5.7
3.0	59.5	6.4	42.5	6.8
4.0	76.8	7.7	53.5	5.0
6.0	98.2	4.9	71.3	2.7
8.0	109.2	0.9	86.9	1.2
10.0	--	--	99.7	1.6

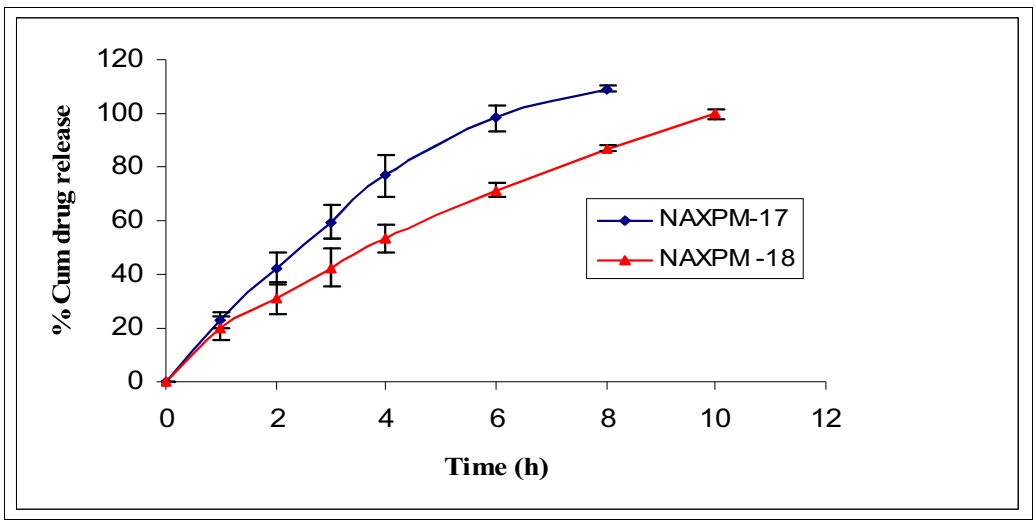


Figure 38: Drug release profile of NAXPM-17 and NAXPM-18

Based on the drug release profile it was planned to use higher viscosity HPMC polymer (100 K cps) alongwith PEO polymer to form strong gel for desired sustained drug release.

4.3.2.3 Wet (Non-Aqueous) Granulation Method (B. No. NAXPM -19)

The tablets were prepared by wet granulation technique. Tablets were prepared by using PVP K 30 dissolved in IPA as granulating agent and using, high viscous HPMC

(100 K cps) and PEO as release modifying agent. Complete drug was released in 8-10 hr (Table 92 and Figure 39).

Table 92: Dissolution Profile of NAXPM -19

Time (h)	% Drug Release	% RSD
1.0	18.6	3.5
2.0	30.0	1.7
4.0	48.5	2.7
6.0	66.0	1.6
8.0	81.9	1.7
10.0	94.0	1.6
12.0	102.7	0.8

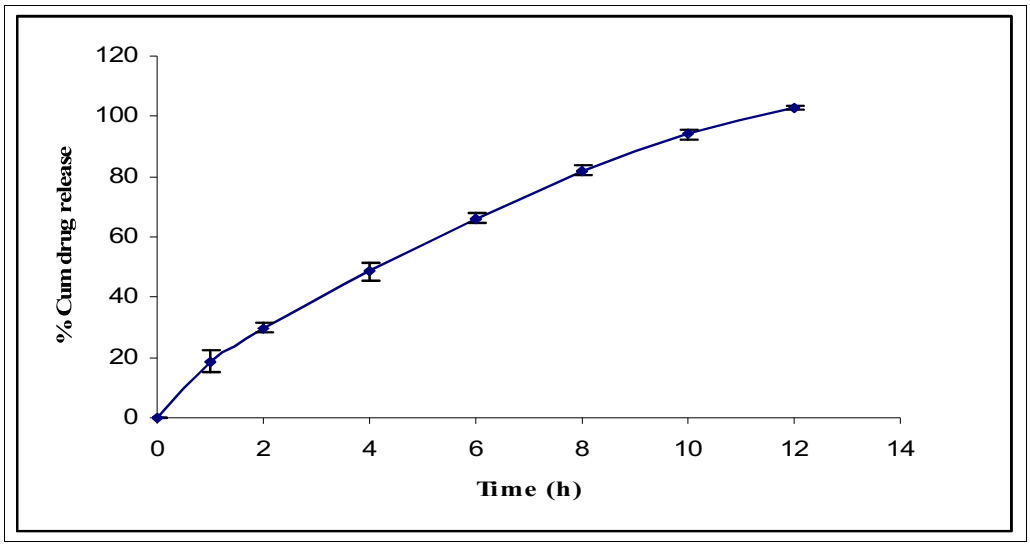


Figure 39: Drug release profile of NAXPM-19

The drug release was as per desired criteria for fast release profile and it was selected to carry out stability and bioavailability studies on this formulation. It was also selected to increase the polymer concentration for getting the sustained release upto 16 hr.

4.3.2.4 Wet (Non-Aqueous) Granulation Method (B. No. NAXPM -20)

The tablets were prepared by wet granulation technique. Tablets were prepared by using PVP K 30 dissolved in IPA as granulating agent and using higher amount of high viscous HPMC (100 K cps) and reducing the amount of PEO as release modifying agent. Complete drug was released in 16-18 hr (Table 93 and Figure 40).

Table 93: Dissolution Profile of NAXPM -20

Time (h)	% Drug Release	% RSD
1.0	17.4	2.5
2.0	25.4	4.8
4.0	38.7	1.7
6.0	51.0	2.1
8.0	63.9	1.1
10.0	73.0	1.2
12.0	82.4	1.3
14.0	89.5	2.0
16.0	97.1	1.1
18.0	100.0	0.8

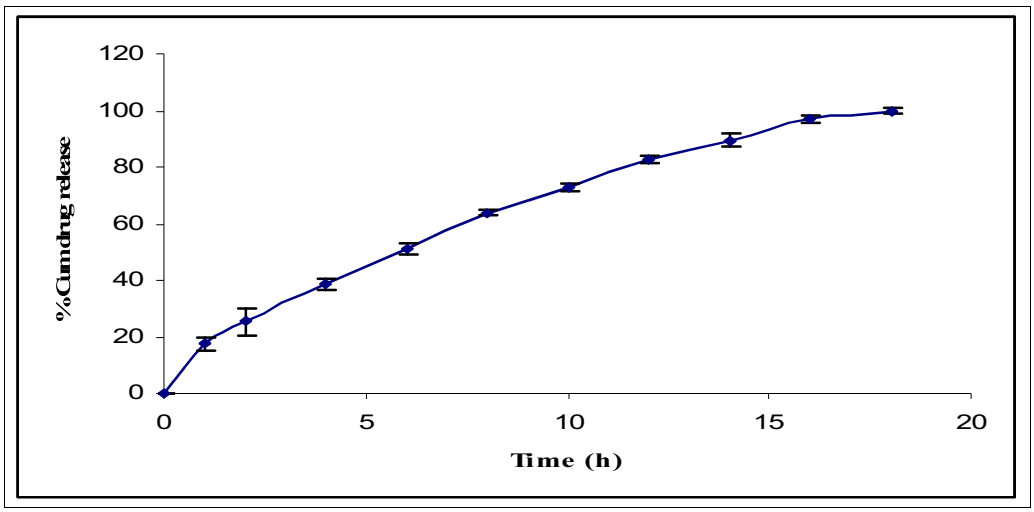


Figure 40: Drug release profile of NAXPM-20

The drug release was as per desired criteria for slow release profile and it was decided to carry out stability and bioavailability studies on this formulation.

4.3.2.5 Stability studies of the developed formulation

The accelerated stability studies of the developed tablet formulation (NAXPM-19) were performed as per ICH guidelines. The tablets were packaged in HDPE bottle and alu-alu blister pack and charged for stability. The result of the accelerated stability study showed (Table 94 and Figure 41) that the tablet is stable in all the packaging material and there is no degradation or change in the drug release behaviour. On the basis of stability results it was decided to carry out *in vivo* bioavailability study of the developed formulation.

Table 94: Stability data of the modified release tablet formulation (B. No. NAXPM-19)

	% Cumulative Drug Release						
	Initial	Pack Type					
		HDPE			Alu-Alu		
Time (Hrs) / Month	Condition	40°C/75%RH			40°C/75%RH		
		1	2	3	1	2	3
1	16.7	17.3	16.4	16.1	17.4	17.5	16.0
2	28.4	28.0	25.4	24.7	28.8	32.2	24.2
4	49.2	51.6	54.1	51.7	52.8	54.5	52.9
6	69.1	71.6	74.8	73.2	72.6	72.2	74.4
8	86.4	87.3	96.0	91.4	89.1	91.2	91.4
10	100.3	98.9	106.1	96.7	99.1	99.8	96.8
12	107.5	105.3	103.3	99.1	105.9	104.1	100.0

Chemical stability							
Pack Type	-	**HDPE**			**Alu-Alu**		
Condition	-	**40°C/75%RH**			**40°C/75%RH**		
Month	Initial	1	2	3	1	2	3
Assay (%)	103.8	99.6	101.0	101.7	100.0	100.5	99.3
SHI (%)	0.02	0.03	0.02	0.04	0.03	0.04	0.04
Total Impurity (%)	0.04	0.04	0.06	0.06	0.06	0.06	0.06
Water (%)	1.2	1.18	1.96	1.55	1.06	1.29	1.21
Hardness	280 N	275 N	281 N	285 N	288 N	280 N	282 N
Friability	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
Thickness	6.57 mm	6.57 mm	6.57 mm	6.57 mm	6.57 mm	6.57 mm	6.57 mm

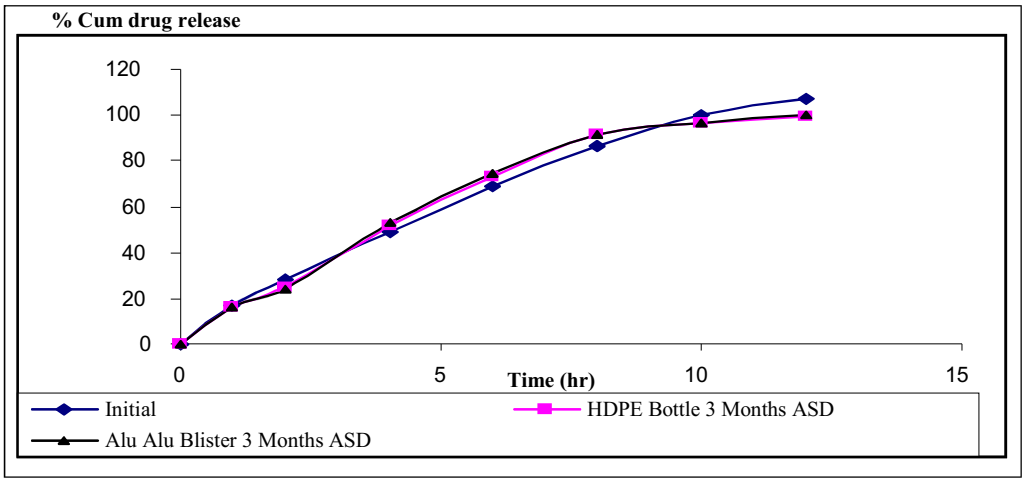


Figure 41: Stability graph of the modified release tablet formulation (B. No. NAXPM-19)

4.3.2.6 Stability studies of the Modified formulation:

The accelerated stability studies of the developed tablet formulation (NAXPM-20) were performed as per ICH guidelines. The tablets were packaged in HDPE bottle and Alu-Alu blister pack and charged for stability. The result of the accelerated stability study showed (Table 95 and Figure 42) that the tablet is stable in all the packaging material and there is no degradation or change in the drug release behaviour. On the basis of stability results it was decided to carry out *in vivo* bioavailability study of the developed formulation.

Table 95: Stability data of the modified release tablet formulation (B.No. NAXPM-20)

	% Cumulative Drug Release						
	Initial	Pack Type					
		HDPE			Alu-Alu		
Time (Hrs) ↓ / Month →	Condition	40°C/75%RH			40°C/75%RH		
		1	2	3	1	2	3
1	13.3	15.0	12.7	12.5	12.7	14.7	13.0
2	20.9	23.5	17.3	18.9	32.8	25.5	19.5
4	37.7	37.8	36.8	35.9	49.5	38.5	36.0
6	51.4	49.6	51.8	50.7	61.9	53.1	51.1
8	61.4	61.7	65.9	64.3	71.7	66.0	63.8
10	72.9	71.8	79.8	78.9	79.6	78.7	78.0
12	85.8	80.8	92.6	91.5	90.7	91.0	90.8
14	92.5	88.6	98.5	97.4	92.8	98.2	96.8
16	96.9	96.8	103.4	100.5	103.3	103.5	100.4
18	102.1	99.5	105.9	101.3	103.2	104.6	101.5

Chemical stability							
Pack Type	-	**HDPE**			**Alu-Alu**		
Condition	-	40°C/75%RH			40°C/75%RH		
Month	Initial	1	2	3	1	2	3
Assay (%)	105.8	99.8	101.5	99.7	99.9	100.8	99.8
SHI (%)	0.02	0.03	0.02	0.04	0.03	0.04	0.04
Total Impurity (%)	0.04	0.05	0.04	0.06	0.06	0.04	0.06
Water (%)	1.25	1.05	1.65	1.35	1.07	1.46	1.58
Hardness	260N	256 N	265 N	258 N	260 N	262 N	260 N
Friability	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
Thickness	7.05 mm	7.05 mm	7.05 mm	7.05 mm	7.05 mm	7.05 mm	7.05 mm

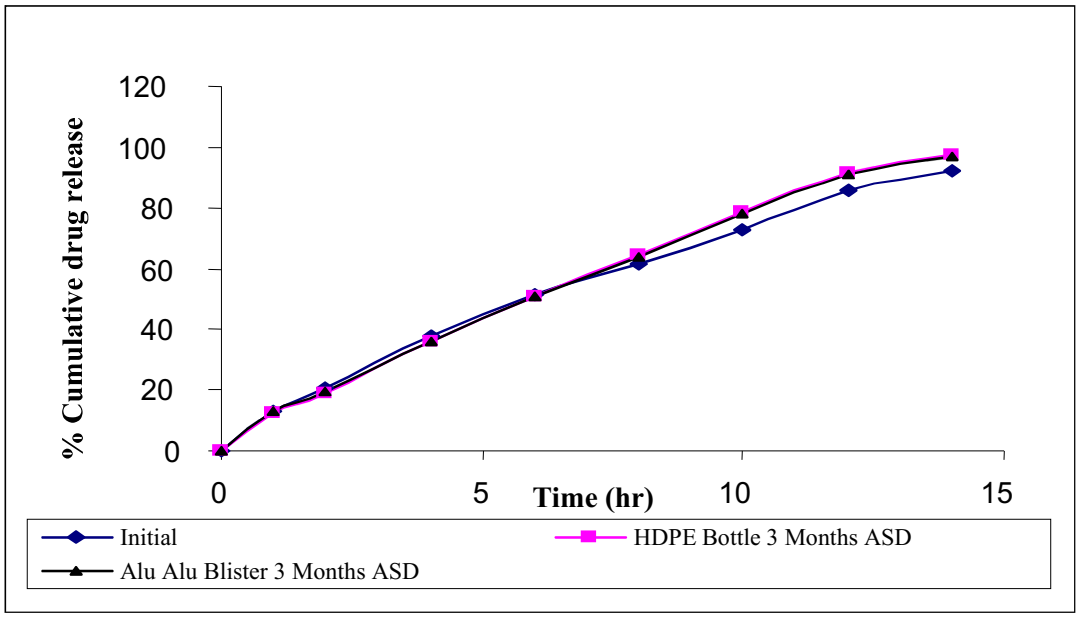


Figure 42: Drug release graph of the modified release tablet formulation at different stages of stability study (B. No. NAXPM-20)

Out of the formulations NAXPM-16 to NAXPM-20, prepared using polymer matrix technology, two formulations were selected for biostudy. These formulations were selected on the basis of their dissolution profiles. In the case of NAXPM-19, about 94% of drug was released in 10 hrs. while in case of NAXPM-20, about 97% drug was released in 16 hrs. The formulations NAXPM-19 and 20 were subjected biostudy III and II respectively in fed and fasted state.

4.3.3 Randomized, single dose, crossover comparative bioavailability study under fasting and fed conditions (Study-II)

A randomized, open label, balanced, three treatment, three period, six sequence, single dose, three way crossover comparative bioavailability study of test MR formulations (Batch No.NAXPM 20) of Mycophenolate Sodium of Panacea Biotec Ltd. under fasting (T1) and fed (T2) conditions with reference formulation under fasting condition was performed in 18 + 6 (Standby) healthy human adult male subjects. The PK parameters are summarized in Table 96. The pharmacokinetic parameters have been obtained by subjecting the plasma concentration time data to Non-Compartmental analysis using WinNonlin 5.2 software. The plasma concentration time data has been summarized in Table 103, 104 and 105.

Table 96: PK Parameters Table

Sub.	Sequence	C_{max} (µg/mL)			T_{max} (hr)			AUC_{0-t} (hr*µg/mL)			$AUC_{0-\infty}$ (hr*µg/mL)			λ_z (1/hr)			$T_{1/2}$ (hr)		
		R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2
S1	T2T1R	30.10	5.40	7.36	0.67	3.00	5.50	43.26	44.19	27.20	44.38	46.42	35.62	0.108	0.062	0.032	6.43	11.13	21.46
S2	T1T2R	13.10	3.41	12.10	0.67	2.00	4.50	52.45	35.97	40.15	55.18	48.85	42.17	0.070	0.060	0.068	9.95	11.48	10.26
S3	RT1T2	8.83	5.01	7.88	0.83	3.00	1.50	21.80	21.28	29.03	23.81	21.82	30.84	0.097	0.258	0.086	7.15	2.69	8.02
S4	T1T2R	10.10	5.62	13.98	0.83	4.50	2.50	51.95	59.76	60.70	55.77	63.60	66.28	0.046	0.054	0.052	14.96	12.76	13.38
S5	T2RT1	33.00	3.96	32.30	0.83	4.00	5.00	73.79	43.11	63.39	75.27	44.83	66.49	0.093	0.113	0.049	7.46	6.13	14.23
S6	T2T1R	17.90	6.49	13.60	0.50	0.33	5.50	37.84	61.17	56.72	39.36	63.33	60.34	0.136	0.066	0.067	5.10	10.46	10.37
S7	T1RT2	19.90	4.25	11.40	0.67	1.00	3.00	43.21	31.19	38.55	45.53	32.78	39.53	0.077	0.091	0.141	9.00	7.63	4.90
S8	T2T1R	14.90	3.17	7.65	0.67	1.25	4.50	34.65	33.64	26.25	43.61	39.54	30.44	0.034	0.041	0.121	20.16	17.00	5.73
S9	T1RT2	8.44	4.77	12.50	0.50	0.50	8.00	68.89	30.41	95.72	131.35	39.50	179.83	0.045	0.081	0.021	15.57	8.53	33.12
S10	RT2T1	30.30	9.01	9.91	0.33	3.50	6.00	127.86	135.54	127.06	297.37	270.15	329.26	0.011	0.014	0.009	65.27	49.37	74.55
S11	T1T2R	38.70	5.79	20.30	0.83	2.50	6.00	49.04	43.23	46.70	57.69	48.75	49.43	0.076	0.054	0.077	9.12	12.81	9.04
S12	RT1T2	25.20	7.13	18.10	0.33	3.50	4.50	54.99	45.26	85.90	69.71	47.74	149.16	0.020	0.065	0.015	34.95	10.67	45.97
S13	RT2T1	34.10	14.10	15.10	1.00	2.00	5.50	156.44	84.17	35.75	206.72	159.87	42.88	0.028	0.014	0.073	24.89	48.14	9.55
S14	T1RT2	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
S15	T2RT1	5.52	5.25	15.60	2.50	2.00	3.50	38.86	40.62	52.43	41.78	77.02	53.93	0.080	0.007	0.089	8.63	96.69	7.80
S16	RT1T2	20.00	5.13	14.70	0.50	2.00	1.25	72.14	54.78	59.78	77.65	61.09	64.75	0.056	0.064	0.057	12.36	10.88	12.07
S17	RT2T1	26.00	4.83	10.80	0.50	0.33	5.00	42.34	21.55	34.15	44.81	23.47	35.99	0.067	0.060	0.081	10.40	11.50	8.57
S18	T2RT1	18.60	6.50	10.60	0.83	2.00	5.50	47.65	42.74	44.78	50.11	46.22	47.20	0.058	0.158	0.058	11.95	4.37	12.00
S19	T1RT2	18.30	6.27	8.93	0.83	2.00	2.50	44.47	50.86	51.16	46.52	54.84	58.86	0.059	0.060	0.031	11.66	11.65	22.05

(Contd…)

Sub.	Sequence	C_{max} (µg/mL)			T_{max} (hr)			AUC_{0-t} (hr*µg/mL)			$AUC_{0-\infty}$ (hr*µg/mL)			λ_z (1/hr)			$T_{1/2}$ (hr)		
		R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2
S20	T1T2R	9.28	5.85	18.40	1.50	2.00	1.00	52.85	45.29	56.11	60.50	48.77	61.38	0.031	0.067	0.049	22.30	10.37	14.16
S21	T2T1R	20.80	5.38	10.70	0.83	2.00	1.50	47.97	41.14	45.17	50.76	43.48	51.46	0.058	0.090	0.036	11.88	7.72	19.21
S22	RT1T2	22.60	6.21	11.50	0.83	2.00	1.50	49.55	50.36	49.68	51.96	58.98	57.89	0.063	0.031	0.033	10.95	22.47	20.98
S23	RT2T1	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
S24	T2RT1	18.70	9.02	9.08	0.83	1.50	1.50	45.04	52.97	53.94	47.77	58.32	59.65	0.081	0.040	0.038	8.51	17.34	18.32
N		22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
Mean		20.20	6.03	13.30	0.81	2.13	3.88	57.14	48.60	53.65	73.53	63.61	73.34	0.063	0.070	0.058	15.39	18.26	17.99
SD		9.16	2.33	5.54	0.45	1.12	1.99	30.27	23.83	23.69	62.95	53.44	67.37	0.030	0.054	0.033	13.17	21.17	15.82
Min		5.52	3.17	7.36	0.33	0.33	1.00	21.80	21.28	26.25	23.81	21.82	30.44	0.011	0.007	0.009	5.10	2.69	4.90
Max		38.70	14.10	32.30	2.50	4.50	8.00	156.44	135.54	127.06	297.37	270.15	329.26	0.136	0.258	0.141	65.27	96.69	74.55
CV%		45.33	38.63	41.66	55.79	52.44	51.22	52.98	49.03	44.16	85.62	84.01	91.87	47.250	75.950	56.270	85.57	115.94	87.93

Table 97: ANOVA Table

Parameter	Unit	Effects	P-value
$Ln(C_{max})$	ug/mL	Sequence	0.469
	ug/mL	Formulation	0.000
	ug/mL	Period	0.837
$Ln(AUC_{0-24})$	hr*ug/mL	Sequence	0.575
	hr*ug/mL	Formulation	0.008
	hr*ug/mL	Period	0.790
$Ln(AUC_{0-t})$	hr*ug/mL	Sequence	0.556
	hr*ug/mL	Formulation	0.160
	hr*ug/mL	Period	0.825
$Ln(AUC_{0-\infty})$	hr*ug/mL	Sequence	0.370
	hr*ug/mL	Formulation	0.366
	hr*ug/mL	Period	0.423

On applying analysis of variance (Table 97), it was observed that no statistically significant effect (P>0.05) was observed, due to periods for $Ln(C_{max})$, $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$. Formulation effect was statistically significant for $Ln(C_{max})$ and $Ln(AUC_{0-24})$ (P<0.05) but for $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$ it was found to be non significant (P>0.05). No significant difference (p>0.10) was observed due to sequence for $Ln(C_{max})$, $Ln(AUC_{0-24})$, $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$. The results of the statistical comparisons performed on the single-dose pharmacokinetic parameter estimates are summarized in Table 98, (Test-1, fasting) (NAXPM-20) and in Table 99, (Test-2, fed) (NAXPM-20).

The value of $AUC_{0-24,}$ AUC_{0-t} and $AUC_{0-\infty}$ for Test 1 (NAXPM-20 in fasting condition) were found to be 19.95%, 13.99 % and 11.61 % lower than that of reference respectively (Table 98), while in case of Test 2 (NAXPM-20 under fed condition) the values were less than 10% lower than that of reference (Table 99); From Table 99, it was observed that Test 2 (NAXPM-20 under fed condition) was bioequivalent to reference w.r.t. AUC_{0-24}, $AUC0_{-t}$ and $AUC_{0-\infty}$. As expected for modified-release products, Test 1 (T1, fasting) (NAXPM-20) and Test 2 (T2, fed) (NAXPM-20) both had lower peak concentrations and longer times to peak concentration compared to the reference (Table 96). On observing food effect it was found that for T2 (in fed condition) C_{max} and T_{max} were on the higher side as compared to T1 (in fasting condition). The mean concentration vs. time chart on linear scale was shown in the Figure 43.

Table 98: Comparison of Pharmacokinetic parameters of Test 1 and Reference

Parameter	Least-Squares Means [1]		Ratio% [2]	CV% [3]	90% Confidence Interval [4]		Power
	Test 1 (T1, fast)	Reference (R)			Lower	Upper	
AUC_{0-24} (hr*µg/mL)	38.9102	49.0616	79.31	-	67.30	91.31	-
AUC_{0-t} (hr*µg/mL)	49.9208	58.2983	85.63	-	70.85	100.41	-
$AUC_{0-\infty}$ (hr*µg/mL)	67.3857	76.8715	87.66	-	68.95	106.37	
C_{max} (µg/mL)	6.1121	20.2621	30.17	-	15.79	44.55	
λ_z (1/hr)	0.0693	0.0626	110.62	-	-	-	-
$T_{1/2}$ (hr)	18.9558	15.7178	120.60	-	-	-	-
Ln-Transformed:							
AUC_{0-24} (hr*µg/mL)	36.7902	45.9599	80.05	22.98	71.33	89.83	0.9384
AUC_{0-t} (hr*µg/mL)	45.2600	52.6255	86.00	27.01	75.15	98.43	0.8613
$AUC_{0-\infty}$ (hr*µg/mL)	54.7009	61.8911	88.38	33.02	75.05	104.09	0.7286
C_{max} (µg/mL)	5.7358	18.0795	31.73	39.53	26.14	38.51	0.6007

1. Least-squares geometric means for Ln-transformed data.
2. Ratio calculated as Test 1 least-squares mean divided by the Reference least-squares mean.
3. Estimated intra-subject coefficient of variation, CV%=100*SQRT (e^{MSE}-1), where MSE is the mean square error term from the ANOVA.
4. Confidence interval on the ratio.

Reference (R): Reference formulation as single oral dose under **fasting** condition.
Test 1 (T1) : MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose under **fasting** conditions (NAXPM-20).
Test 2 (T2) : MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose under **fed** conditions (NAXPM-20).

Table 99: Comparison of Pharmacokinetic parameters of Test 2 and Reference

Parameter	Least-Squares Means [1]		Ratio% [2]	CV% [3]	90% Confidence Interval [4]		Power
	Test 2 (T2, fed)	Reference (R)			Lower	Upper	
AUC_{0-24} (hr*µg/mL)	43.6600	49.0616	88.99	-	76.99	100.99	-
AUC_{0-t} (hr*µg/mL)	54.8219	58.2983	94.04	-	79.25	108.82	-
$AUC_{0-\infty}$ (hr*µg/mL)	76.9970	76.8715	100.16	-	81.45	118.87	-
C_{max} (µg/mL)	13.3083	20.2621	65.68	-	51.30	80.06	-
λ_z (1/hr)	0.0576	0.0626	92.06	-	-	-	-
$T_{1/2}$ (hr)	18.5882	15.7178	118.26	-	-	-	-
Ln-Transformed:							
AUC_{0-24} (hr*µg/mL)	41.6019	45.9599	90.52	22.98	80.66	101.58	0.9384
AUC_{0-t} (hr*µg/mL)	50.3122	52.6255	95.60	27.01	83.54	109.41	0.8613
$AUC_{0-\infty}$ (hr*µg/mL)	60.9957	61.8911	98.55	33.02	83.68	116.07	0.7286
C_{max} (µg/mL)	12.5098	18.0795	69.19	39.53	57.01	83.98	0.6007

1. Least-squares geometric means for Ln-transformed data.
2. Ratio calculated as Test 1 least-squares mean divided by the Reference least-squares mean.
3. Estimated intra-subject coefficient of variation, CV%=100*SQRT(e^{MSE}-1), where MSE is the mean square error term from the ANOVA.
4. Confidence interval on the ratio.

Reference (R): Reference formulation as single oral dose under **fasting** condition.
Test 1 (T1) : MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose under **fasting** conditions (NAXPM-20).
Test 2 (T2) : MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose under **fed** conditions (NAXPM-20).

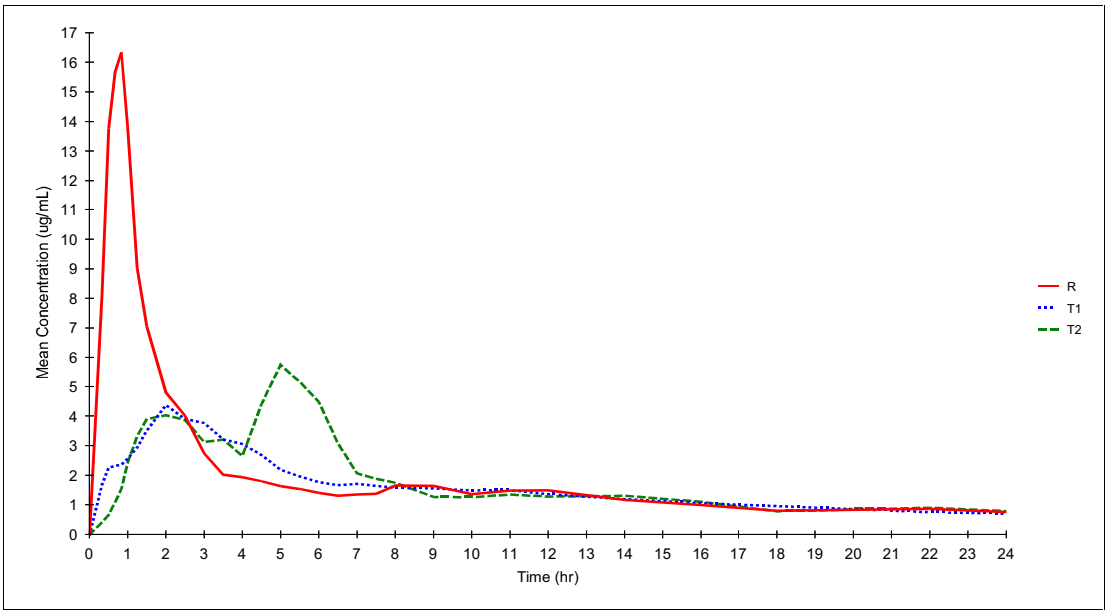


Figure 43: Mean Concentration *vs.* Time Graph (Linear Plot)

Again test samples clearly demonstrate a slower rate of absorption than that of the reference.

Steady State Simulations

Table 100: Simulated Pharmacokinetics of Mycophenolate Sodium for Test 1 (fasted) and Test 2 (fed) and Reference (fasted)

Summary of statistical comparisons of steady-state MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. (Test 1 and Test 2) given every 24 hr and Reference formulation given every 12 hr (Table 100).

Parameter	Test-1	Test-2	Ref.	Test-1/Ref. (%)	Test-2/Ref. (%)
Ln (AUC) *	52.3906	58.9704	57.7252	90.76	102.16
Ln ($AUC_{0-\infty}$) *	69.4991	88.9693	75.7080	91.80	117.52
Ln (C_{max}) *	6.3057	11.4942	7.8190	80.65	147.00
C_{min}(mcg/mL)	1.4676	1.6279	1.7494	83.89	93.05
T_{max} (hr.)#	2.0915	4.1060	1.1081	108.74	370.54
Fluctuation%	257.1298	475.1536	306.0926	84.00	155.23

Note: * Table provides least square mean for Test-1, Test-2 and Reference and Test-to-Reference ratios of least square mean for C_{min}, T_{max} and Fluctuation%. For Ln (AUC) $_{ss}$, Ln ($AUC_{0-\infty}$) $_{ss}$ and Ln (C_{max}) $_{ss}$ geometric least square means and Test-to-Reference ratios of geometric least-squares means are presented.

Least square mean for T_{max} at steady state for Ref. R, Test-1 and Test-2 was observed to be 193.1081, 290.0915 and 292.1060 hr.

The simulated Mycophenolate Sodium mean steady-state concentration chart for T1, T2 and R was shown in the Table 101 and 102, Figure 44.

Table 101: Bioequivalence Table Steady State (Test-1 *vs.* Ref. R)

	Unit	Test-1	Ref. R	Ratio%	CV%	90% CI		Power
						Lower	Upper	
Ln (AUC)	hr*µg /mL	52.3906	57.7252	90.76	35.21	76.28	107.99	0.6827
Ln ($AUC_{0-\infty}$)	hr*µg /mL	69.4991	75.7080	91.80	70.08	66.57	126.59	0.3082
Ln (C_{max})	µg/mL	6.3057	7.8190	80.65	38.67	66.70	97.50	0.6159

Table 102: Bioequivalence Table Steady State (Test-2 *vs.* Ref. R)

	Unit	Test-2	Ref. R	Ratio%	CV%	90% CI		Power
						Lower	Upper	
Ln (AUC)	hr*µg /mL	58.9704	57.7252	102.16	35.21	85.86	121.55	0.6827
Ln ($AUC_{0-\infty}$)	hr*µg /mL	88.9693	75.7080	117.52	70.08	85.22	162.05	0.3082
Ln (C_{max})	µg/mL	11.4942	7.8190	147.00	38.67	121.59	177.33	0.6159

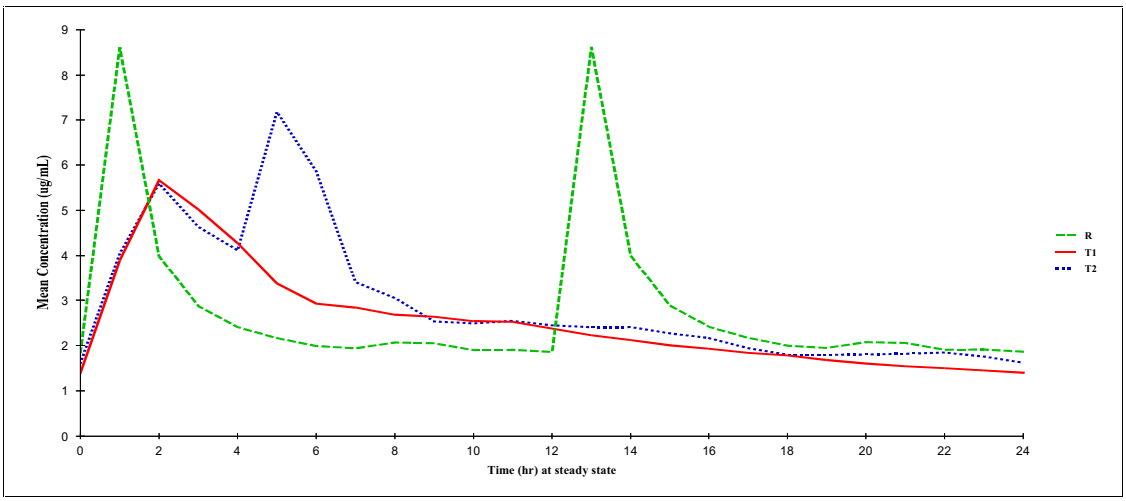


Figure 44: Simulated Mycophenolate Sodium mean concentration at steady state in for Test 1 (T1), Test 1 (T2) and Reference (R)

Test formulation in fasted state (Test 1) (NAXPM-20) had lower average C_{max} as desired. The value for C_{min} for NAXPM-20 in fasted state was found to be 1.467 µg/mL and T/R was found to be 0.84 which met the acceptance criteria. The value of T/R ratio for Ln (AUC) and Ln ($AUC_{0-\infty}$) was more than 0.9 (Table 101) depicting no significant loss of bioavailabilty. The mean percent steady-state fluctuation for Test 1 in fasted state (NAXPM-20) was less than reference (Table 100). From Table 102 it was observed that for test formulation (T2) (NAXPM-20) in fed condition ratio % and 90% CI for Ln (AUC) was meeting the pre-specified limits of bioequivalence *i.e.* 80% to 125%.

Effect of food on the pharmacokinetic parameters of NAXPM20

It could be concluded from Biostudy II that food had a significant effect on the values of C_{max} and AUC of modified release formulation. The values of C_{max} and AUC were significantly higher in case of fed state when compared with that of fasted state. The reason can be attributed to the increase residence time of the modified release tablet in the stomach and variation in absorption of drug from different sites. The variation in absorption in turn is due to pH dependent solubility of the drug.

Table 103: Concentration *vs.* Time Data for Reference (R), (Study-II, Batch No. U0389)

Sub.	Sequence	Reference (R) - Concentration (µg/mL)																
		Time (hr)																
		0.00	**0.33**	**0.50**	**0.67**	**0.83**	**1.00**	**1.25**	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**4.50**	**5.00**	**5.50**	**6.00**
S1	**T2T1R**	0.00	5.94	7.13	30.10	24.30	14.50	8.12	7.44	4.57	2.34	1.58	1.21	0.96	0.90	0.86	0.97	0.86
S2	**T1T2R**	0.00	3.98	12.40	13.10	8.16	9.08	8.22	5.65	4.26	1.52	1.07	1.01	1.49	1.29	1.62	1.41	1.04
S3	**RT1T2**	0.00	0.60	3.39	7.96	8.83	5.83	3.43	2.95	1.28	1.46	1.29	1.19	2.08	2.76	2.24	1.88	1.39
S4	**T1T2R**	0.00	2.59	4.82	7.20	10.10	8.11	7.74	6.63	5.52	5.04	5.53	5.83	4.99	2.43	1.82	1.91	1.69
S5	**T2RT1**	0.00	16.20	25.60	28.10	33.00	32.20	23.40	14.40	8.59	3.50	2.39	2.14	2.92	3.67	3.68	3.46	2.60
S6	**T2T1R**	0.00	12.20	17.90	10.50	9.83	5.88	4.22	4.07	2.50	2.23	1.23	0.82	0.50	0.76	0.38	0.45	0.52
S7	**T1RT2**	0.00	7.82	14.60	19.90	16.50	12.80	8.17	5.59	3.51	3.10	2.31	1.29	1.18	1.10	1.68	1.07	0.82
S8	**T2T1R**	0.00	9.20	10.50	14.90	10.50	7.38	5.89	5.36	3.67	1.53	1.03	0.98	0.66	0.70	0.59	0.55	0.40
S9	**T1RT2**	0.00	1.68	8.44	8.36	8.42	7.31	6.16	6.74	3.43	3.80	3.36	3.74	2.74	2.96	2.61	2.62	2.84
S10	**RT2T1**	0.00	30.30	29.70	19.90	11.60	8.13	6.29	4.85	3.80	2.83	2.72	2.58	2.96	4.04	3.00	2.73	2.59
S11	**T1T2R**	0.00	2.29	14.30	28.40	38.70	28.80	16.70	11.50	3.72	2.53	1.57	1.14	0.89	0.88	0.45	0.51	0.38
S12	**RT1T2**	0.00	25.20	24.70	17.20	13.50	12.20	8.12	6.86	5.09	3.13	2.76	1.80	1.43	1.70	1.32	1.02	1.03
S13	**RT2T1**	0.00	12.00	22.20	19.70	24.20	34.10	27.30	20.10	12.56	13.30	10.40	5.69	4.30	3.27	3.17	3.16	3.72
S15	**T2RT1**	0.00	0.20	1.20	1.55	2.72	2.71	2.35	2.33	4.49	5.52	3.56	2.16	2.38	2.29	1.76	1.60	1.38
S16	**RT1T2**	0.00	18.20	20.00	15.40	15.30	8.58	5.99	5.92	4.11	3.92	2.60	1.45	1.45	1.54	1.45	1.12	1.27
S17	**RT2T1**	0.00	19.10	26.00	25.10	24.30	19.80	10.30	6.88	3.70	3.45	1.13	0.66	0.57	0.57	0.53	0.50	0.36
S18	**T2RT1**	0.00	4.23	11.80	14.30	18.60	16.00	8.07	5.43	4.35	4.10	2.22	1.30	1.29	1.29	1.29	1.32	1.27
S19	**T1RT2**	0.00	4.47	11.50	15.30	18.30	16.40	8.40	5.69	4.57	4.27	2.29	1.25	1.25	1.22	1.22	1.27	1.11
S20	**T1T2R**	0.00	0.19	0.29	0.29	0.47	4.18	5.19	9.28	7.24	8.16	4.36	3.90	3.92	1.66	1.79	1.90	1.75
S21	**T2T1R**	0.00	0.22	12.30	15.20	20.80	15.20	8.93	5.72	4.86	4.33	2.20	1.47	1.51	1.43	1.32	1.28	1.24
S22	**RT1T2**	0.00	0.26	12.80	16.40	22.60	17.40	9.74	6.43	5.32	4.84	2.44	1.60	1.63	1.55	1.50	1.45	1.39
S24	**T2RT1**	0.00	1.67	11.20	15.90	18.70	18.00	5.84	5.39	4.38	3.27	2.25	1.18	1.43	1.56	1.62	1.66	1.28
N		**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**
Mean		0.0	8.1	13.8	15.7	16.3	13.8	9.0	7.1	4.8	4.0	2.7	2.0	1.9	1.8	1.6	1.5	1.4
SD		0.0	8.8	8.2	7.9	9.3	8.7	6.0	3.9	2.3	2.6	2.0	1.5	1.2	1.0	0.9	0.8	0.9
Min		0.0	0.2	0.3	0.3	0.5	2.7	2.4	2.3	1.3	1.5	1.0	0.7	0.5	0.6	0.4	0.4	0.4
Max		0.0	30.3	29.7	30.1	38.7	34.1	27.3	20.1	12.6	13.3	10.4	5.8	5.0	4.0	3.7	3.5	3.7
CV%		M	107.8	59.8	50.7	56.8	63.0	66.9	55.3	47.4	64.2	74.3	73.0	63.9	55.3	53.9	54.7	61.1

(Continued……)

Sub.	Sequence	Reference (R) - Concentration (µg/mL)														
		Time (hr)														
		6.50	7.00	7.50	8.00	9.00	10.00	11.00	12.00	14.00	16.00	18.00	22.00	24.00	36.00	48.00
S1	T2T1R	0.82	0.75	0.82	0.73	0.77	0.93	1.52	1.19	0.98	0.52	0.40	0.52	0.48	0.12	0.00
S2	T1T2R	1.24	1.34	1.02	1.12	1.61	2.06	1.25	2.94	0.98	0.85	0.95	1.12	0.87	0.27	0.19
S3	RT1T2	1.09	0.45	0.69	0.80	0.58	0.63	0.74	0.58	0.45	0.31	0.16	0.20	0.20	0.00	0.00
S4	T1T2R	1.70	1.77	1.74	1.60	1.26	1.05	1.18	0.97	0.79	0.22	0.54	0.62	0.56	0.40	0.18
S5	T2RT1	1.79	1.62	1.50	1.60	1.45	0.85	1.07	1.25	0.39	1.07	0.56	0.44	0.74	0.14	0.00
S6	T2T1R	1.37	3.19	3.21	2.85	2.09	1.46	1.27	1.16	0.74	0.95	0.66	0.57	0.21	0.00	0.00
S7	T1RT2	0.76	1.06	1.05	1.15	0.49	0.87	1.52	1.38	0.74	0.59	0.78	0.42	0.48	0.18	0.00
S8	T2T1R	0.33	0.31	0.27	0.25	0.51	0.91	1.07	1.04	0.88	0.65	0.54	0.48	0.52	0.31	M
S9	T1RT2	2.41	2.67	2.48	2.51	2.40	2.84	2.78	2.84	2.87	2.01	2.58	2.13	2.78	M	M
S10	RT2T1	3.04	3.05	2.97	3.90	3.46	2.57	2.74	2.45	2.21	2.21	2.19	2.35	2.26	1.86	1.80
S11	T1T2R	0.55	0.48	1.22	1.82	1.85	1.66	1.05	0.87	0.50	0.51	0.70	0.42	0.66	0.00	0.00
S12	RT1T2	1.06	0.73	0.54	0.51	0.63	1.01	0.95	1.06	0.59	0.67	0.47	1.35	0.47	0.33	0.29
S13	RT2T1	2.31	2.50	2.19	2.16	2.56	1.74	4.44	4.28	2.97	2.26	2.12	2.66	2.74	1.54	1.40
S15	T2RT1	1.05	1.28	1.03	0.88	1.15	1.45	1.46	1.88	1.63	0.63	0.67	0.77	0.55	0.23	0.00
S16	RT1T2	1.13	0.89	1.23	2.28	2.10	3.08	1.64	1.36	1.26	0.72	0.75	1.51	1.20	0.91	0.31
S17	RT2T1	0.38	0.84	0.29	0.00	0.37	0.35	0.44	0.57	0.35	0.29	0.27	0.39	0.42	0.17	0.00
S18	T2RT1	1.26	1.21	1.17	2.19	2.54	1.04	1.07	1.07	1.04	1.02	0.58	0.48	0.24	0.25	0.14
S19	T1RT2	1.19	1.15	1.05	1.87	2.21	0.90	0.90	0.95	0.88	0.84	0.47	0.39	0.21	0.19	0.12
S20	T1T2R	1.68	1.77	1.74	1.84	1.23	1.28	2.01	1.68	1.79	1.85	0.50	0.52	0.54	0.26	0.24
S21	T2T1R	1.05	0.87	1.28	2.07	2.28	1.13	1.17	1.14	1.18	1.16	0.54	0.49	0.18	0.25	0.16
S22	RT1T2	1.10	0.93	1.41	2.26	2.30	1.14	1.19	1.13	1.16	1.19	0.52	0.47	0.17	M	0.15
S24	T2RT1	1.32	0.88	1.35	1.85	2.31	0.97	1.17	1.02	1.20	1.30	0.63	0.56	0.20	0.22	0.00
N		22	22	22	22	22	22	22	22	22	22	22	22	22	20	20
Mean		1.30	1.35	1.37	1.65	1.64	1.36	1.48	1.49	1.16	0.99	0.80	0.86	0.76	0.38	0.25
SD		0.66	0.83	0.77	0.92	0.86	0.71	0.87	0.89	0.73	0.61	0.64	0.70	0.79	0.49	0.48
Min		0.33	0.31	0.27	0.00	0.37	0.35	0.44	0.57	0.35	0.22	0.16	0.20	0.17	0.00	0.00
Max		3.04	3.19	3.21	3.90	3.46	3.08	4.44	4.28	2.97	2.26	2.58	2.66	2.78	1.86	1.80
CV%		50.36	61.32	56.21	55.57	52.03	52.19	58.45	59.87	62.78	61.01	79.50	81.58	104.77	129.30	191.82

Table 104: Concentration *vs.* Time Data for Test (T1), (Study-II, Batch No. NAXPM-20)

Sub.	Sequence	Test 1 Formulation - Concentration (µg/mL)																
		Time (hr)																
		0.00	0.33	0.50	0.67	0.83	1.00	1.25	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00
S1	T2T1R	0.00	0.43	4.40	3.60	3.77	4.23	3.75	3.27	3.15	3.72	5.40	4.83	4.57	4.58	2.68	1.66	1.39
S2	T1T2R	0.00	0.43	0.23	0.17	0.12	0.10	0.40	0.94	3.41	2.92	2.80	2.70	2.35	1.99	2.27	1.91	2.41
S3	RT1T2	0.00	0.45	1.41	1.87	2.07	2.13	2.47	2.48	2.43	2.85	5.01	3.10	1.96	2.19	1.37	1.27	1.22
S4	T1T2R	0.00	1.27	1.28	1.08	1.11	1.13	1.20	2.52	4.57	4.36	4.00	4.32	4.57	5.62	3.69	3.02	2.97
S5	T2RT1	0.00	0.40	0.46	0.42	0.43	0.37	0.35	0.40	1.43	2.11	2.65	2.27	3.96	2.22	2.84	2.77	2.35
S6	T2T1R	0.00	6.49	5.86	6.29	4.14	4.38	2.93	3.37	3.07	3.39	3.12	3.20	3.64	3.26	3.46	2.68	2.60
S7	T1RT2	0.00	0.84	1.15	3.07	3.83	4.25	3.99	2.97	2.24	1.87	1.88	1.92	1.94	2.03	1.93	1.56	1.53
S8	T2T1R	0.00	0.11	0.22	0.82	1.36	2.81	3.17	3.08	2.01	1.30	1.81	1.60	1.10	1.22	0.79	0.78	0.71
S9	T1RT2	0.00	2.62	4.77	3.58	4.10	3.90	3.96	4.00	2.51	2.58	2.58	2.05	2.21	2.02	1.33	1.14	1.10
S10	RT2T1	0.00	4.14	2.53	2.57	3.13	2.84	2.35	2.01	2.13	2.09	8.03	9.01	8.04	6.71	3.98	4.22	4.14
S11	T1T2R	0.00	4.32	4.13	4.12	4.65	5.07	4.78	4.20	5.11	5.79	4.79	3.01	2.57	2.19	1.95	2.03	1.70
S12	RT1T2	0.00	0.62	0.85	0.73	0.55	0.33	0.52	0.39	0.41	0.26	5.29	7.13	6.05	4.73	2.37	1.53	1.17
S13	RT2T1	0.00	0.56	1.17	1.71	1.47	1.43	1.33	1.37	14.10	13.00	4.44	3.22	2.71	2.09	1.89	1.63	1.56
S15	T2RT1	0.00	3.15	3.49	3.20	3.38	3.92	3.78	3.64	5.25	5.04	4.77	4.45	3.66	2.02	1.75	1.41	1.11
S16	RT1T2	0.00	1.75	1.68	1.88	1.52	1.51	2.50	3.02	5.13	4.65	2.82	3.24	3.14	3.63	3.13	3.44	2.90
S17	RT2T1	0.00	4.83	4.82	3.84	3.96	3.25	2.98	2.34	0.96	0.93	0.94	1.32	1.18	0.97	0.62	0.73	0.88
S18	T2RT1	0.00	0.99	2.56	2.72	2.72	3.15	3.99	6.10	6.50	5.10	4.08	2.19	2.28	2.16	2.21	2.07	1.49
S19	T1RT2	0.00	0.98	2.52	2.69	2.75	3.09	4.00	5.93	6.27	4.44	4.11	2.24	2.16	2.28	2.25	2.07	1.63
S20	T1T2R	0.00	0.95	2.38	2.47	2.47	2.73	3.47	5.81	5.85	4.60	3.83	2.12	2.15	2.09	2.15	1.97	1.57
S21	T2T1R	0.00	1.01	1.82	2.03	1.95	2.46	4.13	5.04	5.38	4.00	3.01	1.57	1.55	1.61	1.62	1.65	1.04
S22	RT1T2	0.00	0.95	1.55	1.72	1.78	2.22	3.50	5.29	6.21	4.56	3.19	1.59	1.73	1.84	1.87	1.86	1.27
S24	T2RT1	0.00	0.00	0.29	0.33	0.48	1.01	5.16	9.02	8.01	6.24	4.30	3.55	3.69	1.62	1.83	1.70	2.12
N		22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
Mean		0.00	1.70	2.25	2.31	2.35	2.56	2.94	3.51	4.37	3.90	3.77	3.21	3.06	2.69	2.18	1.96	1.77
SD		0.00	1.78	1.67	1.48	1.38	1.43	1.41	2.09	2.99	2.58	1.53	1.87	1.66	1.47	0.86	0.84	0.83
Min		0.00	0.00	0.22	0.17	0.12	0.10	0.35	0.39	0.41	0.26	0.94	1.32	1.10	0.97	0.62	0.73	0.71
Max		0.00	6.49	5.86	6.29	4.65	5.07	5.16	9.02	14.10	13.00	8.03	9.01	8.04	6.71	3.98	4.22	4.14
CV%		M	104.98	73.88	63.98	58.84	55.98	47.82	59.50	68.31	66.25	40.54	58.22	54.37	54.84	39.34	42.73	47.09

(Continued……)

Sub.	Sequence	Test 1 Formulation - Concentration (µg/mL)														
		Time (hr)														
		6.50	7.00	7.50	8.00	9.00	10.00	11.00	12.00	14.00	16.00	18.00	22.00	24.00	36.00	48.00
S1	T2T1R	0.92	0.91	0.93	0.96	0.99	1.01	1.33	1.39	1.30	0.50	0.45	0.69	0.62	0.28	0.14
S2	T1T2R	2.09	2.11	1.95	1.51	1.18	0.88	0.81	1.13	1.07	0.80	0.83	0.60	0.35	0.78	0.00
S3	RT1T2	1.23	1.45	1.26	0.71	0.51	0.46	0.30	0.31	0.27	0.21	0.60	0.15	0.14	0.00	0.00
S4	T1T2R	2.60	2.00	1.69	1.51	1.17	1.25	2.52	1.97	2.03	1.83	1.26	1.21	0.77	0.41	0.21
S5	T2RT1	2.26	2.65	2.51	2.98	2.57	2.42	2.06	1.75	0.99	1.07	0.56	0.89	0.85	0.20	0.00
S6	T2T1R	2.05	1.70	1.34	1.15	1.19	1.40	2.31	2.30	1.23	0.41	0.80	0.73	1.05	0.99	0.14
S7	T1RT2	1.46	1.94	2.15	2.00	2.00	1.72	1.20	0.85	0.57	0.39	0.39	0.31	0.41	0.14	0.00
S8	T2T1R	0.72	0.72	0.79	0.69	0.64	1.79	1.08	0.99	0.78	0.80	0.80	0.69	0.67	0.39	0.24
S9	T1RT2	1.37	1.32	1.06	1.01	1.19	0.96	1.10	0.89	0.61	0.88	0.62	0.94	0.74	M	M
S10	RT2T1	3.71	4.39	3.81	3.58	3.55	2.56	2.21	2.64	2.89	2.40	2.93	2.92	2.53	2.41	1.89
S11	T1T2R	1.64	1.62	1.84	1.94	1.21	0.82	0.61	0.51	0.65	0.33	0.36	0.45	0.40	0.43	0.30
S12	RT1T2	1.01	0.79	0.79	0.67	1.68	1.57	1.49	0.80	1.03	0.71	0.51	0.94	0.87	0.61	0.16
S13	RT2T1	1.40	1.66	1.51	1.41	2.12	2.09	2.37	2.06	1.63	1.41	1.36	1.41	1.54	1.26	1.09
S15	T2RT1	1.01	0.95	0.89	1.00	1.44	1.43	1.23	0.79	0.88	0.52	0.47	0.49	0.31	0.28	0.26
S16	RT1T2	2.56	2.72	2.32	2.59	2.69	2.22	2.22	1.92	1.59	0.65	0.76	0.43	0.47	0.49	0.40
S17	RT2T1	0.81	0.79	0.68	0.85	0.78	0.65	0.73	0.48	0.37	0.35	0.40	0.59	0.27	0.12	0.00
S18	T2RT1	1.67	1.65	1.97	1.75	1.75	1.73	1.36	1.46	1.31	1.57	1.46	0.50	0.55	M	M
S19	T1RT2	1.75	1.85	2.08	1.93	1.86	1.92	1.43	1.53	1.38	1.64	1.50	0.49	0.54	0.22	0.24
S20	T1T2R	1.67	1.65	1.85	1.73	1.73	1.66	1.38	1.41	1.31	1.50	1.38	0.47	0.53	0.23	M
S21	T2T1R	1.27	1.29	1.35	1.30	1.28	1.33	1.31	1.48	1.36	1.46	1.45	0.50	0.56	0.21	M
S22	RT1T2	1.36	1.49	1.45	1.53	1.47	1.47	1.47	1.64	1.49	1.62	1.55	0.55	0.62	M	0.27
S24	T2RT1	2.01	1.93	1.91	1.88	1.22	1.30	2.27	1.69	1.49	2.14	0.56	0.59	0.55	0.32	0.21
N		22	22	22	22	22	22	22	22	22	22	22	22	22	19	18
Mean		1.66	1.71	1.64	1.58	1.56	1.48	1.49	1.36	1.19	1.05	0.96	0.75	0.70	0.51	0.31
SD		0.70	0.81	0.72	0.75	0.71	0.56	0.63	0.62	0.58	0.64	0.61	0.56	0.51	0.56	0.47
Min		0.72	0.72	0.68	0.67	0.51	0.46	0.30	0.31	0.27	0.21	0.36	0.15	0.14	0.00	0.00
Max		3.71	4.39	3.81	3.58	3.55	2.56	2.52	2.64	2.89	2.40	2.93	2.92	2.53	2.41	1.89
CV%		42.21	47.48	43.83	47.59	45.88	37.70	42.18	45.33	48.84	60.81	63.79	75.07	72.36	108.18	151.39

Table 105: Concentration *vs.* Time Data for Test (T2), (Study-II, Batch No. NAXPM-20)

Sub.	Sequence	Test 2 Formulation - Concentration (µg/mL)																
		Time (hr)																
		0.00	**0.33**	**0.50**	**0.67**	**0.83**	**1.00**	**1.25**	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**4.50**	**5.00**	**5.50**	**6.00**
S1	**T2T1R**	0.00	0.33	0.22	0.18	0.15	0.15	0.11	0.11	0.13	0.32	0.47	0.51	0.61	6.59	6.11	7.36	4.42
S2	**T1T2R**	0.00	0.24	0.27	0.21	0.21	0.24	0.44	0.56	0.00	0.45	0.44	0.47	1.02	12.10	6.55	4.32	3.52
S3	**RT1T2**	0.00	0.76	1.21	2.31	3.28	4.74	6.27	7.88	6.43	4.86	5.16	5.48	4.17	3.18	1.31	0.74	0.70
S4	**T1T2R**	0.00	0.40	0.38	0.47	0.49	0.62	0.87	1.44	12.30	13.98	12.60	7.94	5.59	6.00	4.01	3.54	1.66
S5	**T2RT1**	0.00	0.11	0.15	0.16	0.16	0.15	0.31	0.73	0.57	0.42	0.34	0.27	0.20	3.44	32.30	15.90	7.78
S6	**T2T1R**	0.00	0.00	0.00	0.00	0.22	0.29	0.23	0.13	0.39	0.48	0.55	0.58	1.74	4.86	6.76	13.60	10.20
S7	**T1RT2**	0.00	0.50	0.42	0.57	1.11	1.51	2.62	2.98	9.83	9.69	11.40	11.40	4.16	2.35	1.58	0.78	0.51
S8	**T2T1R**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.46	0.51	0.49	0.77	7.65	6.27	6.85	4.39
S9	**T1RT2**	0.00	1.70	1.51	1.54	1.94	1.72	2.00	2.54	1.79	1.62	1.67	1.59	1.50	1.65	1.74	1.89	1.96
S10	**RT2T1**	0.00	1.94	2.45	1.82	2.22	1.85	1.72	1.75	2.11	2.94	2.64	2.61	4.76	4.51	4.21	4.88	9.91
S11	**T1T2R**	0.00	0.00	0.00	0.41	0.47	0.34	0.36	0.70	0.53	0.40	0.17	0.00	0.00	0.24	3.67	5.50	20.30
S12	**RT1T2**	0.00	1.34	1.19	1.03	1.43	1.20	1.19	0.90	0.86	0.95	0.97	2.59	5.51	18.10	12.00	6.58	3.67
S13	**RT2T1**	0.00	0.16	0.13	0.15	0.21	0.30	0.44	0.38	0.28	0.42	0.48	0.51	0.58	0.74	8.79	15.10	9.24
S15	**T2RT1**	0.00	0.24	0.62	1.93	3.54	3.84	5.22	5.78	4.96	4.56	5.76	15.60	6.84	3.59	1.87	1.09	0.82
S16	**RT1T2**	0.00	0.00	0.29	0.30	0.43	2.03	14.70	14.00	6.90	4.03	2.44	1.65	0.91	1.09	1.06	1.27	1.21
S17	**RT2T1**	0.00	0.00	0.00	0.13	0.39	0.47	1.18	1.04	1.67	2.41	2.21	3.40	3.58	7.17	10.80	4.20	2.70
S18	**T2RT1**	0.00	0.23	0.22	0.26	0.35	0.43	0.58	0.41	0.30	0.24	0.25	0.21	0.91	6.47	10.10	10.60	7.43
S19	**T1RT2**	0.00	0.17	0.26	0.27	0.43	3.94	5.10	5.71	7.58	8.93	3.87	3.84	3.76	1.55	1.46	1.68	1.79
S20	**T1T2R**	0.00	0.37	4.23	11.10	15.20	18.40	15.00	7.46	5.38	4.53	4.27	2.45	1.38	1.41	1.44	1.32	1.37
S21	**T2T1R**	0.00	0.20	0.27	0.33	0.49	3.95	4.33	10.70	9.20	8.10	3.77	2.62	3.07	1.15	1.36	1.27	1.26
S22	**RT1T2**	0.00	0.23	0.32	0.34	0.56	4.48	4.94	11.50	9.65	9.08	4.21	2.84	3.55	1.31	1.44	1.46	1.45
S24	**T2RT1**	0.00	0.00	0.33	0.33	0.47	2.71	5.48	9.08	7.61	6.14	4.52	3.52	3.68	1.66	1.71	3.60	2.25
N		**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**	**22**
Mean		0.00	0.41	0.66	1.08	1.53	2.43	3.32	3.90	4.03	3.86	3.12	3.21	2.65	4.40	5.75	5.16	4.48
SD		0.00	0.55	1.00	2.34	3.21	3.91	4.27	4.36	4.02	3.95	3.40	3.90	2.01	4.24	6.87	4.72	4.73
Min		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.17	0.00	0.00	0.24	1.06	0.74	0.51
Max		0.00	1.94	4.23	11.10	15.20	18.40	15.00	14.00	12.30	13.98	12.60	15.60	6.84	18.10	32.30	15.90	20.30
CV%		M	136.11	151.78	215.67	209.41	161.07	128.46	111.72	99.66	102.09	108.89	121.71	75.93	96.44	119.42	91.50	105.55

(Continued……)

Sub.	Sequence	Test 2 Formulation - Concentration (µg/mL)														
		Time (hr)														
		6.50	7.00	7.50	8.00	9.00	10.00	11.00	12.00	14.00	16.00	18.00	22.00	24.00	36.00	48.00
S1	T2T1R	2.68	1.55	1.09	0.79	0.49	0.38	0.32	0.32	0.40	0.42	0.47	0.49	0.38	0.27	0.00
S2	T1T2R	2.11	1.54	1.63	0.95	0.72	0.71	0.89	1.09	0.93	0.89	0.61	0.64	0.69	0.41	0.14
S3	RT1T2	0.49	0.46	0.46	0.56	0.73	0.82	0.46	0.36	0.29	0.26	0.15	0.27	0.16	0.00	0.00
S4	T1T2R	1.32	1.03	0.93	0.74	0.43	0.52	0.37	0.58	1.50	0.74	0.74	1.19	0.76	0.33	0.29
S5	T2RT1	4.67	3.43	2.14	1.70	1.65	1.30	1.33	1.45	1.93	0.97	0.91	1.19	0.49	0.25	0.15
S6	T2T1R	5.73	2.88	2.03	1.25	1.26	0.82	1.96	2.27	1.79	1.40	0.41	1.29	0.99	0.28	0.24
S7	T1RT2	0.58	0.39	0.28	0.25	0.63	1.21	1.74	0.85	0.45	0.52	0.47	0.34	0.14	0.00	0.00
S8	T2T1R	4.18	2.47	0.80	0.56	0.29	0.23	0.37	0.56	0.68	0.64	0.30	0.85	0.51	M	M
S9	T1RT2	2.15	2.48	9.27	12.50	5.90	3.64	2.73	2.25	2.91	2.86	2.27	1.98	2.59	1.76	0.00
S10	RT2T1	8.86	7.02	5.34	3.32	2.41	2.70	3.05	2.79	3.27	2.50	2.12	2.62	2.35	2.15	1.88
S11	T1T2R	13.70	6.31	3.32	2.00	1.20	1.33	1.26	0.91	1.35	0.79	0.85	0.32	0.48	0.21	M
S12	RT1T2	2.10	1.87	1.56	1.55	1.42	1.55	1.66	1.97	2.17	1.76	1.48	1.64	1.37	1.20	0.95
S13	RT2T1	3.94	1.84	0.99	0.64	0.33	0.34	0.46	0.63	0.54	0.14	0.50	0.40	0.60	0.52	0.00
S15	T2RT1	0.59	0.53	0.64	0.68	0.94	1.18	1.19	1.10	0.92	0.41	0.42	0.70	1.13	0.29	0.13
S16	RT1T2	1.43	1.33	1.46	1.76	1.98	2.22	2.19	1.71	1.45	1.25	1.15	1.22	1.10	0.47	0.29
S17	RT2T1	1.66	0.60	0.46	0.54	0.51	0.63	0.64	0.57	0.65	0.95	0.63	0.52	0.33	0.15	0.00
S18	T2RT1	3.83	2.17	1.64	1.27	0.91	1.21	1.06	1.81	1.00	0.70	0.51	1.00	0.56	0.22	0.14
S19	T1RT2	1.69	1.72	1.68	1.78	1.18	1.17	1.98	1.69	1.79	1.68	0.54	0.52	0.55	0.28	0.24
S20	T1T2R	1.31	0.99	1.32	1.22	2.29	2.65	1.06	1.14	1.09	1.10	1.06	0.96	0.51	0.23	0.26
S21	T2T1R	1.25	1.32	1.17	1.18	0.65	0.83	1.29	1.07	0.97	1.19	0.49	0.53	0.55	0.28	0.23
S22	RT1T2	1.42	1.44	1.35	1.30	0.67	0.91	1.49	1.20	1.14	1.26	0.50	0.58	0.60	0.30	0.27
S24	T2RT1	2.00	1.95	1.78	1.89	1.24	1.25	2.10	1.82	1.46	1.88	0.55	0.59	0.52	M	0.22
N		22	22	22	22	22	22	22	22	22	22	22	22	22	20	20
Mean		3.08	2.06	1.88	1.75	1.27	1.26	1.35	1.28	1.30	1.11	0.78	0.90	0.79	0.48	0.27
SD		3.09	1.69	1.98	2.50	1.20	0.85	0.78	0.68	0.78	0.70	0.55	0.59	0.62	0.56	0.43
Min		0.49	0.39	0.28	0.25	0.29	0.23	0.32	0.32	0.29	0.14	0.15	0.27	0.14	0.00	0.00
Max		13.70	7.02	9.27	12.50	5.90	3.64	3.05	2.79	3.27	2.86	2.27	2.62	2.59	2.15	1.88
CV%		100.43	81.93	105.13	142.95	94.77	68.10	57.59	53.41	59.58	62.99	70.52	65.52	79.08	117.42	159.85

4.3.4 Randomized, single dose, crossover comparative bioavailability study under fasting and fed conditions (Study-III)

A randomized, open label, balanced, three treatment, three period, six sequence, single dose, three way crossover comparative bioavailability study of test MR formulations (NAXPM19) of Mycophenolate Sodium of Panacea Biotec Ltd. under fasting (T1) and fed (T2) conditions with reference formulation under fasting condition was performed in 18+6 (Standby) healthy human adult male subjects. The pharmacokinetic parameters are summarized in Table 106. These pharmacokinetic parameters have been obtained by subjecting the plasma concentration time data to Non-Compartmental analysis using WinNonlin 5.2 software. The data plasma concentration *vs* time has been summarized in Table 113, 114 and 115.

Table 106: PK Parameters Table

Sub.	Sequence	C_{max} (µg/mL)			T_{max} (hr)			AUC_{0-t} (hr*µg/mL)			$AUC_{0-\infty}$ (hr*µg/mL)			λ_z (1/hr)			$T_{½}$ (hr)		
		R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2
S1	T2T1R	14.30	6.83	15.00	1.50	0.50	1.50	43.49	46.88	41.16	45.95	49.53	43.64	0.104	0.096	0.072	6.69	7.24	9.61
S2	T1T2R	20.40	10.70	16.90	0.33	2.00	2.50	53.03	53.41	58.40	59.62	56.05	61.31	0.049	0.103	0.058	14.05	6.73	11.87
S3	RT1T2	17.50	3.40	7.71	0.67	0.33	2.50	38.59	29.22	37.63	49.76	34.33	68.47	0.036	0.053	0.012	19.31	13.13	58.74
S4	T1T2R	11.20	3.18	12.70	0.83	0.67	1.50	38.80	35.73	47.27	44.38	38.96	53.12	0.110	0.061	0.066	6.30	11.35	10.58
S5	T2RT1	19.00	4.62	9.40	0.50	2.00	4.50	38.14	33.84	23.89	42.91	42.93	26.79	0.067	0.044	0.276	10.34	15.71	2.52
S6	T2T1R	15.10	6.33	6.64	0.83	1.50	2.00	33.25	36.39	30.37	35.51	40.97	32.14	0.089	0.084	0.136	7.78	8.27	5.10
S7	T1RT2	30.50	3.48	12.30	0.50	0.83	5.50	54.68	37.49	45.49	57.59	40.79	48.85	0.081	0.078	0.073	8.56	8.84	9.50
S8	T2T1R	45.00	5.94	7.14	0.50	0.67	2.00	41.74	19.42	32.91	44.71	20.03	35.98	0.147	0.179	0.083	4.73	3.87	8.39
S9	T1RT2	5.74	31.50	14.50	1.50	0.50	3.00	51.84	65.95	55.57	55.57	70.01	57.62	0.066	0.072	0.084	10.53	9.61	8.25
S10	RT2T1	27.40	6.72	12.40	1.00	1.25	6.00	70.78	78.28	62.09	73.52	81.23	65.28	0.062	0.078	0.072	11.20	8.95	9.67
S11	T1T2R	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
S12	RT1T2	19.80	3.33	8.03	0.50	2.00	5.50	35.47	27.31	26.37	37.19	33.07	27.62	0.063	0.066	0.111	10.93	10.48	6.27
S13	RT2T1	19.90	10.40	7.08	1.00	0.83	6.50	42.88	21.81	23.60	44.34	24.95	27.58	0.084	0.073	0.046	8.24	9.46	15.10
S14	T1RT2	22.90	8.41	26.20	0.83	0.83	5.00	55.92	32.07	39.11	59.12	33.65	46.71	0.069	0.086	0.043	10.03	8.05	15.96
S15	T2RT1	16.30	4.78	8.91	0.33	1.50	3.00	52.80	23.25	40.82	53.56	36.78	43.07	0.171	0.050	0.091	4.04	13.77	7.60
S16	RT1T2	14.70	8.80	4.63	1.00	1.25	1.50	39.36	39.07	36.70	41.31	48.27	39.57	0.059	0.029	0.052	11.78	23.98	13.25
S17	RT2T1	19.50	13.70	14.60	1.25	1.25	6.50	67.27	90.77	72.99	69.11	96.78	74.36	0.072	0.062	0.101	9.63	11.14	6.84
S18	T2RT1	21.70	3.73	16.60	1.25	7.00	5.00	45.10	35.65	44.24	48.00	38.15	47.28	0.078	0.088	0.080	8.91	7.91	8.64
S19	T1RT2	18.10	5.18	10.10	0.67	0.50	1.25	57.93	33.97	33.76	62.75	36.48	44.17	0.083	0.074	0.059	8.34	9.39	11.79

Sub.	Sequence	C_{max} (µg/mL)			T_{max} (hr)			AUC_{0-t} (hr*µg/mL)			$AUC_{0-\infty}$ (hr*µg/mL)			λ_z (1/hr)			$T_{½}$ (hr)		
		R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2	R	T1	T2
S20	**T1T2R**	35.50	14.10	19.50	1.25	1.50	1.50	135.20	59.01	105.03	137.35	64.18	142.77	0.094	0.103	0.023	7.41	6.73	30.63
S21	**T2T1R**	17.60	22.70	9.73	0.83	1.50	4.00	47.21	116.24	39.43	48.80	142.26	46.47	0.098	0.027	0.042	7.07	26.07	16.37
S22	**RT1T2**	32.10	3.49	26.70	0.50	2.00	4.50	88.92	52.54	73.35	98.21	67.68	81.92	0.056	0.037	0.048	12.40	18.56	14.52
S23	**RT2T1**	16.20	2.88	11.80	0.67	2.00	4.50	38.87	34.07	76.82	48.33	36.15	85.41	0.027	0.066	0.046	26.13	10.47	14.96
S24	**T2RT1**	45.40	6.65	20.50	3.00	3.00	6.50	92.26	60.06	51.28	100.32	67.28	55.10	0.048	0.101	0.114	14.43	6.86	6.10
N		**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**
Mean		21.99	8.30	13.00	0.92	1.54	3.75	54.94	46.19	47.75	59.04	52.20	54.58	0.079	0.074	0.078	10.38	11.16	13.14
SD		9.89	6.89	5.96	0.57	1.36	1.85	23.54	23.68	19.79	23.91	27.11	25.32	0.033	0.032	0.052	4.81	5.42	11.43
Min		5.74	2.88	4.63	0.33	0.33	1.25	33.25	19.42	23.60	35.51	20.03	26.79	0.027	0.027	0.012	4.04	3.87	2.52
Max		45.40	31.50	26.70	3.00	7.00	6.50	135.20	116.24	105.03	137.35	142.26	142.77	0.171	0.179	0.276	26.13	26.07	58.74
CV%		44.97	82.97	45.83	61.66	88.63	49.44	42.85	51.26	41.45	40.50	51.94	46.40	41.750	42.830	67.230	46.30	48.61	86.94

Table 107: ANOVA Table

Parameter	Unit	Effects	P-value
$Ln(C_{max})$	ug/mL	Sequence	0.328
	ug/mL	Formulation	0.000
	ug/mL	Period	0.247
$Ln(AUC_{0-24})$	hr*ug/mL	Sequence	0.938
	hr*ug/mL	Formulation	0.010
	hr*ug/mL	Period	0.634
$Ln(AUC_{0-t})$	hr*ug/mL	Sequence	0.906
	hr*ug/mL	Formulation	0.027
	hr*ug/mL	Period	0.737
$Ln(AUC_{0-\infty})$	hr*ug/mL	Sequence	0.951
	hr*ug/mL	Formulation	0.129
	hr*ug/mL	Period	0.796

On applying analysis of variance (Table 107), it was observed that no statistically significant effect ($P>0.05$) was observed, due to periods for $Ln(C_{max})$, $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$. Formulation effect was statistically significant for $Ln(C_{max})$, $Ln(AUC_{0-24})$ and $Ln(AUC_{0-t})$ ($P<0.05$) but for $Ln(AUC_{0-\infty})$ it was found to be non significant ($P>0.05$). No significant difference ($p>0.10$) was observed due to sequence for $Ln(C_{max})$, $Ln(AUC_{0-24})$, $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$. The results of the statistical comparisons performed on the single-dose pharmacokinetic parameter estimates are summarized in Table 108 (Test-1, fasting) (NAXPM-19) and in Table 109 (Test-2, fed) (NAXPM-19).

The values of $AUC_{0-24,}$ AUC_{0-t} and $AUC_{0-\infty}$ for the test1 (NAXPM-19) in fasting condition were found to be 19.90%, 19.45% and 15.53 % lower than that of reference respectively (Table 108). While in case of test 2 (NAXPM-19) under fed condition, the values were less than 15%lower than that of reference (Table 109). As expected for modified-release products, Test 1 (T1, fast) (NAXPM-19) and Test 2 (T2, fed) (NAXPM-19) both had lower peak concentrations and longer times to peak concentration compared to the reference (Table 106). On observing food effect it was found that for T2 (in fed condition) C_{max} and T_{max} were on the higher side as compared to T1 (in fasting condition). The mean concentration vs. time chart on linear scale was shown in the Figure 45.

Table 108: Comparison of Pharmacokinetic parameters of Test 1 and Reference

Parameter	Least-Squares Means [1]		Ratio% [2]	CV% [3]	90% Confidence Interval [4]		Power
	Test 1 (T1, fast)	Reference (R)			Lower	Upper	
AUC_{0-24} (hr*µg/mL)	38.9888	46.7822	83.34	-	70.8	95.88	-
AUC_{0-t} (hr*µg/mL)	45.6574	54.4996	83.78	-	69.11	98.44	-
$AUC_{0-\infty}$ (hr*µg/mL)	51.6037	58.594	88.07	-	71.90	104.24	-
C_{max} (µg/mL)	8.0354	21.8214	36.82	-	19.56	54.09	-
λ_z (1/hr)	0.0752	0.0793	94.87	-	-	-	-
$T_{1/2}$ (hr)	11.0268	10.2613	107.46	-	-	-	-
Ln-Transformed:							
AUC_{0-24} (hr*µg/mL)	35.5266	44.3546	80.10	24.05	71.20	90.11	0.9301
AUC_{0-t} (hr*µg/mL)	41.1345	51.0663	80.55	26.88	70.65	91.84	0.8775
$AUC_{0-\infty}$ (hr*µg/mL)	46.6747	55.2546	84.47	27.93	73.72	96.79	0.8558
C_{max} (µg/mL)	6.4897	19.8541	32.69	55.70	25.25	42.31	0.4119

1. Least-squares geometric means for Ln-transformed data.
2. Ratio calculated as Test 1 least-squares mean divided by the Reference least-squares mean.
3. Estimated intra-subject coefficient of variation, CV%=100*SQRT (e^{MSE}-1), where MSE is the mean square error term from the ANOVA.
4. Confidence interval on the ratio.

Reference (R): Reference formulation as single oral dose under **fasting** condition.
Test 1 (T1): MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose under **fasting** conditions (NAXPM-19).
Test 2 (T2): MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose under **fed** conditions (NAXPM-19).

Table 109: Comparison of Pharmacokinetic parameters of Test 2 and Reference

Parameter	Least-Squares Means [1]		Ratio% [2]	CV% [3]	90% Confidence Interval [4]		Power
	Test 2 (T2, fed)	Reference (R)			Lower	Upper	
AUC_{0-24} (hr*µg/mL)	40.1176	46.7822	85.75	-	73.21	98.29	-
AUC_{0-t} (hr*µg/mL)	47.2776	54.4996	86.75	-	72.08	101.42	-
$AUC_{0-\infty}$ (hr*µg/mL)	54.0578	58.594	92.26	-	76.09	108.43	-
C_{max} (µg/mL)	12.9032	21.8214	59.13	-	41.87	76.4	-
λ_z (1/hr)	0.0783	0.0793	98.76	-	-	-	-
$T_{1/2}$ (hr)	13.0038	10.2613	126.73	-	-	-	-
Ln-Transformed:							
AUC_{0-24} (hr*µg/mL)	38.1323	44.3546	85.97	24.05	76.42	96.72	0.9301
AUC_{0-t} (hr*µg/mL)	43.9794	51.0663	86.12	26.88	75.53	98.2	0.8775
$AUC_{0-\infty}$ (hr*µg/mL)	49.842	55.2546	90.20	27.93	78.72	103.36	0.8558
C_{max} (µg/mL)	11.7364	19.8541	59.11	55.70	45.66	76.52	0.4119

1. Least-squares geometric means for Ln-transformed data.
2. Ratio calculated as Test 1 least-squares mean divided by the Reference least-squares mean.
3. Estimated intra-subject coefficient of variation, CV%=100*SQRT(e^{MSE}-1), where MSE is the mean square error term from the ANOVA.
4. Confidence interval on the ratio.

Reference (R): Reference formulation as single oral dose under **fasting** condition.
Test 1 (T1): MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose under **fasting** conditions (NAXPM-19).
Test 2 (T2): MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. as single oral dose under **fed** conditions(NAXPM-19).

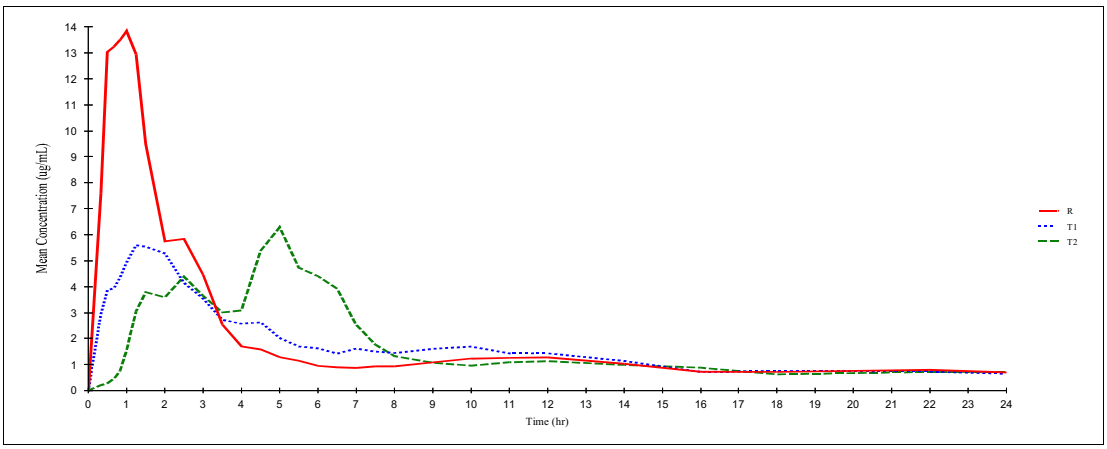


Figure 45: Mean Concentration *vs*. Time Graph (Linear Plot)

Once again the test formulation demonstrated a slower rate of absorption than that of the reference.

Steady State Simulations

Summary of statistical comparisons of steady-state MR tablet of Mycophenolate Sodium of Panacea Biotec Ltd. (Test 1 and Test 2) given every 24 hr and Reference formulation given every 12 hr.

Table 110: Simulated Pharmacokinetics of Mycophenolate Sodium for Test 1 and Test 2 and Reference

Parameter	Test-1	Test-2	Ref.	Test-1/Ref. (%)	Test-2/Ref. (%)
Ln (AUC) *	44.8363	48.2635	50.2602	89.21	96.03
Ln ($AUC_{0-\infty}$) *	57.6438	62.6094	60.0245	96.03	104.31
Ln (C_{max}) *	6.3201	10.1610	7.8961	80.04	128.68
C_{min}(µg/mL)	0.7590	0.7908	1.0000	75.91	79.08
T_{max}(hr.)#	1.7054	3.7925	1.1410	149.46	332.38
Fluctuation%	332.5767	507.2282	351.7084	94.56	144.22

Note: * Table provides least square mean for Test-1, Test-2 and Reference and Test-to-Reference ratios of least square mean for C_{min}, T_{max} and Fluctuation%. For Ln (AUC) $_{ss}$, Ln ($AUC_{0-\infty}$) $_{ss}$ and Ln (C_{max}) $_{ss}$ geometric least square means and Test-to-Reference ratios of geometric least-squares means are presented.

Least square mean for T_{max} at steady state for Ref. R, Test-1 and Test-2 was observed to be 181.1410, 217.7054 and 219.7925 hr.

Table 111 Bioequivalence Table Steady State (Test-1 *vs.* Ref. R)

	Unit	Test-1	Ref. R	Ratio%	CV%	90% CI		Power
						Lower	Upper	
Ln (AUC)	hr*µg/mL	44.8363	50.2602	89.21	27.83	77.89	102.17	0.8581
Ln ($AUC_{0-\infty}$)	hr*µg/mL	57.6438	60.0245	96.03	42.27	78.52	117.46	0.5722
Ln (C_{max})	µg/mL	6.3201	7.8961	80.04	45.55	64.52	99.30	0.5240

Table 112: Bioequivalence Table Steady State (Test-2 *vs.* Ref. R)

	Unit	Test-2	Ref. R	Ratio%	CV%	90% CI		Power
						Lower	Upper	
Ln(AUC)	hr*µg/mL	48.2635	50.2602	96.03	27.83	83.85	109.98	0.8581
Ln($AUC_{0-\infty}$)	hr*µg/mL	62.6094	60.0245	104.31	42.27	85.28	127.57	0.5722
Ln(C_{max})	µg/mL	10.1610	7.8961	128.68	45.55	103.72	156.65	0.5240

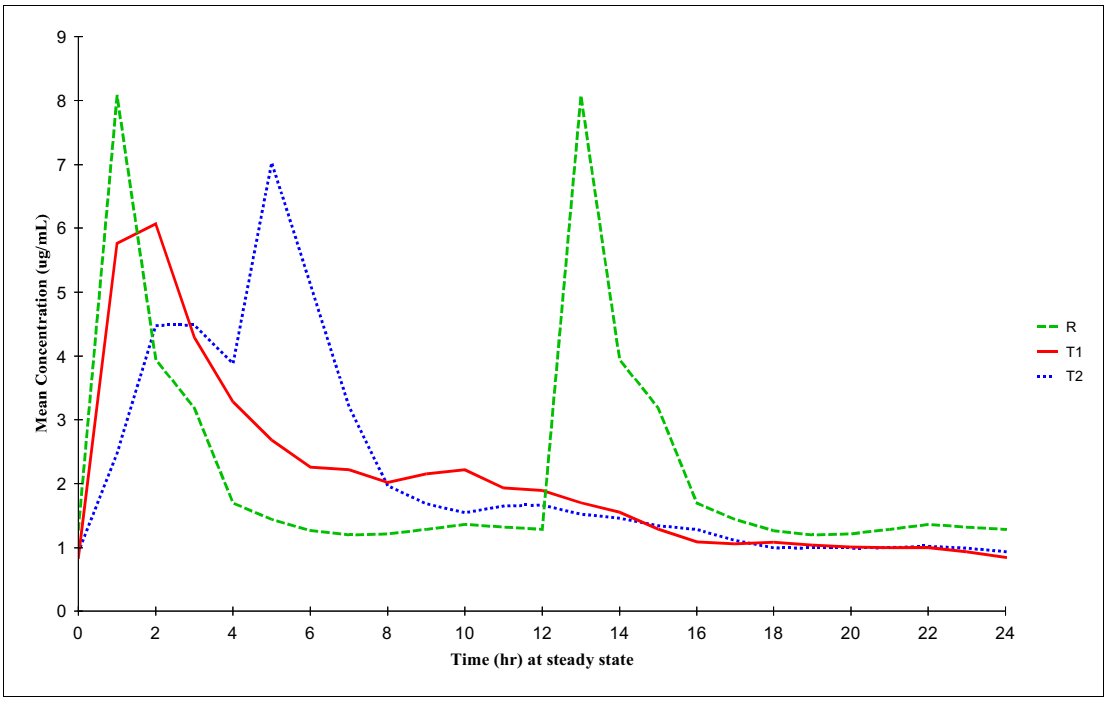


Figure 46: Simulated Mycophenolate Sodium mean concentration at steady state in for Test 1 (T1), Test 1 (T2) and Reference (R)

The simulated Mycophenolate Sodium mean steady-state concentration chart for T1, T2 and R was shown in the Figure 46. Test formulation in fasted state (Test 1) (NAXPM-19) had lower average C_{max} as desired. However, the C_{min} concentration was lower than the reference formulation (Table 110) and failed to meet the acceptance criteria. The mean percent steady-state fluctuation for test in fasted state Test 1 (NAXPM-19) was slightly less than reference (Table 111). From Table 112 it was observed that for test formulation (T2) in fed condition ratio% and 90% CI for Ln(AUC) was meeting the pre-specified limits of bioequivalence *i.e.* 80% to 125% with adequate power 0.8581.

Effect of food on the pharmacokinetic parameters of NAXPM19

It could be interpreted from biostudy III that Food had a significant effect on the values of C_{max} and AUC of modified release formulation. The values of C_{max} and AUC were significantly higher in case of fed state when compared with that of fasted state. The reason can be attributed to the increase residence time of the modified release tablet in the stomach and variation in absorption of drug from different sites. The variation in absorption in turn is due to pH dependent solubility of the drug.

Table 113: Concentration *vs.* Time Data for Reference (R), (Study-III, Batch No. U0389)

Sub.	Sequence	Reference (R) - Concentration (µg/mL)																
		Time (hr)																
		0.00	**0.33**	**0.50**	**0.67**	**0.83**	**1.00**	**1.25**	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**4.50**	**5.00**	**5.50**	**6.00**
S1	**RT1T2**	0.00	0.42	5.07	5.17	5.98	6.75	11.50	14.30	10.40	10.40	6.50	3.19	1.95	2.22	1.96	1.70	1.12
S2	**T1RT2**	0.00	20.40	16.50	12.10	10.60	6.48	5.08	4.68	2.40	1.52	1.22	1.15	0.84	0.62	0.54	0.67	0.87
S3	**T1T2R**	0.00	1.41	5.72	17.50	15.60	13.80	14.50	7.90	3.41	1.63	1.00	0.70	0.54	0.49	0.36	0.34	0.30
S4	**T2RT1**	0.00	0.63	7.05	8.02	11.20	10.10	7.26	6.33	6.73	3.81	1.74	2.33	1.86	2.95	1.84	1.41	1.08
S5	**RT2T1**	0.00	0.00	19.00	13.00	7.12	5.87	4.40	5.37	2.51	3.27	1.66	1.18	0.85	0.86	1.14	1.24	0.87
S6	**T2T1R**	0.00	2.42	8.73	13.10	15.10	12.80	7.30	5.57	2.33	3.11	2.63	2.12	1.70	1.76	1.28	0.87	0.60
S7	**T1T2R**	0.00	22.50	30.50	24.90	26.40	20.60	13.40	8.10	2.92	2.62	1.12	0.87	0.97	1.12	1.35	1.60	1.77
S8	**RT2T1**	0.00	21.70	45.00	24.20	16.20	9.59	7.63	6.38	3.89	2.69	2.08	1.63	1.48	0.92	0.54	0.42	0.32
S9	**T2RT1**	0.00	0.00	2.88	4.44	4.82	5.25	5.22	5.74	5.28	4.18	3.43	3.55	3.36	3.65	3.29	2.88	2.25
S10	**RT1T2**	0.00	0.00	2.67	8.57	11.60	27.40	26.70	16.50	11.90	6.42	2.92	1.89	1.68	3.17	3.34	2.83	2.41
S12	**T2RT1**	0.00	19.00	19.80	14.30	13.10	6.27	9.15	5.42	2.29	1.31	0.94	0.75	0.60	0.85	1.03	1.21	0.97
S13	**T1RT2**	0.00	8.85	10.60	12.50	14.60	19.90	17.10	11.20	4.10	2.24	1.53	1.35	0.82	0.61	0.52	0.77	0.50
S14	**RT1T2**	0.00	2.16	5.53	13.40	22.90	22.60	19.90	10.60	5.73	4.16	2.81	1.44	1.63	1.22	0.64	0.46	0.46
S15	**T2T1R**	0.00	16.30	16.30	14.70	12.80	10.30	9.67	8.30	3.48	2.91	2.09	1.85	1.79	1.98	1.10	0.78	0.67
S16	**T1T2R**	0.00	1.94	4.85	6.09	8.26	14.70	14.00	10.10	7.48	6.56	2.43	1.31	0.80	0.54	0.36	0.34	0.28
S17	**RT2T1**	0.00	0.18	4.97	9.49	12.20	16.20	19.50	15.60	12.60	9.35	7.97	3.14	1.98	1.43	0.81	0.62	0.50
S18	**T1RT2**	0.00	1.77	5.47	8.91	11.80	20.80	21.70	19.10	5.39	2.43	1.72	1.05	0.81	0.82	0.71	0.61	0.58
S19	**T2T1R**	0.00	8.47	14.50	18.10	9.61	6.89	4.92	3.23	3.29	2.21	1.81	1.45	1.26	1.36	1.07	0.73	1.07
S20	**T1RT2**	0.00	12.50	16.70	14.00	15.00	24.40	35.50	26.20	13.80	9.96	7.13	4.90	3.67	2.59	1.94	1.80	1.63
S21	**T1T2R**	0.00	8.74	14.90	16.60	17.60	15.50	13.10	9.72	7.42	7.18	2.36	1.32	0.99	1.41	1.50	1.34	0.90
S22	**T2RT1**	0.00	18.00	32.10	26.10	27.20	25.20	15.70	4.43	4.55	2.53	1.54	1.17	0.95	0.91	1.58	1.76	1.38
S23	**RT1T2**	0.00	6.90	9.85	16.20	15.60	11.70	9.34	6.46	3.60	1.13	0.65	0.51	0.41	0.24	0.15	0.30	0.26
S24	**RT2T1**	0.00	0.42	1.02	2.94	5.21	5.12	5.04	6.79	6.61	42.60	45.40	19.50	8.00	4.61	2.23	1.53	0.98
N		**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**
Mean		0.00	7.60	13.03	13.23	13.50	13.84	12.94	9.48	5.74	5.84	4.46	2.54	1.69	1.58	1.27	1.14	0.95
SD		0.00	8.17	10.87	6.29	5.98	7.12	7.83	5.55	3.45	8.49	9.14	3.84	1.60	1.14	0.86	0.73	0.60
Min		0.00	0.00	1.02	2.94	4.82	5.12	4.40	3.23	2.29	1.13	0.65	0.51	0.41	0.24	0.15	0.30	0.26
Max		0.00	22.50	45.00	26.10	27.20	27.40	35.50	26.20	13.80	42.60	45.40	19.50	8.00	4.61	3.34	2.88	2.41
CV%		M	107.51	83.45	47.55	44.33	51.45	60.51	58.59	60.00	145.43	204.66	151.51	94.64	71.93	67.68	64.16	63.51

(Continued……)

Sub.	Sequence	Reference (R) - Concentration (µg/mL)														
		Time (hr)														
		6.50	7.00	7.50	8.00	9.00	10.00	11.00	12.00	14.00	16.00	18.00	22.00	24.00	36.00	48.00
S1	RT1T2	0.88	0.78	0.64	0.46	0.62	1.03	1.00	0.88	0.92	0.82	0.33	0.37	0.26	M	M
S2	T1RT2	1.24	1.54	1.73	2.36	2.32	1.79	1.88	1.52	0.70	0.90	0.91	1.28	0.57	0.34	0.33
S3	T1T2R	0.29	0.36	0.47	0.56	0.58	0.59	0.83	0.91	0.99	0.83	0.37	0.64	0.65	0.40	0.00
S4	T2RT1	0.84	0.91	1.40	1.39	2.37	1.38	1.13	0.94	0.59	0.56	0.57	0.85	0.61	M	M
S5	RT2T1	0.76	0.96	1.23	1.22	1.28	1.29	1.09	0.77	0.62	1.31	0.28	1.00	0.52	0.32	M
S6	T2T1R	0.51	0.48	0.36	0.40	0.26	0.49	0.90	1.00	1.07	0.69	0.46	0.83	0.20	M	0.00
S7	T1T2R	1.92	1.99	1.83	1.45	1.28	1.20	1.05	0.91	0.71	0.47	0.53	0.69	0.18	0.24	0.00
S8	RT2T1	0.42	0.33	0.29	0.26	0.25	0.27	0.41	0.63	0.76	0.44	0.51	0.58	0.44	M	M
S9	T2RT1	2.21	2.09	2.67	1.67	1.56	1.57	1.24	1.05	1.51	0.96	0.69	0.96	0.39	0.38	0.25
S10	RT1T2	1.98	1.54	1.47	1.45	1.70	1.53	1.20	1.21	1.25	0.48	0.81	1.14	0.75	0.40	0.17
S12	T2RT1	0.81	0.67	0.65	0.64	0.60	0.65	0.65	0.71	0.67	0.31	0.26	0.33	0.48	0.11	0.00
S13	T1RT2	0.95	0.95	0.65	0.45	0.53	1.72	1.24	0.92	0.48	0.41	0.38	0.24	0.67	0.12	0.00
S14	RT1T2	0.52	0.55	0.55	0.45	0.25	0.27	0.33	0.80	1.08	0.51	0.40	0.52	1.16	0.58	0.22
S15	T2T1R	0.46	0.46	0.39	0.26	0.95	1.48	1.99	1.32	1.18	0.82	0.37	1.27	1.21	0.13	0.00
S16	T1T2R	0.27	0.45	0.62	0.61	0.53	0.30	0.50	0.54	0.41	0.36	0.47	0.55	0.49	0.28	0.12
S17	RT2T1	0.33	0.42	0.58	0.71	0.91	1.61	2.20	2.63	1.05	0.52	0.71	0.68	1.33	0.18	0.13
S18	T1RT2	0.45	0.51	0.71	0.94	1.02	1.50	0.98	0.85	0.64	0.36	0.38	0.77	0.46	0.23	0.00
S19	T2T1R	1.09	0.94	1.26	1.18	1.56	3.13	3.17	3.19	2.22	1.59	0.96	1.36	0.80	0.40	0.00
S20	T1RT2	1.57	1.42	1.58	2.18	3.47	2.91	3.12	3.78	2.65	1.90	4.44	1.99	2.27	1.03	0.20
S21	T1T2R	0.78	0.62	0.70	1.16	1.12	1.02	0.72	0.76	0.56	0.28	0.22	0.64	0.48	0.16	M
S22	T2RT1	1.14	0.89	0.69	0.67	0.94	1.58	2.32	2.50	1.32	0.72	0.82	0.76	0.91	1.86	0.52
S23	RT1T2	0.28	0.45	0.49	0.43	0.33	0.35	0.28	0.68	1.04	0.68	0.52	0.29	0.38	0.54	0.25
S24	RT2T1	0.76	0.60	0.45	0.46	0.45	0.52	0.54	0.77	1.16	0.59	1.03	0.55	0.79	0.39	0.00
N		23	23	23	23	23	23	23	23	23	23	23	23	23	19	18
Mean		0.89	0.86	0.93	0.93	1.08	1.23	1.25	1.27	1.03	0.72	0.71	0.79	0.69	0.42	0.12
SD		0.57	0.51	0.60	0.60	0.80	0.77	0.82	0.88	0.53	0.41	0.85	0.41	0.46	0.41	0.15
Min		0.27	0.33	0.29	0.26	0.25	0.27	0.28	0.54	0.41	0.28	0.22	0.24	0.18	0.11	0.00
Max		2.21	2.09	2.67	2.36	3.47	3.13	3.17	3.78	2.65	1.90	4.44	1.99	2.27	1.86	0.52
CV%		63.62	59.58	64.93	64.45	74.39	62.70	65.75	69.01	52.08	57.00	118.38	51.51	66.21	96.03	123.98

Table 114: Concentration *vs.* Time Data for Test (T1), (Study-III, Batch No. NAXPM-19)

Sub.	Sequence	Test 1 Formulation - Concentration (µg/mL)																
		Time (hr)																
		0.00	**0.33**	**0.50**	**0.67**	**0.83**	**1.00**	**1.25**	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**4.50**	**5.00**	**5.50**	**6.00**
S1	**RT1T2**	0.00	0.00	6.83	5.37	5.46	5.06	4.89	3.70	3.94	3.48	4.29	3.25	2.78	2.47	1.70	1.33	1.51
S2	**T1RT2**	0.00	0.88	1.70	1.31	0.90	2.46	3.41	3.35	10.70	5.60	4.76	3.44	2.59	2.88	2.22	2.36	2.64
S3	**T1T2R**	0.00	3.40	3.12	3.13	2.05	2.33	2.01	2.07	1.92	1.98	1.68	1.36	1.73	0.94	1.65	1.14	2.30
S4	**T2RT1**	0.00	2.76	2.79	3.18	2.98	3.18	2.97	2.79	2.42	3.00	2.28	1.18	0.82	0.79	0.77	0.70	0.56
S5	**RT2T1**	0.00	1.47	1.20	0.63	0.90	2.38	2.96	2.52	4.62	2.65	2.47	2.19	2.05	1.91	1.12	0.94	0.64
S6	**T2T1R**	0.00	3.13	4.97	5.32	6.15	5.53	5.73	6.33	4.76	3.56	2.57	2.65	2.29	2.28	1.47	1.19	1.42
S7	**T1T2R**	0.00	0.64	3.16	3.25	3.48	3.41	3.14	3.02	2.27	2.33	2.79	2.44	2.91	2.87	1.89	1.98	1.81
S8	**RT2T1**	0.00	3.99	4.96	5.94	5.03	4.78	5.74	3.43	3.35	3.19	3.16	3.00	2.29	1.60	0.76	0.56	0.28
S9	**T2RT1**	0.00	27.00	31.50	27.30	22.80	14.00	8.07	6.08	3.19	2.33	2.00	1.54	2.18	3.10	3.44	2.46	2.03
S10	**RT1T2**	0.00	3.69	3.24	3.47	5.33	5.65	6.72	5.67	3.83	2.83	1.95	2.78	3.42	4.34	3.34	2.84	2.43
S12	**T2RT1**	0.00	1.06	2.86	2.41	2.63	2.58	3.04	2.95	3.33	2.94	1.92	1.92	1.60	1.65	1.40	1.23	1.37
S13	**T1RT2**	0.00	0.38	0.71	2.45	10.40	8.63	6.74	5.95	3.60	3.17	2.06	1.03	1.11	1.19	1.14	0.84	0.84
S14	**RT1T2**	0.00	1.34	1.31	4.24	8.41	7.92	5.74	3.81	3.03	2.35	2.11	1.61	1.80	2.67	2.06	1.65	1.43
S15	**T2T1R**	0.00	1.01	0.63	0.40	0.22	1.04	3.06	4.78	3.35	2.16	2.22	2.16	1.59	1.52	0.96	0.76	0.61
S16	**T1T2R**	0.00	0.00	0.20	0.43	0.86	6.62	8.80	5.86	5.35	4.73	4.79	2.73	2.69	2.21	1.59	1.71	0.87
S17	**RT2T1**	0.00	2.86	3.06	4.14	4.41	10.10	13.70	12.80	11.40	7.99	5.94	4.30	2.91	3.91	3.98	4.05	3.79
S18	**T1RT2**	0.00	1.27	1.19	1.30	1.39	1.45	1.45	1.44	1.49	1.45	1.42	1.38	1.43	1.38	1.51	1.37	1.60
S19	**T2T1R**	0.00	2.60	5.18	4.79	4.44	3.43	3.23	2.99	1.74	2.43	2.49	2.41	2.51	2.41	1.35	1.15	1.22
S20	**T1RT2**	0.00	1.60	1.83	2.24	2.60	2.91	6.32	14.10	13.80	12.20	7.71	4.97	4.86	3.16	2.81	2.42	1.56
S21	**T1T2R**	0.00	1.92	1.75	1.70	2.03	11.80	21.80	22.70	21.40	13.90	11.50	7.38	7.54	9.19	4.36	2.41	2.29
S22	**T2RT1**	0.00	2.78	2.73	2.78	2.99	3.27	3.30	3.44	3.49	3.22	2.66	2.25	2.09	1.89	2.09	2.05	2.36
S23	**RT1T2**	0.00	0.16	0.21	0.31	0.43	0.55	0.88	2.35	2.88	2.43	2.21	1.78	1.75	1.77	1.65	1.41	1.33
S24	**RT2T1**	0.00	4.03	4.10	4.57	4.67	4.53	5.05	5.28	5.70	5.67	6.65	5.20	4.18	4.41	3.04	2.30	2.36
N		**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**
Mean		0.00	2.96	3.88	3.94	4.37	4.94	5.60	5.54	5.29	4.16	3.55	2.74	2.57	2.63	2.01	1.69	1.62
SD		0.00	5.40	6.27	5.37	4.76	3.49	4.52	4.85	4.73	3.17	2.42	1.51	1.43	1.74	1.01	0.84	0.83
Min		0.00	0.00	0.20	0.31	0.22	0.55	0.88	1.44	1.49	1.45	1.42	1.03	0.82	0.79	0.76	0.56	0.28
Max		0.00	27.00	31.50	27.30	22.80	14.00	21.80	22.70	21.40	13.90	11.50	7.38	7.54	9.19	4.36	4.05	3.79
CV%		M	182.62	161.49	136.12	108.82	70.73	80.73	87.56	89.47	76.33	68.30	55.26	55.47	66.16	50.39	49.48	51.17

(Continued……)

Sub.	Sequence	Test 1 Formulation - Concentration (µg/mL)														
		Time (hr)														
		6.50	7.00	7.50	8.00	9.00	10.00	11.00	12.00	14.00	16.00	18.00	22.00	24.00	36.00	48.00
S1	RT1T2	1.63	1.76	1.60	1.43	1.76	1.78	1.59	1.55	1.38	0.81	0.98	1.05	0.70	0.25	M
S2	T1RT2	2.10	1.76	1.57	1.54	1.89	2.06	1.68	1.80	1.24	0.82	0.91	1.05	1.07	0.27	M
S3	T1T2R	1.69	2.06	1.50	1.11	1.01	1.11	1.22	0.97	0.66	0.13	0.46	0.60	0.46	0.27	0.00
S4	T2RT1	0.56	0.65	0.67	0.60	2.14	2.98	1.79	1.51	0.93	0.76	0.37	0.39	0.40	0.39	0.20
S5	RT2T1	0.96	1.38	1.39	1.44	2.26	1.67	0.99	0.67	0.69	0.38	0.49	0.80	0.61	0.40	0.00
S6	T2T1R	1.43	1.99	1.44	1.89	1.68	1.29	1.01	0.86	1.05	0.77	0.57	0.65	0.38	0.00	0.00
S7	T1T2R	1.62	1.83	2.40	2.00	1.47	1.17	1.29	1.40	1.29	0.62	0.56	0.68	0.41	0.26	0.00
S8	RT2T1	0.25	0.17	0.15	0.00	0.12	0.22	0.32	0.34	0.19	0.00	0.12	0.22	0.11	0.00	0.00
S9	T2RT1	1.35	2.50	1.49	2.06	1.98	2.03	1.39	1.52	0.85	0.67	0.89	0.74	0.80	0.29	0.00
S10	RT1T2	2.34	2.18	2.21	2.21	2.48	1.82	1.61	1.86	1.97	1.11	3.64	0.99	1.47	0.92	0.23
S12	T2RT1	1.11	1.11	1.37	1.25	1.13	0.72	0.60	0.51	0.75	0.60	0.28	0.33	0.27	0.38	0.00
S13	T1RT2	0.32	0.25	0.21	0.17	0.27	0.65	0.51	0.58	0.29	0.61	0.23	0.25	0.23	0.00	0.00
S14	RT1T2	1.11	1.31	1.11	0.98	0.58	0.46	0.73	0.45	0.60	0.47	0.28	0.43	0.79	0.14	0.00
S15	T2T1R	0.38	0.49	0.60	0.55	0.62	1.05	0.87	1.24	0.93	0.52	0.61	0.46	0.68	M	M
S16	T1T2R	0.31	0.30	0.26	0.11	0.28	1.02	1.40	1.51	0.37	0.29	0.21	0.59	0.54	0.43	0.27
S17	RT2T1	3.57	3.90	4.47	4.56	4.76	3.30	2.61	2.29	2.20	1.30	0.96	0.81	0.84	0.81	0.37
S18	T1RT2	1.78	3.73	3.14	2.79	2.56	2.08	1.85	1.29	1.20	0.38	0.51	0.84	0.52	0.22	0.00
S19	T2T1R	1.16	1.27	1.62	1.57	1.65	1.21	1.01	0.99	0.91	0.46	0.65	0.71	0.42	0.19	0.00
S20	T1RT2	1.34	1.17	1.00	0.95	1.24	2.07	2.65	2.57	1.51	0.88	0.95	0.94	0.53	M	M
S21	T1T2R	1.55	1.82	1.26	1.10	2.64	5.08	2.85	2.87	1.88	1.35	0.89	1.95	1.31	1.01	0.69
S22	T2RT1	2.35	2.04	1.84	1.49	1.23	1.12	1.14	1.54	1.55	1.51	0.82	0.90	0.81	0.52	0.57
S23	RT1T2	1.10	0.97	0.92	1.04	0.93	1.12	1.24	1.43	0.76	0.49	0.46	0.53	0.68	0.44	0.14
S24	RT2T1	2.51	2.34	2.12	2.25	2.06	2.77	2.38	3.04	2.86	1.45	1.33	0.87	0.73	0.00	0.00
N		23	23	23	23	23	23	23	23	23	23	23	23	23	21	19
Mean		1.41	1.61	1.49	1.44	1.60	1.69	1.42	1.43	1.13	0.71	0.75	0.73	0.64	0.34	0.13
SD		0.81	0.98	0.97	0.99	1.02	1.08	0.69	0.74	0.65	0.41	0.70	0.36	0.33	0.29	0.21
Min		0.25	0.17	0.15	0.00	0.12	0.22	0.32	0.34	0.19	0.00	0.12	0.22	0.11	0.00	0.00
Max		3.57	3.90	4.47	4.56	4.76	5.08	2.85	3.04	2.86	1.51	3.64	1.95	1.47	1.01	0.69
CV%		57.45	60.77	64.99	68.80	63.92	64.08	48.64	52.16	57.08	56.99	94.02	49.88	50.85	83.53	162.79

Table 115: Concentration *vs.* Time Data for Test (T2), (Study-III, Batch No. NAXPM-19)

Sub.	Sequence	Test 2 Formulation - Concentration (µg/mL)																
		Time (hr)																
		0.00	**0.33**	**0.50**	**0.67**	**0.83**	**1.00**	**1.25**	**1.50**	**2.00**	**2.50**	**3.00**	**3.50**	**4.00**	**4.50**	**5.00**	**5.50**	**6.00**
S1	**RT1T2**	0.00	0.33	0.41	0.93	3.04	8.86	14.20	15.00	10.60	8.31	6.10	3.32	2.82	2.28	0.90	0.64	0.44
S2	**T1RT2**	0.00	0.21	0.34	0.52	0.61	0.69	0.87	2.00	8.03	16.90	7.84	6.90	3.42	2.68	3.75	2.70	1.85
S3	**T1T2R**	0.00	0.14	0.39	0.45	0.39	0.36	0.82	0.80	3.57	7.71	5.05	3.57	2.48	2.41	2.61	2.54	0.70
S4	**T2RT1**	0.00	0.00	0.14	0.35	0.35	0.40	4.74	12.70	9.26	5.25	2.57	7.63	3.49	4.48	1.77	1.13	0.90
S5	**RT2T1**	0.00	0.34	0.00	0.00	0.13	0.22	0.39	0.71	0.50	0.31	0.21	0.15	2.02	9.40	5.63	5.08	3.65
S6	**T2T1R**	0.00	0.17	0.23	0.28	0.37	0.48	2.36	4.73	6.64	4.19	5.08	4.75	5.79	6.02	3.36	1.79	1.03
S7	**T1T2R**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.15	0.68	6.06	12.10	12.30	10.40
S8	**RT2T1**	0.00	0.19	0.35	0.68	1.25	3.47	4.23	5.89	7.14	5.35	1.96	0.12	4.80	4.94	2.97	1.75	0.93
S9	**T2RT1**	0.00	0.00	0.00	0.00	0.26	0.53	1.92	3.80	4.66	7.67	14.50	11.00	5.52	3.15	2.60	1.86	1.63
S10	**RT1T2**	0.00	0.11	0.00	0.00	0.13	1.10	1.54	1.69	1.47	1.33	1.12	1.15	0.94	1.10	7.84	10.60	12.40
S12	**T2RT1**	0.00	0.00	0.14	0.15	0.10	0.15	0.12	0.12	0.20	0.20	0.21	0.18	0.20	0.24	2.87	8.03	7.17
S13	**T1RT2**	0.00	0.29	0.40	0.42	0.46	0.53	0.42	0.46	0.51	0.61	0.65	0.70	0.60	0.92	1.06	2.17	4.10
S14	**RT1T2**	0.00	0.00	0.00	0.19	0.34	0.22	0.24	0.19	0.10	0.10	0.00	0.00	0.00	0.16	26.20	9.98	5.52
S15	**T2T1R**	0.00	0.00	0.00	0.16	0.21	0.67	1.63	1.72	6.11	7.31	8.91	6.75	6.18	5.44	3.70	1.54	0.87
S16	**T1T2R**	0.00	0.73	0.87	1.14	1.36	1.31	3.73	4.63	3.79	2.60	2.24	1.18	1.62	1.86	1.59	1.06	0.91
S17	**RT2T1**	0.00	0.30	0.62	0.67	0.52	0.39	0.29	0.34	0.59	0.46	0.42	0.27	0.24	0.78	4.91	7.08	11.10
S18	**T1RT2**	0.00	0.00	0.00	0.20	0.27	0.21	0.14	0.13	0.16	0.19	0.24	0.49	0.44	10.70	16.60	10.10	7.21
S19	**T2T1R**	0.00	0.00	0.36	1.89	4.34	7.82	10.10	8.17	6.28	5.98	4.54	2.69	1.41	1.48	0.83	0.90	0.90
S20	**T1RT2**	0.00	1.54	1.60	1.47	1.88	5.71	18.80	19.50	6.66	14.60	9.87	6.38	8.27	11.30	4.31	2.75	2.39
S21	**T1T2R**	0.00	0.00	0.00	0.11	0.14	0.17	0.28	0.27	1.36	1.40	1.77	1.30	9.73	9.42	9.71	5.23	3.64
S22	**T2RT1**	0.00	0.14	0.21	0.30	0.44	0.58	2.29	2.12	1.11	0.45	0.35	0.17	0.13	26.70	21.00	10.90	6.83
S23	**RT1T2**	0.00	0.22	0.25	0.29	0.32	0.32	0.61	1.53	3.59	9.69	9.81	10.10	9.87	11.80	7.76	6.29	4.24
S24	**RT2T1**	0.00	0.00	0.00	0.20	0.67	1.38	0.86	0.89	0.50	0.46	0.46	0.53	0.55	0.54	0.40	2.40	12.60
N		**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**	**23**
Mean		0.00	0.20	0.27	0.45	0.76	1.55	3.07	3.80	3.60	4.39	3.65	3.02	3.10	5.39	6.28	4.73	4.41
SD		0.00	0.34	0.37	0.49	1.04	2.49	4.85	5.28	3.36	4.82	4.09	3.47	3.12	5.99	6.78	3.84	4.02
Min		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.40	0.64	0.44
Max		0.00	1.54	1.60	1.89	4.34	8.86	18.80	19.50	10.60	16.90	14.50	11.00	9.87	26.70	26.20	12.30	12.60
CV%		M	166.22	135.17	108.87	136.60	161.37	158.01	138.94	93.33	109.62	112.00	114.82	100.82	111.15	107.89	81.12	91.26

(Continued……)

Sub.	Sequence	Test 2 Formulation - Concentration (µg/mL)														
		Time (hr)														
		6.50	7.00	7.50	8.00	9.00	10.00	11.00	12.00	14.00	16.00	18.00	22.00	24.00	36.00	48.00
S1	RT1T2	0.33	0.29	0.34	0.24	0.24	0.71	0.80	0.80	0.64	0.28	0.28	0.52	0.38	0.18	0.00
S2	T1RT2	1.40	1.13	1.11	1.10	0.99	1.65	2.23	2.12	1.35	0.67	0.69	0.98	0.69	0.37	0.17
S3	T1T2R	0.50	0.39	0.32	0.29	0.23	0.36	0.61	0.96	0.83	0.93	0.50	0.50	0.51	0.48	0.36
S4	T2RT1	0.62	0.65	1.02	1.77	2.25	2.22	1.52	1.14	0.60	1.30	0.39	0.85	0.41	0.38	0.00
S5	RT2T1	4.01	2.37	2.19	1.40	0.93	0.81	0.84	0.79	0.80	M	M	M	M	0.00	M
S6	T2T1R	0.61	0.47	0.33	0.36	0.46	0.59	0.54	0.53	0.47	0.78	0.34	0.43	0.24	0.00	0.00
S7	T1T2R	7.38	2.87	1.56	1.06	0.53	0.45	0.59	0.59	1.24	1.30	0.93	0.65	0.60	0.25	0.00
S8	RT2T1	1.22	0.79	0.46	0.34	1.01	0.73	0.90	1.30	0.32	0.30	0.86	0.36	0.15	0.25	0.00
S9	T2RT1	1.37	1.30	1.54	1.77	1.89	1.83	1.70	1.73	0.93	1.46	0.57	0.46	0.98	0.17	0.00
S10	RT1T2	7.56	3.85	2.43	1.85	0.98	0.89	1.23	1.44	1.34	1.09	0.79	1.28	1.28	0.57	0.23
S12	T2RT1	4.21	2.94	2.39	1.13	0.57	0.31	0.23	0.20	0.37	0.46	0.36	0.59	0.60	0.14	0.00
S13	T1RT2	7.08	3.51	2.52	1.73	0.86	0.42	0.47	0.48	0.42	0.30	0.36	0.34	0.33	0.18	0.00
S14	RT1T2	3.36	2.33	1.61	1.05	0.64	0.51	0.84	0.91	0.72	0.45	0.36	0.36	0.50	0.33	0.00
S15	T2T1R	0.57	0.40	0.30	0.26	0.36	0.88	1.53	1.40	0.57	0.57	0.31	0.83	0.51	0.21	0.00
S16	T1T2R	0.65	0.63	0.81	1.18	1.48	1.27	1.38	1.50	0.82	0.84	0.55	0.39	0.44	0.52	0.15
S17	RT2T1	14.60	13.90	5.53	3.43	1.51	0.90	0.81	1.34	1.55	1.75	1.45	1.04	1.58	0.72	0.14
S18	T1RT2	3.35	1.97	1.06	0.76	0.63	0.75	1.03	1.11	0.93	0.81	0.43	0.72	0.68	0.24	0.00
S19	T2T1R	0.52	0.42	0.38	0.50	1.17	1.05	1.45	1.13	0.79	0.54	0.49	0.60	0.61	M	0.00
S20	T1RT2	1.62	1.43	1.28	1.28	2.92	2.47	2.79	2.53	1.68	1.15	1.10	1.80	1.47	1.04	0.85
S21	T1T2R	1.74	0.95	0.70	0.74	0.69	0.63	0.94	0.99	0.97	0.46	0.46	0.50	0.56	0.30	0.00
S22	T2RT1	4.53	2.35	1.73	0.98	0.65	0.52	0.75	1.15	1.71	0.80	0.72	1.16	1.42	0.51	0.41
S23	RT1T2	2.66	1.93	1.08	0.95	0.76	0.64	0.91	1.22	2.34	1.84	0.85	0.65	1.21	0.65	0.40
S24	RT2T1	20.50	11.10	9.77	6.15	2.77	1.34	0.82	0.57	1.18	1.21	0.89	0.71	0.43	0.00	M
N		23	23	23	23	23	23	23	23	23	22	22	22	22	22	21
Mean		3.93	2.52	1.76	1.32	1.07	0.95	1.08	1.13	0.98	0.88	0.62	0.71	0.71	0.34	0.13
SD		4.94	3.35	2.09	1.27	0.75	0.59	0.59	0.53	0.50	0.46	0.30	0.36	0.42	0.26	0.22
Min		0.33	0.29	0.30	0.24	0.23	0.31	0.23	0.20	0.32	0.28	0.28	0.34	0.15	0.00	0.00
Max		20.50	13.90	9.77	6.15	2.92	2.47	2.79	2.53	2.34	1.84	1.45	1.80	1.58	1.04	0.85
CV%		125.71	132.80	118.83	96.67	70.62	61.69	54.42	47.45	51.35	52.56	48.26	50.33	59.28	75.32	169.60

4.4 Modeling of Drug Release Kinetics

The data obtained for dissolution profiles for different batches was fitted into various kinetic models which were Zero order, First order, Higuchi model, Hixon Crowell model and Korsmeyer model. The regression coefficient, constant values were determined for each model and reported in Table 116.

Based on the values of r^2 obtained for first order, Korsmeyer Peppas and Hixon Crowell it could be interpreted that two mechanistic phenomenon take place: the swelling and erosion of the polymer. The release kinetics of the drug are dependent upon the relative magnitude of the rate of polymer swelling at the moving rubbery/glassy front and the rate of polymer erosion at the swollen polymer/ dissolution medium front. The results are consistent with a release process where the fickian release mechanism plays an important role along with the matrix erosion, which is considered a characteristic of the system based on hydrophilic polymers.

Table 116: Behavior of various batches in different Kinetic models

Kinetic Models	Regression/ constant	NAXLM 1	NAXLM 2	NAXLM 3	NAXLM 4	NAXLM 5	NAXLM 6
Zero order	r^2	0.7805	0.7675	0.7860	0.8255	0.7428	0.8913
	K	11.22	4.13	5.12	1.82	7.09	7.04
First order	r^2	0.9901	0.9774	0.9934	0.9656	0.9561	0.9205
	K	-0.51	-0.21	-0.25	-0.15	-0.41	-0.28
Higuchi	r^2	0.5110	0.8810	0.8932	0.9114	0.8557	0.8760
	K	35.62	20.20	23.17	6.68	29.67	0.05
Korsmeyer-Peppas	r^2	1.0000	1.0000	1.0000	-	1.0000	1.0000
	K	1.99	1.99	1.99	-	1.99	1.94
	n	1.58	1.59	1.54	-	1.28	1.52
Hixson-Crowell	r^2	0.9995	0.9764	0.6946	0.9686	0.6944	0.8760
	K	-1.40	-0.32	0.07	0.10	0.07	0.05

r^2 = Regression Coefficient
K = Dissolution rate constant

(Continued……)

Kinetic Models	Regression/ constant	NAXLM 7a	NAXLM 7b	NAXLM 7c	NAXLM 7d	NAXLM 8	NAXLM 9
Zero order	r^2	0.6703	0.8567	0.8631	0.8797	0.9265	0.9871
	K	1.85	8.62	7.04	19.88	20.28	26.27
First order	r^2	0.8970	0.8970	0.9993	0.9879	0.8970	0.9773
	K	0.08	0.08	-0.11	2.01	0.13	2.02
Higuchi	r^2	0.6894	0.9649	0.9492	0.9595	0.9869	0.9899
	K	12.35	31.56	33.65	-15.91	-17.69	-4.47
Korsmeyer-Peppas	r^2	-	0.9904	0.9634	0.9514	0.9802	0.9871
	K	-	4.06	8.29	8.42	6.61	3.68
	n	-	0.7667	1.27	1.14	0.8645	0.624
Hixson-Crowell	r^2	0.9986	0.9595	0.9807	0.9805	0.9753	0.9938
	K	0.01	0.12	-0.24	4.59	1.05	-0.25

r^2 = Regression Coefficient
K = Dissolution rate constant

(Continued……)

Kinetic Models	Regression/ constant	NAXPM 10	NAXPM 11	NAXPM 12	NAXPM 13	NAXPM 14	NAXPM 15
Zero order	r^2	0.9703	0.9322	0.9850	0.9935	0.9259	0.9388
	K	7.68	7.32	9.25	4.86	4.49	5.63
First order	r^2	0.9693	0.9916	0.9382	0.8809	0.9606	0.9165
	K	-0.14	-0.07	0.01	-0.06	-0.08	-0.10
Higuchi	r^2	0.9886	0.9817	0.9984	0.9814	0.9889	0.9791
	K	32.88	33.90	39.04	24.78	25.61	29.43
Korsmeyer-Peppas	r^2	0.9684	0.9981	0.9970	0.9851	0.9981	0.9997
	K	3.36	8.57	5.67	7.40	5.78	6.54
	n	0.477	0.0732	0.7955	1.11	0.7353	0.7181
Hixson-Crowell	r^2	0.9830	0.9830	0.8126	0.9571	0.9990	0.9774
	K	-0.21	-0.21	-0.52	-0.15	-0.16	29.43

r^2 = Regression Coefficient
K = Dissolution rate constant

(Continued……)

Kinetic Models	Regression/ constant	NAXPM 16	NAXPM 17	NAXPM 18	NAXPM 19	NAXPM 20
Zero order	r^2	0.9979	0.9826	0.9893	0.9757	0.9860
	K	15.39	15.09	8.90	4.97	16.59
First order	r^2	0.9923	0.8976	0.7717	0.8973	0.9710
	K	-0.14	-0.32	-0.23	-0.08	2.31
Higuchi	r^2	0.9856	0.9972	0.9966	0.9957	0.9957
	K	53.21	52.90	37.92	27.26	-13.06
Korsmeyer-Peppas	r^2	0.9958	1.0000	0.9977	0.9990	0.9964
	K	5.65	4.33	9.94	5.91	5.73
	n	0.7282	0.8659	0.7183	0.7038	0.621
Hixson-Crowell	r^2	0.8242	0.9704	0.9304	0.9750	0.9750
	K	-1.11	-0.61	-0.37	-0.18	4.67

r^2 = Regression Coefficient

K = Dissolution rate constant

The values of r2 for First order and Hixson-Crowell was found to be higher for both lipid matrix and Polymer matrix technology. The value of n was more than 0.5 during the data treatment of the formulations by Korsmeyer Peppas. All the results indicate that the release of the drug due to relaxation of polymer chain leading to the diffusion and erosion technology. The drug releases by nearly zero order

4.5 IVIVC Correlation

IVIVC was calculated using IVIVC toolkit of WinNonlin software. Level A correlation was established between fractions absorbed (F_{abs}) and fraction dissolved (F_{diss}). R^2 values were greater than 0.9 for all the batches (Table 117) which shows good correlation between *in vitro* and *in vivo* results. Levy plots for Biostudy I, II and III are represented in Figures 47, 48 and 49 respectively.

Table 117: IVIVC Correlation

Biostudy	Batch No.	R^2
1	**NAXPM-15**	0.976
	NAXLM-8	0.976
2	**NAXLM-20**	0.934
3	**NAXLM-19**	0.961

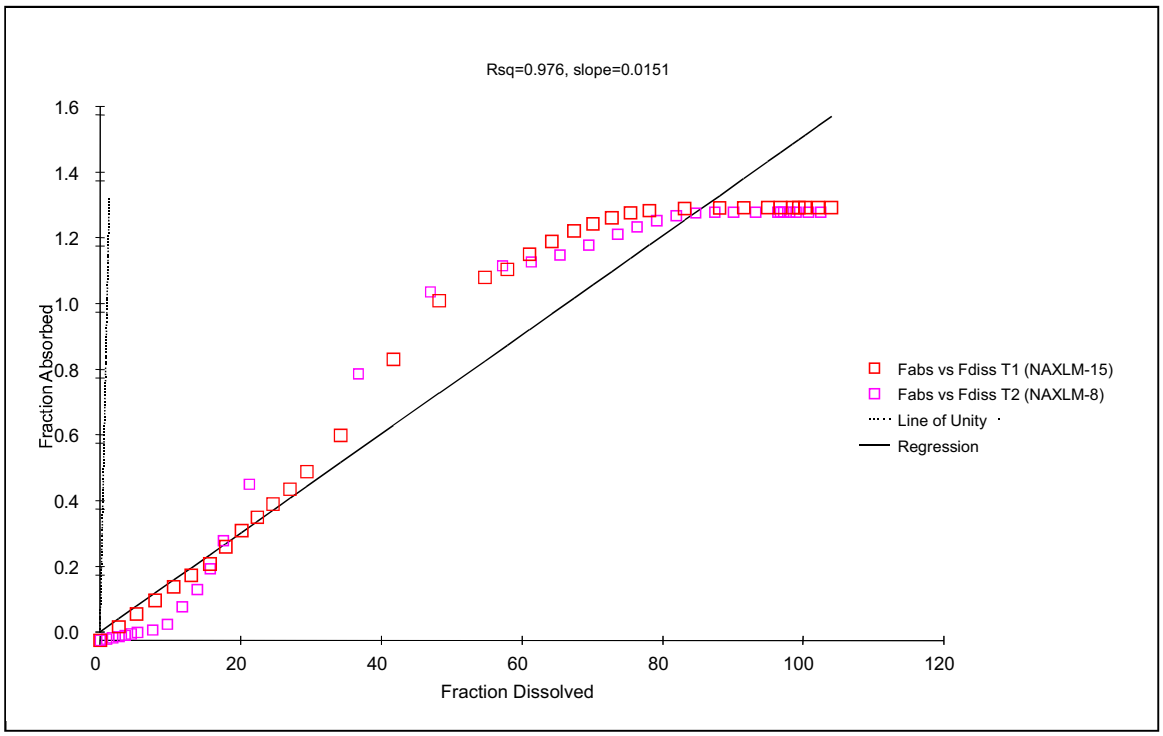


Figure 47: Levy plots for NAXPM-15, NAXLM-8

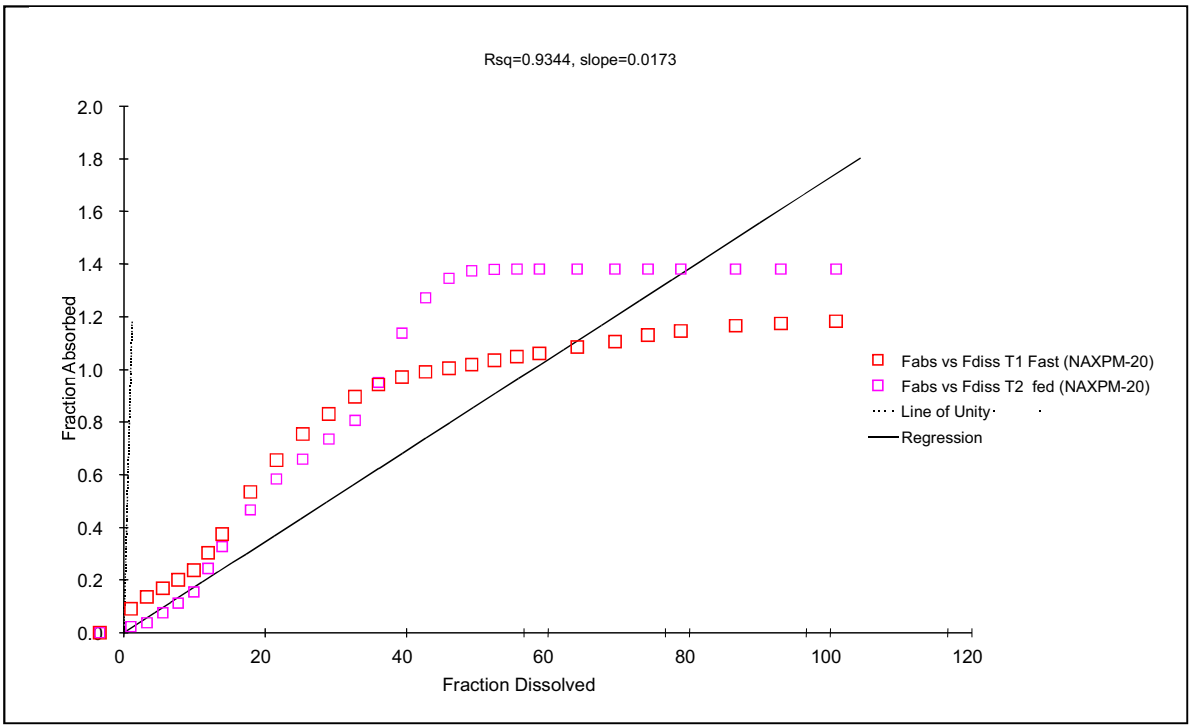


Figure 48: Levy plots for NAXPM-20

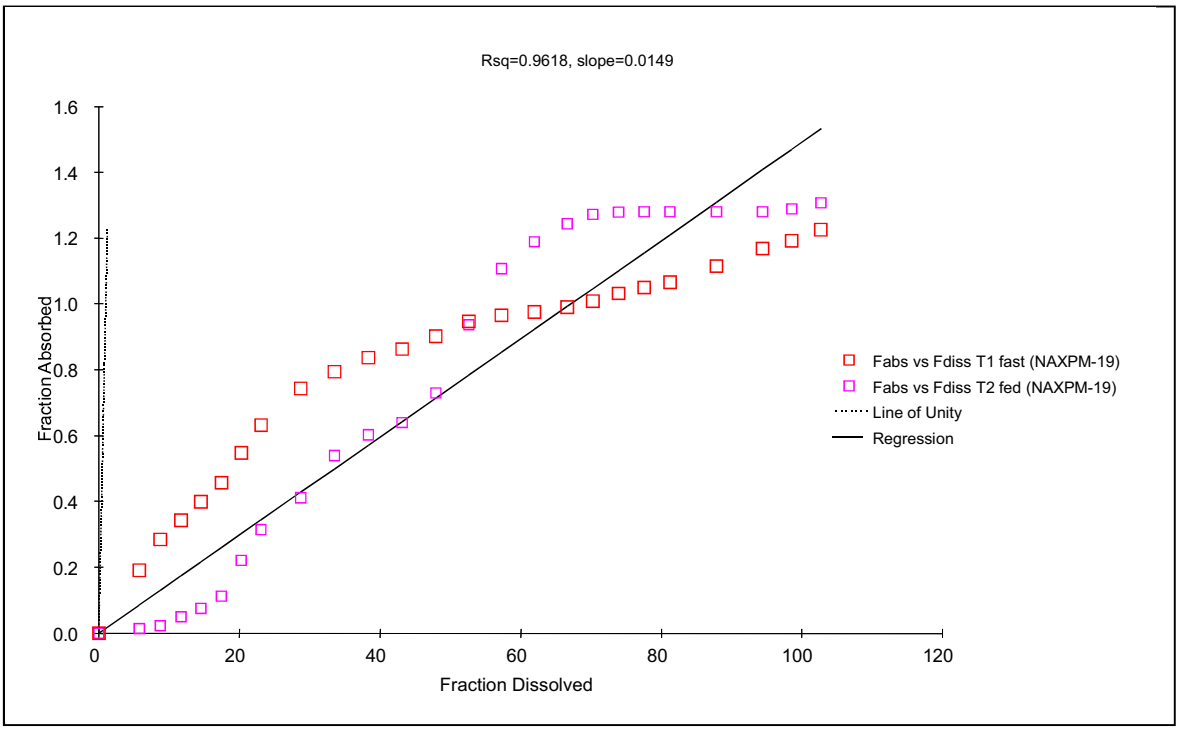


Figure 49: Levy plots for NAXPM-19

5.0
Conclusions and Future Scope of Work

5.0 CONCLUSIONS AND FUTURE SCOPE OF WORK

5.1 Conclusions

Objectives: Transplantation is important for thousands of people in the world. The improvement of today's medicine in the field of grafting has raised a new hope for patients and opened new perspectives for the vital and urgent needs of the patients. However, transplantation itself is not enough; there is a need to balance the amount of immunosuppression necessary to assure graft survival with the potential toxicity of the immunosuppressive agents. A great success has been obtained in combination with the immunosuppressants *viz.,* inosine monophosphate dehydrogenase (IMPDH) inhibitors [8], as Mycophenolic acid *etc.,* In current clinical practice the oral formulations of available immunosuppressants are generally administered on a twice daily basis. Poor compliance has been shown to be one of the factors associated with late graft loss. The efficacy of these immunosuppressants has also been associated with adverse effects comprises of anemia, gastrointestinal effects viz., constipation, diahhorea, nausea, dyspepsia, vomiting. It was envisaged to prepare modify release formulation of MPS after extensive literature survey about the pharmacokinetics and business potential of the molecule. MPS is presently available in the market in the form of conventional tablets and prescribed twice daily. The drug follows linear pharmacokinetics. Thus, it was planned to design once a day tablet formulation of the Mycophenolate Sodium. Based on the FDA approved dosing of MPA and marketed products, it was decided to prepare 720 mg (as MPA) strength of mycophenolate sodium. The target product profile of the proposed drug product was prepared to achieve the above mentioned objective.

Preformulation studies: Preformulation studies of Mycophenolate Sodium viz., solubility studies, LOD, bulk density, Hausner ratio, compressibility index, angle of repose, particle size and drug excipient compatibility studies were carried out.

Analytical method development and validation: In order to evaluate the formulations, the analytical method of Mycophenolate Sodium was developed and validated using HPLC for assay and dissolution samples. During forced degradation studies, the Mycophenolate Sodium peak was found to be separated from placebo peaks and the peak purity index for each sample was within acceptable limits. The values of RSD for precision, accuracy and repeatability were found be less than 5 in

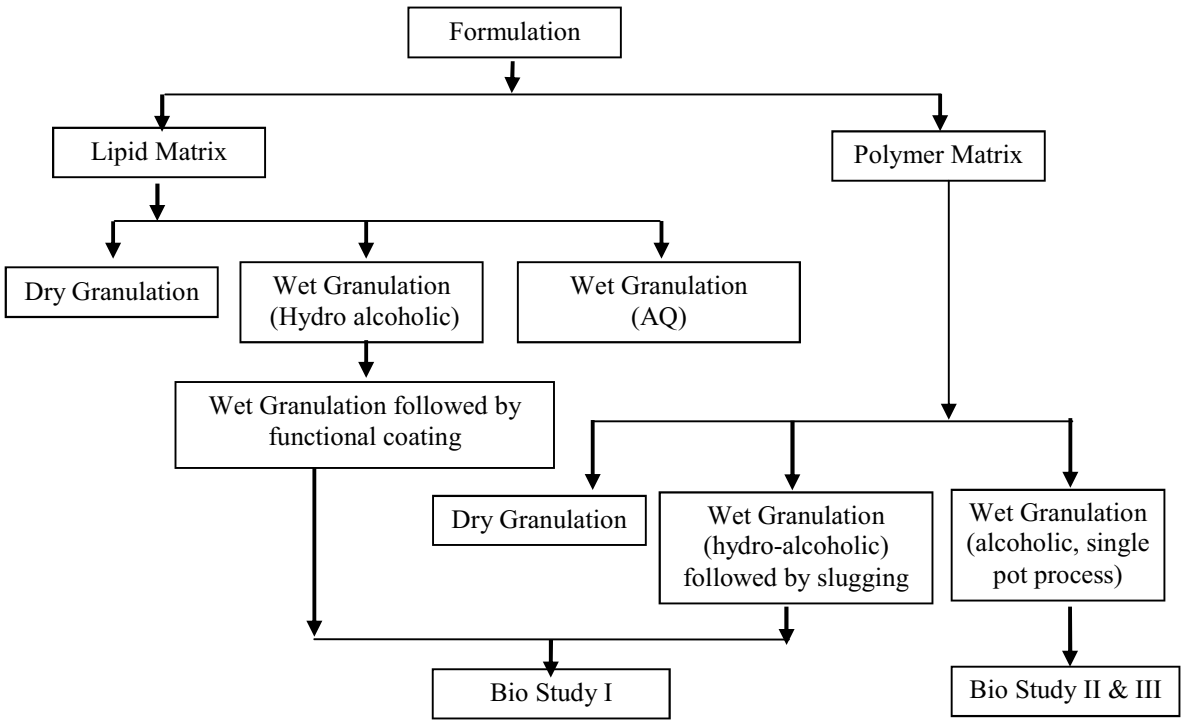
all the cases. The method for determination of metabolite of Mycophenolate Sodium was also developed and validated by LCMS in human plasma for biostudies.

Formulation Development

The formulations of Mycophenolate Sodium were prepared using lipid and polymer matrix technology. For lipid matrix technology, medium chain triglycerides (Compritol 888) were selected as release rate retarding material as it is hydrophobic and approved from the regulatory authorities for oral use. The formulations were prepared using lipid matrix technology with functional coating. Formulations were also prepared using polymer matrix technology. Various strategies such as dry granulation method, wet granulation method, non-aqueous granulation using DCM: IPA and ethanol: water as solvents were tried. The sticking characteristics of the tablet to the punches which was observed during dry granulation method could be overcome using wet granulation technique. The strength of the granules prepared using DCM: IPA solvent was less than when Ethanol: Water was used as a solvent. However, the release of drug from dry granulation and wet granulation strategies was fast. Hence, it was decided to exploit rate controlling coating with a combination of Kollicoat IR and Kollicoat SR. The amount or ratio of Kollicoat IR and SR had a significant effect on initial and total duration of sustained release of the drug. With an increase in the amount of Kollicoat IR initial release was obtained while on increasing the amount of Kollicoat SR, the initial release was slower down and prolonged release was obtained. The optimum amount of Kollicoat SR and Kollicoat IR were found to be 7.5:1 with percent weight gain of 5%. With rate controlling coating strategy, the release of the drug was retarded for about 14 to 16 hr. The release of drug from lipid matrix technology with coating was based on diffusion controlled system whereas the release of drug from polymer matrix technology was based on swelling and erosion of the matrix. With polymer matrix technology strategy also, various methods were employed *viz.,* dry granulation, wet granulation and non-aqueous granulation. Batches NAXPM 9 to NAXPM 20 were prepared using polymer matrix technology. In dry granulation method, sticking was observed due to poor flow properties of the drug. It was observed that a single swellable polymer in composition could not provide desired prolonged drug release profile. Therefore, a combination of hydrophilic, swellable, and erodible polymers were used. Based on the values of r^2 obtained for first order, Korsmeyer Peppas and Hixon Crowell it could be interpreted that two

mechanistic phenomenon take place: the swelling and erosion of the polymer. The release kinetics of the drug are dependent upon the relative magnitude of the rate of polymer swelling at the moving rubbery/glassy front and the rate of polymer erosion at the swollen polymer / dissolution medium front. The results are consistent with a release process where the fickian release mechanism plays an important role along with the matrix erosion, which is a considerable characteristics of system based on hydrophilic polymers.

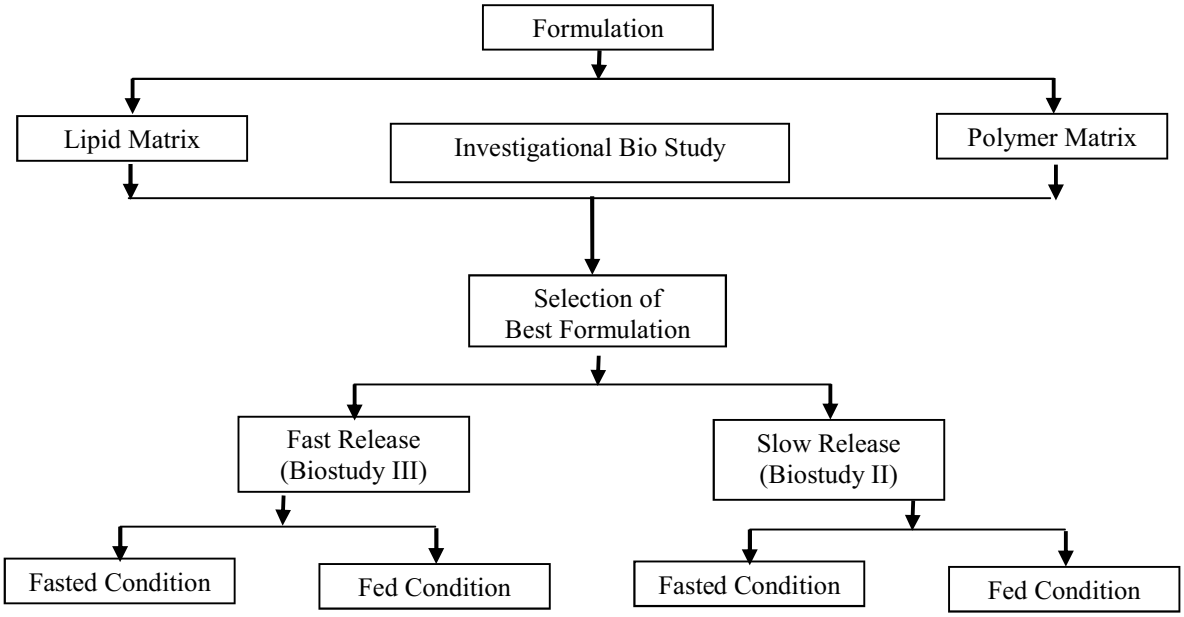
The over all formulation strategy employed in the present investigation has been described in the following flow chart:



Stability Studies: The drug excipient stability studies of the formulations were carried out using DSC. No significant change in the melting behaviour of the drug was observed in the formulation of the tablet. XRD studies of drug substance, drug product and placebo revealed that the drug was in the same polymorphic form in the product. The stability of the optimised formulations using lipid matrix and polymer matrix technology was conducted under accelerated conditions *i.e.,* 40ºC±2ºC and 75%±5%RH. The formulations were found to be stable with respect to assay, rs and

dissolution at accelerated conditions in three packaging systems *i.e.,* ALU-ALU and PVDC-PVC blister and HDPE bottles.

Biostudies Strategy: The following flow chart describes the biostudy strategy adopted in the present investigation to select best formulation:



One formulation each from lipid matrix technology (NAXLM 9) and polymer matrix technology (NAXPM 15) was selected for biostudy I.

Stability Studies

The drug excipient stability studies of the formulations were carried out using DSC. No significant change in the melting behaviour of the drug was observed in the formulation of the tablet. XRD studies of drug substance, drug product and placebo revealed that the drug was in the same polymorphic form in the product. The stability of the optimised formulations using lipid matrix and polymer matrix technology was conducted under accelerated conditions i.e., 40ºC+2ºC and 75%+5%RH. The formulations were found to be stable with respect to assay, Related substances and dissolution at accelerated conditions in three packaging systems i.e., ALU-ALU, HDPE, PVDC-PVC.

Biostudy I: When the above mentioned two formulations each of lipid matrix technology and polymer matrix technology having similar drug release profiles were subjected to bioavailability studies, it was observed that the values of AUC for lipid

matrix technology formulation were less as compared to that of polymer matrix technology. The mean values of $C_{max,}$ $T_{max,}$, AUC_{0-t} and AUC_{inf} were found to be 4.28 µg/mL, 3.5 hr, 27.78 hr*µg/mL and 32.45 hr*µg/mL respectively for polymer matrix formulation. For lipid matrix formulation in fasting condition, the average $C_{max,}$ $T_{max,}$ AUC_{0-t} and AUC_{inf} were found to be 4.389 µg/mL, 4.556 h, 26.197 hr*µg/mL and 28.675 h*µg/mL respectively. The values of AUC_{0-t} and $AUC_{0-\infty}$ for polymer matrix formulation were found to be 13.88 % and 16.71 % lower than that of reference respectively. While in case of lipid matrix formulation, the values were more than 20 % lower than that of reference. It could be interpreted from steady state simulations that both formulations had lower peak concentrations C_{max}, and longer times to peak concentration compared to the reference which is a characteristic of modified release formulation. However, both the formulations failed to meet the acceptance criteria for $C_{min.}$ The value of C_{min} was lower (Polymer matrix 0.17 µg/mL and lipid matrix 0.064 µg/mL) than that was observed with reference formulation (0.608 µg/mL). On applying analysis of variance, it was observed that no statistically significant effect ($P>0.05$) was observed, due to periods for $Ln(C_{max})$, $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$. Formulation effect was statistically significant for $Ln(C_{max})$ ($P<0.05$) but for $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$ it was found to be non significant ($P>0.05$). No significant difference ($p>0.10$) was observed due to sequence for $Ln(C_{max})$, $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$. The following table summarizes the parameters obtained for biostudy I as per acceptance criteria.

Parameters obtained from Biostudy I

Parameters	Acceptance Criteria	Results	
		T1 (NAXPM-15) (Polymer based)	T2 (NAXLM-8) (Lipid based)
AUC: T/R	>0.8	0.816	0.709*
C_{max}: T/R	Preferably ≤ 1 but not more than 1.25	0.280	0.105
Fluctuation: T/R	Preferably ≤ 1 but not more than 1.25	0.783	0.887
Inter individual variation for test	Less than that of reference	Less than reference	Less than reference
C_{min}:	0.8µg/mL or more	0.170*	0.064*

*Fails to meet acceptance criteria.

It was concluded after biostudy I that AUC for lipid matrix technology formulation was less than that of polymeric matrix technology. Though AUC T/R ratio for Polymer technology was more than 0.8, however the value C_{min} achieved was less than that of acceptance criteria set up for proposed target product profile. Based on the above mentioned results, it was decided to use polymer matrix technology for future development to achieve the desired values of AUC and C_{min}.

Biostudy II: Finally, a single pot method with PEO, HPMC and PVP K 90 for preparation of formulation was developed which could provide us the desired drug release profile. Two formulations NAXPM-19 (fast release) and NAXPM-20 (slow release) were selected for biostudy III and II respectively under fasted and fed conditions. The values of $AUC_{0-24,}$ AUC_{0-t} and $AUC_{0-\infty}$ for NAXPM-20 in fasting condition were found to be 19.95%, 13.99 % and 11.61 % lower than that of reference, respectively. While in case of NAXPM-20 under fed condition, the values were less than 10% lower than that of reference. It was observed that NAXPM-20 under fed condition was bioequivalent to reference w.r.t. AUC_{0-24}, $AUC0_{-t}$ and $AUC_{0-\infty}$. As expected for modified-release products, NAXPM-20 under fasting and fed had lower peak concentrations and longer times to peak concentration compared to the reference. On observing food effect it was found that in fed condition C_{max} and T_{max} were on the higher side as compared to fasting condition. Steady state simulations revealed that NAXPM-20 in fasted state had lower average C_{max} as desired. The value of C_{min} for NAXPM-20 was found to be 1.46µg/mL and T/R ratio was found to be 0.83 which meets the acceptance criteria. The mean percent steady-state fluctuation for NAXPM-20 in fasted state was also less than reference. It was observed that for NAXPM-20 in fed condition ratio % and 90% CI for Ln (AUC) were within the limits of bioequivalence *i.e.* 80% to 125%. On applying analysis of variance, it was observed that no statistically significant effect ($P>0.05$) was observed, due to periods for $Ln(C_{max})$, Ln (AUC_{0-t}) and $Ln(AUC_{0-\infty})$. Formulation effect was statistically significant for $Ln(C_{max})$ and $Ln(AUC_{0-24})$ ($P<0.05$) but for $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$ it was found to be non significant ($P>0.05$). No significant difference ($p>0.10$) was observed due to sequence for $Ln(C_{max})$, $Ln(AUC_{0-24})$, $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$.

Predicted Steady state PK Parameters for Study-II.

Parameters	Acceptance Criteria	Results (NAXPM-20)
AUC: T/R	>0.8	0.907
AUC: Lower level of 90% CI	>0.7	0.762
C_{max}: T/R	Preferably ≤ 1 but not more than 1.25	0.806
Fluctuation: T/R	Preferably ≤ 1 but not more than 1.25	0.84
Inter individual variation for test	Less than that of reference	Less than Reference
C_{min} T/R for C_{min}	0.8 to 1µg/mL or more >0.8	1.46 µg/mL 0.83

From above table it was observed that for formulation NAXPM-20, T/R ratio for AUC, C_{max} and Fluctuation was found to be 0.907, 0.806 and 0.84 respectively. The values of C_{min} and lower level of 90% CI for AUC were found to be 1.46µg/mL and 0.762 respectively. On comparing the inter-individual variation, it was observed that test formulation (NAXPM-20) is less variable than reference formulation. On the basis of results given in table, it was observed that formulation NAXPM-20 meets all the set acceptance criteria for steady state pharmacokinetic parameters.

Biostudy III: Formulation (NAXPM-19 with release profile of 12 hr) was subjected to randomized, single dose, three way cross over comparative bioavailability study under fasting and fed conditions. The values of $AUC_{0-24,}$ $AUC0_{-t}$ and $AUC_{0-\infty}$ for NAXPM-19 in fasting condition were found to be 19.90%, 19.45% and 15.53 % lower than that of reference respectively. While in case of NAXPM-19 under fed condition, the values were less than 15% lower than that of reference. On observing food effect it was found that for in fed condition C_{max} and T_{max} were on the higher side as compared to fasting condition. On applying analysis of variance, it was observed that no statistically significant effect (P>0.05) was observed, due to periods for $Ln(C_{max})$, $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$. Formulation effect was statistically significant for Ln (C_{max}), $Ln(AUC_{0-24})$ and $Ln(AUC_{0-t})$ (P<0.05) but for Ln $(AUC_{0-\infty})$ it was found to be non significant (P>0.05). No significant difference (p>0.10) was observed due to sequence for $Ln(C_{max})$, $Ln(AUC_{0-24})$, $Ln(AUC_{0-t})$ and $Ln(AUC_{0-\infty})$.

Predicted Steady state PK Parameters for Study-III.

Parameters	Acceptance Criteria	Results (NAXPM-19)
AUC: T/R	>0.8	0.892
AUC: Lower level of 90% CI	>0.7	0.778
C_{max}: T/R	Preferably ≤ 1 but not more than 1.25	0.800
Fluctuation: T/R	Preferably ≤ 1 but not more than 1.25	0.94
Inter individual variation for test	Less than that of reference	More than Reference
C_{min}	0.8 to 1µg/mL or more	0.75 µg/mL

From above table it was observed that for fast release formulation (NAXPM-19) in Biostudy-III, T/R ratio for AUC, C_{max} and Fluctuation was found to be 0.892, 0.800 and 0.94 respectively. C_{min} value and lower level of 90% CI for AUC was found to be 0.75µg/mL and 0.778 respectively. On comparing the inter-individual variation, it was observed that test formulation (NAXPM-19) is more variable than reference formulation.

On the basis of results given in table, it was observed that formulation NAXPM-19 meets the set acceptance criteria for steady state PK parameters i.e. AUC, C_{max}, fluctuation and lower level of 90% CI of AUC but acceptance criteria was not met for C_{min} and inter individual variation.

Formulation (NAXPM-20 with release profile of 16 hrs) was found to be best among the three formulations.

***In Vitro In vivo* correlation**

IVIVC was calculated by using IVIVC toolkit of WinNonlin® software. Level A correlation was established between fractions absorbed (F_{abs}) and fraction dissolved (F_{diss}). A correlation between *in vitro* and *in vivo* data (IVIVC) was established in order to reduce development time and optimize the formulation. Level A correlation represents a point-to-point relationship between *in vitro* dissolution and the *in vivo* input rate (e.g., the *in vivo* dissolution of the drug from the dosage form). In a linear correlation, the *in vitro* dissolution and *in vivo* input curves may be directly super imposable. The R^2 value was found to be more than 0.90 which clearly shows establishment of Level A correlation.

Biostudy	Batch No.	R2
1	NAXPM-15	0.976
	NAXLM-8	0.976
2	NAXPM-20	0.934
3	NAXPM-19	0.961

5.1.1 Summary

This thesis demonstrates several aspects of development strategy of controlled release tablet formulation of Mycophenolate Sodium.

- Preformulation studies viz., solubility studies, LOD, bulk density, Hausner ratio, compressibility index, angle of repose and particle size and drug excipient compatibility studies were carried out.
- An HPLC method for analysis of Mycophenolate Sodium was developed for assay and dissolution samples. The method was validated for accuracy, precision and repeatability.
- Bio-analytical method of Mycophenolate Sodium was developed by LCMS/MS and evaluated with respect to selectivity, matrix effect, accuracy and precision.
- Formulations were developed using lipid matrix and polymer matrix technology employing strategies viz., dry granulation method, wet granulation method, non-aqueous granulation.
- The formulations were found to be stable with respect to assay, RS and dissolution at accelerated conditions in three packaging systems *i.e.,* ALU-ALU, PVDC-PVC blister packs and HDPE bottle.
- Three biostudies were conducted on optimized formulations and one of the formulations was found to be suitable
- As per the set acceptance criteria for steady state Pharmacokinetic parameters, slow release test formulation (NAXPM-20) was found to be best amongst the

two test formulations i.e. slow release (NAXPM-20) and fast release (NAXPM-19).

- Effect of food on the bioavailability of developed MR formulation was investigated. It was observed that food had a significant effect on the AUC and C_{max} of MR formulation.
- Level A *in vitro in vivo* correlation was established from the dissolution profile and plasma time concentration data
- Based on the novelty, innovation and non obviousness of the developed formulation, three patents were filed which are in different stages of prosecution.
- The possibilities for commercialization of the controlled release tablet formulation of Mycophenolate Sodium were foreseen. When the formulation will be developed for commercial use, it will be a breakthrough in the treatment of organ transplant patients.

The present investigation gives an insight into the requirement of the studies for the development and commercialization of controlled release oral formulation and inspires further work in this direction, which ultimately is going to be beneficial to the society. But before commercialization or regulatory filing a few more studies are required which are recommended below:

5.2 Future scope of work:

Following work is recommended for the formulations

- Multiple Dose studies should be conducted to determine the pharmacokinetic parameters viz., C_{min}, AUC, swing, percentage fluctuation
- Phase II Clinical trial for organ transplant patients.
- Regulatory approvals and commercialization for the benefit of the society.

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7.0 List of Patents and Presentations

7.0 LIST OF PATENTS AND PRESENTATIONS

7.1 Jain Rajesh, Singh Sukhjeet and Devarajan Sampath Kumar, Indian Application No. 1714/DEL/2007 entitled "Extended release compositions comprising Mycophenolate sodium and processes thereof", filed on 13/08/2007.

7.2 Jain Rajesh, Singh Sukhjeet and Devarajan Sampath Kumar, PCT Application No. PCT/IN2008/000505 entitled "Extended release compositions comprising Mycophenolate sodium and processes thereof", filed on 11/08/2008.

Abstract

EXTENDED RELEASE COMPOSITIONS COMPRISING MYCOPHENOLATE SODIUM AND PROCESSES THEREOF

Extended release pharmaceutical compositions comprising Mycophenolate sodium as the active agent, wherein the said composition exhibits a characteristic release profile when subjected to in-vitro dissolution study, and wherein said Mycophenolate sodium is released in a sustained manner in-vivo for a prolonged duration in such quantities that substantially alleviates or at least reduces the chances of causing any associated gastrointestinal side effect(s) without compromising the bioavailability of the said active agent are provided. The present invention also provides process of preparing such dosage form compositions and prophylactic and/or therapeutic methods of using such dosage form. The composition of the present invention are safe, effective and well-tolerated, and are useful for the management such as prophylaxis, amelioration and/or treatment of immunosuppressant indicated disease(s)/disorder(s) especially for the treatment or prevention of organ, tissue or cellular allograft or Xenograft rejection, e.g. after transplant, or the management of immune-mediated diseases (autoimmune diseases).

7.3 Jain Rajesh, Singh Sukhjeet and Devarajan Sampath Kumar, Indian Application No. 2107/DEL/2007 entitled "High dose solid unit oral pharmaceutical dosage form of Mycophenolate sodium and process of making the same", filed on 8/10/2007

7.4 Jain Rajesh, Singh Sukhjeet and Devarajan Sampath Kumar, PCT Application No. PCT/IN2008/000651 entitled "High dose solid unit oral pharmaceutical dosage form of Mycophenolate sodium and process of making the same", filed on 8/10/2008.

Abstract

HIGH DOSE SOLID UNIT ORAL PHARMACEUTICAL DOSAGE FORM OF MYCOPHENOLATE SODIUM AND PROCESS FOR MAKING SAME

Provided are high dose solid unit oral pharmaceutical dosage form compositions comprising Mycophenolate sodium as active agent in an amount of from greater than about 720 mg to about 1500 mg, preferably from about 800 mg to about 1440 mg calculated as Mycophenolic acid, and one or more pharmaceutically acceptable excipient(s). Particularly the dosage form compositions are meant for once-a-day or twice-a-day administration and provide the active agent in an extended release form which is released in a sustained manner in-vivo for a prolonged duration. The invention is also directed to process of manufacturing the high dose formulations, and prophylactic and/or therapeutic methods of using such dosage forms. The composition of the present invention are easy to formulate, safe, effective and well-tolerated, and are useful for the management such as prophylaxis, amelioration and/or treatment of immunosuppressant indicated disease(s)/disorder(s) especially for the treatment or prevention of organ, tissue or cellular allograft or xenograft rejection, e.g. after transplant, or the management of immune-mediated diseases (autoimmune diseases).

7.5 Jain Rajesh, Singh Sukhjeet, Indian Application No. 549/DEL/2008 entitled "Modified release pharmaceutical compositions comprising Mycophenolate and processes thereof" filed on 05/03/2008.

7.6 Jain Rajesh, Singh Sukhjeet PCT Application No. PCT/IN2009/000148 entitled "Modified release pharmaceutical compositions comprising Mycophenolate and processes thereof" filed on 04/03/2009.

Abstract

MODIFIED RELEASE PHARMACEUTICAL COMPOSITIONS COMPRISING MYCOPHENOLATE AND PROCESSES THEREOF

Modified release pharmaceutical compositions comprising Mycophenolate as the active agent or its pharmaceutically acceptable salts, esters, polymorphs, isomers, prodrugs, solvates, hydrates, or derivatives thereof, wherein the said composition exhibits a biphasic release profile when subjected to in-vitro dissolution and/or upon administration in-vivo are provided. The composition provides a drug release in a manner such that the drug levels are maintained above the therapeutically effective concentration (EC) constantly for an extended duration of time. Further, the difference between the maximum plasma concentration of the drug (Cmax) and the minimum plasma concentration of the drug (Cmin), and in turn the flux defined as ((Cmax – Cmin)/Cavg) is minimal. The present invention also provides process of preparing such dosage form compositions and prophylactic and/or therapeutic methods of using such compositions.

7.7 Presentation

Sukhjeet Singh, Shubhrangshu Shekher Sarkar, Sanju Dhawan, Roop K. Khar **"Design and Development of Once a Day Formulation for Immunosuppressant".** National Conference on Emerging Trends in Life Sciences Research, March 6-7, 2009, Biological science Group & Pharmacy Group of Birla Institute of Technology & Science Pilani, Rajasthan.

Abstract

The field of transplantation remains one of the most innovative and pioneering areas in medicine today. The objective is to prolong life. However, transplantation itself is not enough; there is a need to balance the amount of immunosuppression necessary to assure graft survival with the potential toxicity of the immunosuppressive agents. Cyclosporine is the basis for many immunosuppressive regimens, and great success has been obtained in combination with other immunosuppressants *viz.,* inosine monophosphate dehydrogenase (IMPDH) inhibitors, as Mycophenolic acid etc [1, 2]. In current clinical practice the oral formulations of available immunosuppressants are generally administered on a twice daily basis. Poor compliance has been shown to be one of the factors associated with late graft loss. Hence, there is a need to develop once a day formulations for these agents which not only increase patient compliance but also reduce the adverse effects [3].

Mycophenolate Sodium is a relatively new immunosuppressive drug. It inhibits inosine monophosphate dehydrogenase, a key enzyme in the *de novo* pathway of

purine synthesis, and thus causes lymphocyte-selective immunosuppression [4]. In current clinical practice the oral formulations of available Mycophenolate Sodium, at least two tablets are generally administered on a twice daily basis which leads to patient compliance concerns. Hence, developing a patient compliant dosage form of selected immunosuppressant is the need of the hour. The modified product will obviate this need. Moreover, the adverse events seem to be related to C_{max}, whereas immunosuppressant activity is related to total exposure *i.e.,* AUC. Therefore, it is envisaged that with MR product, Minimum Effective Concentration (MEC) levels will be maintained for longer duration of time suggesting very low probability of rejection.

In the present investigation, it was intended to exploit lipid as well as polymer matrix system to achieve controlled release of Mycophenolate Sodium. The study aims at examining the range of polymers/combinations for preparation of Mycophenolate Sodium tablets with various evaluation parameters. In addition to the formulation development, the plan of the study included: solubility determination, preformulation studies, analytical method development and validation for the quantitation of Mycophenolate Sodium for assay and dissolution samples, formulation development, *in vitro* dissolution tests and effects of diluents on drug release profile, fitting of the dissolution profile into mathematical models to ascertain the mechanism of drug release and stability studies of the optimized formulations. The bioavailability studies of the selected formulations were conducted in human volunteers. The mean values of $C_{max,}$ $T_{max,}$ $AUC_{0\text{-}24}$ and AUC_{inf} were found to be 8.23 µg/mL, 1.54 h, 39.344 h*µg/mL and 52.196 h*µg/mL respectively for test formulation. For reference formulation in fasted condition, the average $C_{max,}$ $T_{max,}$ $AUC_{0\text{-}24}$ and AUC_{inf} were found to be 29.993 µg/mL, 0.924 h, 47.034 h*µg/mL and 59.039 h*µg/mL respectively. There results revealed that there was statistically significant difference for C_{max} $(P<0.0001)^{***}$ and T_{max} $(P<0.05)^{*}$ for the formulations. On comparing $AUC_{0\text{-}24}$ and AUC_{inf}, both the formulations were comparable with P-values 0.168 and 0.3689 respectively. A significant effect of food was observed and the average values for $C_{max,}$ $T_{max,}$ $AUC_{0\text{-}24}$ and AUC_{inf} were found to be increased by 36.18%, 58.93%, 2.5% and 4.35% respectively.

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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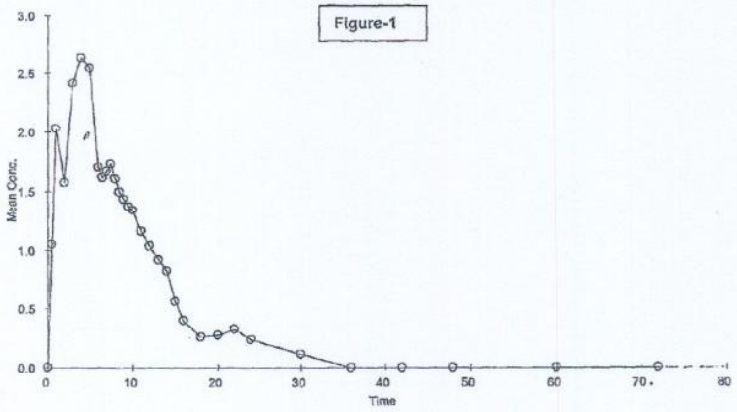
[Continued on next page]

(54) Title: EXTENDED RELEASE COMPOSITIONS COMPRISING MYCOPHENOLATE SODIUM AND PROCESSES THEREOF

Figure-1



(57) Abstract: Extended release pharmaceutical compositions comprising mycophenolate sodium as the active agent, wherein the said composition exhibits a characteristic release profile when subjected to in-vitro dissolution study, and wherein said mycophenolate sodium is released in a sustained manner in-vivo for a prolonged duration in such quantities that substantially alleviates or at least reduces the chances of causing any associated gastrointestinal side effect(s) without compromising the bioavailability of the said active agent are provided. The present invention also provides process of preparing such dosage form compositions and prophylactic and/or therapeutic methods of using such dosage form. The composition of the present indention are safe, effective and well-tolerated, and are useful for the management such as prophylaxis, amelioration and/or treatment of immunosuppressant indicated disease(s)/disorder(s) especially for the treatment or prevention of organ, tissue or cellular allograft or xenograft rejection, e.g. after transplant, or the management of immune-mediated diseases (autoimmune diseases).

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(74) Agent: **GUPTA, Bhartee**; Panacea Biotec Limited, B-1 Extn./A-27Mohan Co-operative Industrial Estate, Mathura Road, New Delhi 110044 (IN).

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(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *of inventorship (Rule 4.17(iv))*

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(54) Title: HIGH DOSE SOLID UNIT ORAL PHARMACEUTICAL DOSAGE FORM OF MYCOPHENOLATE SODIUM AND PROCESS FOR MAKING SAME

(57) Abstract: Provided are high dose solid unit oral pharmaceutical dosage form compositions comprising mycophenolate sodium as active agent in an amount of from greater than about 720 mg to about 1500 mg, preferably from about 800 mg to about 1440 mg calculated as mycophenolic acid, and one or more pharmaceutically acceptable excipient(s). Particularly the dosage form compositions are meant for once-a-day or twice-a-day administration and provide the active agent in an extended release form which is released in a sustained manner in-vivo for a prolonged duration. The invention is also directed to process of manufacturing the high dose formulations, and prophylactic and/or therapeutic methods of using such dosage forms. The composition of the present invention are easy to formulate, safe, effective and well-tolerated, and are useful for the management such as prophylaxis, amelioration and/or treatment of immunosuppressant indicated disease(s)/disorder(s) especially for the treatment or prevention of organ, tissue or cellular allograft or xenograft rejection, e.g. after transplant, or the management of immune-mediated diseases (autoimmune diseases).

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(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **JAIN, Rajesh** [IN/IN]; B-1Extn. A/27, Mohan Co-operative Industrial Estate, Mathura Road, New Delhi 110 044 (IN). **SINGH, Sukhjeet** [IN/IN]; B-1 Estn. A/27, Mohan Co-operative Industrial Estate, Mathura Road, New Delhi 110 044 (IN).

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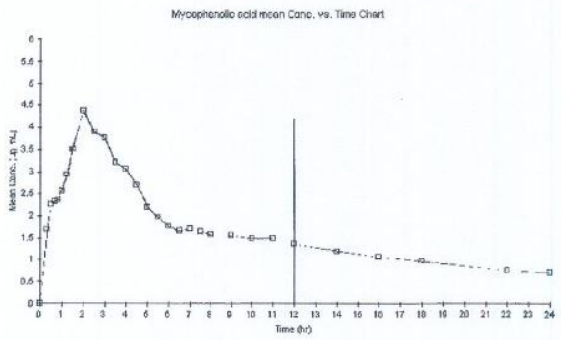
(54) **Title**: MODIFIED RELEASE PHARMACEUTICAL COMPOSITIONS COMPRISING MYCOPHENOLATE AND PROCESSES THEREOF

Fig 8: The plasma concentration vs. time chart for the example 3 as mentioned hereinabove is reproduced below:



(57) **Abstract**: Modified release pharmaceutical compositions comprising mycophenolate as the active agent or its pharmaceutically acceptable salts, esters, polymorphs, isomers, prodrugs, solvates, hydrates, or derivatives thereof, wherein the said composition exhibits a biphasic release profile when subjected to in- vitro dissolution and/or upon administration in- vivo are provided. The composition provides a drug release in a manner such that the drug levels are maintained above the therapeutically effective concentration (EC) constantly for an extended duration of time. Further, the difference between the maximum plasma concentration of the drug (Cmax) and the minimum plasma concentration of the drug (Cmjn), and in turn the flux defined as ((Cmax - Cmjn)/Cavg) is minimal. The present invention also provides process of preparing such dosage form compositions and prophylactic and/or therapeutic methods of using such compositions.

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SUKHJEET SINGH

AT PRESENT:

Since November 2006, Vice President, Research and Development of Panacea Biotec Ltd., a state-of-the-art research facility located at Lalru, Punjab.

PROFESSIONAL EDUCATION: M. Pharma (Pharmaceutics) from Jamia Hamdard University, Hamdard Nagar, New Delhi, INDIA (1992); PG Diploma in Marketing and Sales Management (1991) and B. Pharma from Delhi University, INDIA (1990).

PROFESSIONAL CAREER: Started professional career in Max Pharma Ltd., New Delhi, India (1992-1995); further worked with esteemed organizations as Panacea Biotec Ltd., New Delhi – India (1995-2005), Strides Arcolab Limited, Bangalore, India (2005-2006).

RESEARCH INTERESTS: More than 15 years of active research experience in the field of New Drug Delivery System. The area of research is primarily focused on development of products based on highly specialized drug delivery technologies including Site Specific Drug Delivery, Self Nano-emulsifying injectable System, Depot injectable Preparations, Pulmonary Drug Delivery and Oral Controlled Release Systems.

PROFESSIONAL RECOGNITIONS:

Member of ***American Association of Pharmaceutical Scientists*** (*AAPS*)**,** European Federation for Pharmaceutical Sciences (*EUFEPS*), American Chemical Society **(ACS)**, USA.

PATENTS AND PUBLICATIONS: 26 world wide patents granted and more than 10 Research Publications / poster presentations in the area of formulation research and development.

PROFESSOR ROOP KRISHEN KHAR

Professor Roop Krishen Khar is a renowned academician and research scientist. He obtained B. Pharm and M. Pharm degree from Dr. Hari Singh Gour Vishvavidyalaya (SAGAR UNIVERSITY) in the years 1971 and 1973 respectively and was the University gold medalist for standing 1st in B. Pharm. He was selected by Ministry of H.R.D., Govt. of India for a Ph.D. Fellowship under Indo-Bulgaria cultural exchange programme during 1982-86. During his cultural exchange fellowship he visited most of the east and west European countries, as a part of his academic programme. Dr. Khar has developed a research school in Controlled Drug Delivery Systems and Pharmaceutical Product Development at Department of Pharmaceutics in Jamia Hamdard. Dr. Khar has supervised 45 Ph.D, 65 M. Pharm theses and published more than 220 research papers in International and National journals. He is the Principal Investigator of a number of research projects funded by various government agencies and Pharmaceutical Industries. Dr. Khar has filed 9 Indian and one US Patents in the last few years. He has published nine text and reference books so far and is the member of Editorial Board of Indian Journal of Pharmaceutical Sciences and Indian Journal of Pharmaceutical Education and Research. He is a widely traveled person in India, European Countries, South Africa, UAE and USA. Dr. Khar has been the Dean, Faculty of Pharmacy, Jamia Hamdard, and at present he is Professor, Head of Department of Pharmaceutics and Dean, Students welfare of Jamia Hamdard (Hamdard University). He takes very active interest in professional matters and has been the President of Indian Pharmaceutical Association (Delhi Branch) during the years 2000-2004, Treasurer of IPCA during 2000-2003 and Co-Chairman of 53rd local organizing committee of Indian Pharmaceutical Congress held at Pune in Dec 2002 and at Hydrabad in Dec. 2005 and from time to time at various International and National conferences and Symposia. He is a member of important selection committees of many Universities, UPSC and an Expert member of various statutory bodies of AICTE and PCI. Recently he has been nominated by Ministry of Health Govt. of India as an Expert member of "Dosage Form and Formulation Committee" of Indian Pharmacopoeia Commission and as a member of Unani Pharmacopoeia Committee.