Design and Development of Extended Release Formulations of Anti-diabetic Drugs

THESIS

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by
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Under the Supervision of
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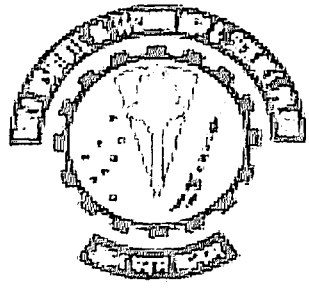

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CERTIFICATE

This is to certify that the thesis entitled "**Design and Development of Extended Release Formulations of Anti-diabetic Drugs,**" and submitted by YATISH KUMAR, ID No. 2004PHXF437 for award of Ph.D. Degree of the institute, embodies original work done by him under my supervision.

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"Thanking may be just a formality, but if done inwardly, it surely does reflect your noblest thoughts within."- Louis Philippe II

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SUMMARY

Diabetes is a disease characterized by excess sugar in the blood (hypoglycemia). While type 1 diabetes is due to lack of insulin, type 2 (which affects 90% of all diabetics) is the result of an inappropriate production of insulin and the insulin-resistance of certain tissues, particularly the liver and muscle. According to a study carried out by WHO, there will be about 300 million diabetics worldwide in 2025. Lack of exercise and richer and fatter food resulting in weight gain, explain the increase in numbers. Type 2 diabetes, an insidious disease that develops over the years without any apparent major symptoms, is usually diagnosed between the ages of 40 and 50, particularly in people who are overweight. Diabetes leads to numerous complications that not only affect the quality of life but can also be fatal.

Phenformin was the first biguanide available for clinical use. However, it was withdrawn from clinical use in the USA and other countries in 1977, following reports of its association with the relatively high incidence of lactic acidosis. Biguanides made resurgence in the 1990's following synthesis of some less toxic compounds of which metformin (MFH) is preferred by the clinicians. Another newer biguanide, buformin, is also in clinical use in certain countries.

MFH has a valuable role in the treatment of diabetes mellitus because it can exert its hypoglycemic effect even in absence of insulin. By virtue of this property, MFH has a distinct advantage over more commonly used sulphonylureas. MFH is the drug of choice in obese diabetics whose hyperglycemia is more due to insulin resistance as MFH does not cause weight gain and does not provoke attacks of hypoglycemia. It has further advantage over other sulphonylureas in treatment of obese diabetics. Moreover, MFH is frequently used in combination with other anti-diabetic drugs (like sulphonylurea) in treatment of type 2 diabetes mellitus when treatment with a drug alone is ineffective.

An obstacle to more successful use of MFH therapy is the high incidence of concomitant gastrointestinal (GI) symptoms such as abdominal discomfort, nausea and diarrhea that specially occur during the initial weeks of treatment. Side effects and the need for administration two or three times per day when large doses are required can also decrease patient compliance. Therefore there is a strong need to develop an extended release (ER)

formulation of MFH alone and in combination with other diabetic drugs as a single unit dosage form that would maintain plasma level of drug for 8 to 12 hours, sufficient for once daily dosing. Modified release preparations can also, avoid the problem of dose dumping, i.e., sudden release and absorption of a large amount of drug. This has great significance because dose dumping in case of oral hypoglycemic can lead to hypoglycemia.

Therefore, the aim of the research work was to design and develop a stable and efficacious platform technology for MFH extended release tablet, which can further be combined with other anti-diabetic drugs [like gliclazide (GLZ) and glimiperide (GPD) etc.] in a single dosage unit for better patient compliance.

To support the research work, various stability indicating UV-spectroscopic and HPLC analytical methods were developed and validated for estimation of MFH, GLZ and GPD in bulk, formulations, dissolution samples and human plasma. Preformulation studies were carried out so as to quantitate various physicochemical properties such as solubility, pH stability, intrinsic dissolution rate, drug stability, photo-stability, particle size analysis, hygroscopicity and drug-excipient incompatibility study at various stress conditions. Most of the excipients showed no incompatibility problems with MFH, GLZ and GPD at various studied conditions. MFH was found to be highly water soluble whereas, GLZ and GPD were practically insoluble in water and had high pH dependant solubility.

Biodegradable polymers in various proportions, alone and in combinations were tried to achieve predetermined dissolution profile for MFH ER tablets. Various viscosity grades of the polymer were used to optimize the formulation. Based on the results of physical parameters, drug content and dissolution profile, the best formulation was selected and subjected to accelerated stability study as per ICH guidelines. The formulation was stable w.r.t. physical and chemical parameters at studied conditions in the required pack (aluminum strip).

The single dose pharmacokinetics of MFH ER tablet in 12 healthy volunteers under fasting condition was compared with the currently marketed immediate release, (IR) MFH tablet using cross over design. The mean bioavailability from MFH ER tablet was approx. 115%, relative to immediate release (IR) product. C_{max} values were lower (550 ±

130 ng/ml) and t_{max} values were greater (5.6 ± 1.1 hour) for the ER formulation compared with the IR product (C_{max} = 740 ± 180 ng/ml and t_{max}= 3.5 ± 0.7 hours).

This developed platform technology was used as a stepping stone for preparing a single dosage unit tablet for rational use of various anti-diabetic drug combinations, either in immediate release form (GPD) or extended release form (GLZ). Various novel technological approaches (drug loading, tab-in-tab, multi-layered tablets) were tried. Based on the experimental trials the best results were obtained with bi-layered tablet formulation. This technology was more sensitive to machine variables, therefore a number of trials were taken to decide the ideal machine parameters [pre-compression force, compression force and speed of the machine (rpm)] to achieve tablets of the desired specifications.

Formulation of the GLZ ER and GPD IR portions were finalized separately based on number of experimental trials, their evaluation and stability studies. The combination bi-layered tablets (MFH ER + GLZ ER and MFH ER + GPD IR) were studied at accelerated (40°C/75% RH) and controlled temperature conditions (25°C/60% RH). The stability results were satisfactory w.r.t. physical parameters, drug content and dissolution profile.

GLZ portion was developed in extended release formulation to meet the clinical needs, such as patient's age and renal impairment etc.

The bioavailability of single unit dosage (i.e. MFH ER + GLZ ER) was compared with IR formulation of commercially available market preparation of GLZ in 12 healthy volunteers under fasting conditions in a cross-over design. The plasma samples were analyzed for MFH content also in addition to GLZ. The pharmacokinetic parameters of MFH ER remained unchanged when administered alone, in combination with GLZ ER (co-administration) and from single unit dosage tablet. The relative bioavailability of ER formulation was approx. 109%, lower C_{max} (2.24±0.55μg/ml) and extended t_{max} (9.1± 3.2 hours) as compared to the IR formulation (C_{max} = 4.89±1.19 μg/ml and t_{max}= 3.61±1.148 hours). It was concluded that GLZ ER tablet has good sustained release property when combined with MFH ER tablet in a single unit dosage tablet. Moreover there was no interference in the pharmacokinetics parameters when drugs were administered alone and in combination.

The technology of making bi-layered was perfectly standardized to get reproducible results making the manufacturing process robust and can be explored for commercial manufacturing. In order to protect the product and product technology, various national and international patents were applied.

The developed platform technologies can be combined with other identified rational anti-diabetic drugs (not studied in this research work) in multiple-layered tablets (bi-layered or tri-layered tablets). The same platform technology can be used for combining other strengths of the same drugs or combining drugs of other therapeutic categories e.g. anti-hypertensive drugs (not studied in this research work). Tri-layered technology can be used for combining more than two drugs.

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LIST OF ABBREVIATIONS / SYMBOLS

Abbreviation / Symbol	Meaning
ACN	Acetonitrile
Approx.	Approximately
ANDA	Abbreviated New Drug Application
ANOVA	Analysis of Variance
AUC	Area Under the Curve
b.i.d	Twice a Day
BMI	Body Mass Index
B.P.	British Pharmacopoeia
C_{max}	Concentration Maximum of Drug
CR	Controlled Release
CRO	Contract Research Organization
CRT	Controlled Room Temperature
DM	Diabetes Mellitus
D.R	Drug Release
DSC	Differential Scanning Calorimetry
DUREDAS	Dual Release Drug Absorption System
E.P.	European Pharmacopoeia
f_2	Similarity Factor
FFA	Free Fatty Acids
FT	Fridge Temperature
GCP	Good Clinical Practices
GIT	Gastro Intestinal Tract
GLP	Good Laboratory Practices
GLZ	Gliclazide
GPD	Glimepiride
HPC	Hydroxy Propyl Cellulose
HPLC	High Performance Liquid Chromatography

Abbreviation / Symbol	Meaning
HPMC	Hydroxy Propyl Methyl Cellulose
HPMCP	Hydroxy Propyl Methyl Cellulose Phthalate
I.C.H	International Conference on Harmonization
ID	Internal Diameter
IDF	International Diabetes Federation
IDDM	Insulin Dependent Diabetes Mellitus
IND	Investigational New Drug
I.P.	Indian Pharmacopoeia
I.P.A	Iso Propyl Alcohol
IR	Infra Red
i.v.	Intravenous
K_{el}	Elimination Rate Constant
K_{deg}	Degradation Rate Constant
LC	Liquid Chromatography
LC- UV	Liquid Chromatography Ultra Violet
L-HPC	Low Substituted Hydroxy Propyl Cellulose
LOD	Limit Of Detection
Lod	Loss on drying
LOQ	Limit Of Quantification
LV	Low Viscosity
MALA	Metformin Associated Lactic Acidosis
MCC	Micro Crystalline Cellulose
MFH ER	Metformin Hydrochloride Extended Release
MFH IR	Metformin Hydrochloride Immediate Release
MFH WS	Metformin Hydrochloride Working Solution
M1	Cyclohexyl Hydroxy Derivative of MFH
M2	Carboxyl Derivative of MFH
NaOH	Sodium Hydroxide
NDA	New Drug Application

Abbreviation / Symbol	Meaning
NIDDM	Non Insulin Dependent Diabetes Mellitus
NIR	Near Infra Red
NSAIDS	Non Steroidal Anti Inflammatory Drugs
NMT	Not More Than
OCRS	Oral Controlled Release System
OD	Once a Day
PEG	Poly Ethylene Glycol
P.Talc	Purified Talc
PVC	Polyvinyl Chloride
PVP	Poly Vinyl Pyrollidone
q.i.d	Four times a Day
R	Range
R	Correlation
RH	Relative Humidity
Rpm	Revolutions Per Minute
RSD	Relative Standard Deviation
RT	Retention Time
SD	Standard Deviation
SGF	Simulated Gastric Fluid
SLS	Sodium Lauryl Sulphate
SOP	Standard Operating Procedures
SSG	Sodium Starch Glycollate
T.i.d	Thrice a Day
T2DM	Type 2 Diabetes Mellitus
$t_{1/2}$	Biological Half Life
T_{max}	Time at which maximum drug concentration has reached
THF	Tetrahydrofuran
TZD	Thiazolidenediones
UKPDS	United Kingdom Prospective Diabetes Study

Abbreviation / Symbol	Meaning
USFDA	United States Food and Drug Administration
USP	United states Pharmacopoeia
UV	Ultraviolet
WHO	World Health Organization
w.r.t.	with regard to
~	Approximately

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Chapter 1

Introduction

1.1 Diabetes:

India a developing country, with fast industrialization and rapid progress on all fronts, is making big strides towards global recognition. The flip side is that the economic prosperity and modern way of life is translating into an increase in lifestyle related diseases. Home to nearly millions of diabetic today, India is fast becoming the diabetic capital of the world [1].

Diabetes is one of the costliest health problems in the world. Globally, diabetes is likely to be the fourth leading cause of death [2]. Diabetes mellitus is a disorder of metabolism. Under normal conditions, a proper balance of sugar is restored by the action of Insulin- a hormone produced by the pancreas. Insulin helps the way the body uses or converts food for energy and growth. Low levels or ineffectiveness of insulin results in the blood sugar levels remaining high indicates the development of diabetes mellitus [2].

Most diabetics have no symptoms. It only gets diagnosed when people go in for insurance or job employment health check-ups. The commonest symptom of diabetes is "no symptom". Since the beginning is insidious, there is an average delay of three to five years in diagnosis. By the time the condition is diagnosed minimal changes like abnormal lipid profile, hypertension, retinal changes are already present [3].

Diabetes is one of the largest therapeutic segments of global pharmaceutical sales and during the last 10 years it has grown by a compound average growth rate of nearly 20% from around US$4 billion in 1995 to over US$17 billion in 2005. Overall, anti-diabetic drugs sales are expected to grow dramatically over the next five years to over US$22 billion in 2012 [3,4,5]. The growth is attributed to increase in the number of addressable patients and new oral premium priced products.

1.2 Types of diabetes

There are two main types of diabetes – type 1diabetes and type 2 diabetes [1]. Other two types of diabetes discussed here are very rare and very less prevalent.

Type 1 diabetes: It was previously called insulin-dependant diabetes-mellitus (IDDM) or juvenile-onset diabetes. Type I diabetes develops when the body's immune system destroys pancreatic beta cells, the only cells in the body that make the hormone insulin

that regulates blood glucose. To survive, people with type I diabetes must have insulin delivered by injection or a pump. This form of diabetes usually strikes children and young adults, although onset of disease can occur at any age.

Type 2 diabetes: It was previously called non-insulin-dependant diabetes-mellitus (NIDDM) or adult-onset diabetes. Approximately 90% of people with diabetes have type 2 diabetes as shown in Fig. 1.1 [1]. It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises; the pancreas gradually loses its ability to produce insulin. Type II diabetes is associated with older age, obesity, family history of gestational diabetes, impaired glucose metabolism, physical inactivity and race/ ethnicity [6].

Gestational diabetes: Another type of diabetes is gestational diabetes which is a temporary form. It is a form of glucose intolerance diagnosed in some women during pregnancy. It usually disappears after pregnancy. It occurs more frequently among African Americans, Hispanic/Latino Americans and American Indians [7,8]. It is also more common among obese women and women with a family history of diabetes.

Others: Other type of diabetes result from specific genetic conditions (such as maturity – onset diabetes of youth), surgery, drugs malnutrition, infections and other illnesses.

"Diabetes care for everyone" was the slogan for the year, 2006 "World Diabetes Day". Doctors and medical practitioners the world over feel that the power to prevent and control diabetes is in our hands [2].

1.3. National estimates on diabetes:

Diabetes Prevalence:

Diabetes is a life-threatening condition. It is a silent killer that kills one person every 10 seconds. Worldwide, 3.2 millions deaths are attributable to diabetes every year [9]. At least one in ten deaths among adults between 35-64 years old is attributable to diabetes.

India has the largest diabetics in the world and every fourth diabetic is in India. In India diabetes is growing at an epidemic rate [1].

Current estimate [2] indicates that worldwide about 170 million people suffer from diabetes, which is projected to reach 366 million by 2030, that means there is estimated rise of whooping 115% in the diabetic patients.

World Health Organization says there are 33 million diabetics in India and at this rate it is estimated to touch 80 million by 2030 [7]. Close to 30% of the population is unaware of the fact that they are suffering from some type of diabetic condition which is quite alarming.

The importance of age on the prevalence of diabetes is as illustrated in Fig. 1.2 which shows sex-specific estimates of diabetes prevalence by age [1].

A comparison of diabetes prevalence between the two sexes suggests that diabetes affects significantly more men than women and also that the prevalence increases after the age of seventy five. By 2030, it is estimated that the number of people with diabetes will be more than 82 million after 64 years of age in developing countries and it will be more than 48 million in developed countries [2].

The top 3 countries estimated to have the highest number of people with diabetes in 2000 and 2030 are India, China & US [2].

The International Diabetes Federation (IDF) estimates direct costs of diabetes to be approximately 6% of the total health budget of economically developed countries. A combination of demographic and lifestyle factors is forecast to more than double the incidence of diabetes between 2002 and 2030, according to the WHO. In 2002, the US direct cost was 92 billion US dollars and the direct and indirect cost (in terms of loss of manpower) totaled 132 billion dollars [5] as shown below.

Cost of diabetes in the USA ($ billions)

Costs	1997	2002	% Growth
Direct medical	44	92	109.1
Indirect	54	40	-25.9
Total	98	132	34.7

1.4. Management of the disease: Treatment options:

Patients with type 1 diabetes mellitus (DM) should receive insulin replacement therapy. On at least a temporary basis, the use of intermediate-or long-acting insulin for controlling fasting plasma glucose, alone or in addition to oral agents, should be considered for patients with type 2 DM in whom oral agents have proven ineffective, intolerable, or are contraindicated, rapid restoration of euglycemia is desirable (e.g., patients with persistent symptoms of diabetes or with hyperglycemia in per operative and/or critical care settings) and where pregnancy is desired or has already occurred [10]. Also it should be considered for patients in whom relative insulin deficiency is suggested by weight loss and persistent, non-fasting ketosis. Diet and exercise and lifestyle modification should be encouraged [11,12].

Control of diet and exercise is usually the appropriate initial management in patients with new onset type 2 diabetes, depending upon the severity of the symptoms, psychosocial evaluation, and overall health status. If treatment goals are not achieved with diet and exercise alone, drug mono-therapy should be initiated [13].

Initial mono-therapy with a sulfonylurea or biguanide (i.e., metformin) should be used as first line drug therapy. Sulfonylurea can be considered for most patients with type 2 diabetes; however, for those who are significantly overweight (body mass index [BMI] >25), initial monotherapy with a biguanide may be preferable [14,15]. Thiazolidinediones (TZDs) are not recommended as monotherapy for patients with type 2 DM, unless there is documented and unacceptable intolerance to metformin and available sulfonylurea agents [16].

Other oral agents, while less effective, are still appropriate first line agents if the desired increase in HbA_1c is proportionally less or if there are additional contraindications to the other first line medications [17,18]. If the glycemic target level is not achieved with one oral agent alone, combination oral and/or insulin therapy is recommended [15,19]. Stepwise approach for diabetes treatment is given Fig.1.3.

1.5. Oral therapeutic agents:

The oral route of drug administration is the most important method of administering drugs for systemic effects. Except in cases of insulin therapy, the parentral route is not

routinely used for self administration of medication [10]. Topical route of administration has only recently been employed to deliver drugs to the body for systemic effects but is limited in its ability to allow effective drug absorption for systemic drug action. It is probable that at least the oral route administers 90% of all drugs used to produce systemic effects. The significance of the oral formulations can be gauged from the Fig.1.4, which represents 76% of the market value being occupied by oral formulations while non-oral formulations contribute only 24% to the total market value [5]. This can be attributed to the ease of administration of oral products and hence, greater patient compliance. Of the drugs those are administered orally, solid dosage forms represent the preferred class of product. The reason for this preference is, tablets and capsules represent unit dosage forms in which one usual dose of the drug has been accurately placed. Fig.1.5 indicates treatment statistics for diabetes mellitus [15].

1.5.1 Oral Monotherapy:

Pharmacotherapy for type 2 diabetes is necessary to contain the sustained $HbA1_c$ drifts by the uptitration of existing drug(s). The conventional approach comprises introducing monotherapy upon failure of nonpharmacological measures such as diet and exercise. Various drugs act by different mechanism to lower hyperglycemia. Biguanides like metformin act preferentially on the glucose production from the liver. Metformin is often used in obese patients until contraindicated or not tolerated. Sulfonylureas like gliclazide and glimepiride increase insulin secretion and are prescribed in leaner patients. Newer additions like thiazolinediones act on the insulin resistance state of muscles and adipocytes. Glucosidase inhibitors act in the intestine to block the action of enzymes that are responsible for breaking down carbohydrates into simple sugars. Oral anti diabetics are categorized into five classes as given Table 1.1 [20].

1.5.2 Oral combination therapy

Despite many advances in the development of oral hypoglycemic agents, an ideal drug for treating Type 2 diabetes is still a distant reality. Today, physicians can choose from among a variety of medications targeting numerous facets of disease, but each drug class poses some problems. The age-old molecules such as sulfonylureas and biguanides are

still considered drugs of choice because of their well-studied mode of action, safety, better tolerability and ideal pharmacodynamic effects [15,21]. Until we find an ideal drug for type 2 diabetes, there is much scope and interest for pharmaceutical companies to modify the pharmacokinetics of older molecules in order to better suit larger sections of patients [15,22]. This compilation is an attempt to describe the advances in drug delivery of oral hypoglycemic agents, particularly the extended and sustained release formulations of individual and metformin combinations, both of which have great promise in treatment of type 2 diabetes mellitus.

Combination oral therapy becomes an obvious choice when glycemic control is not achieved with conventional monotherapy. The advantages of oral dose combinations as compared to their components which are taken alone are lower cost and better patient compliance [22,23].

Combination therapy has been shown to achieve greater blood glucose lowering than mono-therapy because different classes have different and complimentary mechanisms of action. Therefore, it is more logical to add another drug than replace the existing drug. The rapid introduction of combination therapy with two or three complementary oral anti diabetics help in targeting the dual effect and also reduced adverse effects [23].

Type 2 diabetes pathophysiology knowledge leads us to the combination therapy concept but progress in pharmaceutical industry may rapidly move us from a tritherapy to a penta or perhaps hexatherapy. The combination of an insulin secretagogue and metformin seems to be logical [14,23]. Thus due to their promise for future clinical success and because they exhibit mechanisms of action distinct from current therapies, Metformin and its combinations have been studied here.

Combination of insulin secretion- enhancing drugs and Metformin:

The combination of sulfonylurea and metformin is largely used because both drugs are ancient. A large number of studies have demonstrated their synergistic effects. An improvement in blood glucose level and $HbA1_c$ was solely observed with the association of both drugs. The association of glinides with metformin is also interesting. Glinides (repaglinide, nateglinide) are rapid and short- acting insulin secretagogues. They lead to a new insulin drug-induced profile, different from the sulfonylurea profile. The association

with metformin is complimentary as glinides act in the postprandial state and metformin in the basal state. Studies report an improvement of 1.4% ± 0.2 in HbA_{1c} levels with both drugs in comparison with monotherapy. This result confirms the synergistic effect of an association that acts on two different sites of patho-physiologic abnormalities of type 2 diabetes [14].

Combination of metformin with thiazolidinediones:

Both metformin and thiazolidinediones are drugs of the insulin resistance state but metformin acts preferentially on the glucose production from the liver while thiazolidinediones act on the insulin resistance state of muscles and adipocytes. The clinical application of the action of these drugs at different sites has been well demonstrated. Metformin alone and thiazolidinedione alone share the same glycemic effect with similar results on blood glucose level in the post absorptive or post prandial state. Both drugs together improve glucose level by 1.5 m.mol/l. Both drugs are synergistic. These two effects on two different sites of insulin action lead to the same results in terms of blood glucose level when the drugs were used separately, but when they were used together, their effects and their results were summed up [16,24,25].

Combination of insulin secratogogues with thiazolidinediones:

This combination is quite new as thiazolidinediones were introduced recently. Since their use in human studies, this association in many different trials has reported an improvement of 1 to 2% in $HbA1_c$ with biotherapy in comparison with monotherapy. The glinide- thiazolidinedione combination has been studied and similar results as sulfonylurea- thiazolidinedione combination were reported [14,24,25].

If treatment goals are not achieved with diet and exercise alone, drug therapy should be initiated. Initial monotherapy with the sulfonylurea or biguanide (i.e., metformin) should be as first line drug treatment [26,27]. Sulfonylurea can be considered for most patients with type 2 diabetes; however, for those who are significantly overweight (body mass index [BMI] > 25), initial monotherapy with biguanide (i.e. metformin) may be preferred, approved in the 1950s in Europe [25] and recently approved in the USA , 1995.

Metformin has a valuable role in treatment of diabetes mellitus because of its distinct advantages [28,29].

- It can exert its hypoglycemic effect even in absence of insulin.
- Metformin is a drug of choice in obese diabetics whose hyperglycemia is more due to insulin resistance.
- Metformin does not cause weight gain and does not provoke attacks of hypoglycemia.

Moreover, metformin is frequently used in combination with a sulfonylurea in treatment of type 2 diabetes mellitus when treatment with a sulfonylurea alone is ineffective [30]. Thiazolidinediones (TZDs) are an alternative if metformin is contraindicated or a trial of metformin has failed to achieve the target HbA1c [31].

In a conventional release dosage form metformin can be used in a wide dosage range varying from 500mg to 2.5gm daily. It is best to start with the lowest possible dose, i.e. 500mg / day single dose with breakfast and continue it for several days. During this period one has to look out for gastro-intestinal complaints (anorexia, nausea, vomiting, abdominal discomfort, diarrhea), which are common with metformin [32]. The dose can be increased to 1500mg / day if the hyperglycemia persists and there are no gastro-intestinal symptoms. Same total daily dose can also be administered as 850mg twice daily with meals. Maximum permissible total daily dose of metformin is 850mg thrice a day. A single dose should not be in excess of 850mg, as this will increase the chances of gastrointestinal side effects [32,33].

Although there is absorption over the whole range of the intestine, the main part of the drug appears to be absorbed from the small intestine. Metformin is incompletely absorbed. The absorption is slower than the elimination. Oral bioavailability of usual dose is 50 to 60% [32]. This absorption is marginally reduced when metformin is taken with food. Therefore, it is rational to develop modified release preparations for metformin to avoid dose dumping in turn causing hypoglycemia. Clinical effectiveness of metformin does not increase proportionally with increase of dose. In fact it works best in lower dosage and increasing the dose does not increase hypoglycemic action [32]. Keeping this factor in mind, a modified release preparation of metformin has better therapeutic utility

compared to a conventional release formulation alone or in combination with other hypoglycemic agents where polychemotherapy is strongly recommended.

1.6. Oral Controlled Release Drug Delivery System

In the world market, oral drug delivery constitutes 70% to 80% of the drug delivery market share (Fig1.6) [5]. The reason that oral drug delivery has evolved is that its biggest benefit is patient compliance, which translates to better compliance. From a manufacturing point of view, oral delivery is also the least expensive due to ease of manufacturing and drug stability. All these factors make oral drug delivery the most convenient form of delivery [33,34]. The growth rate for oral controlled release drug delivery systems is expected to increase 9% annually [35]. Worldwide market revenues are at US $21.6 billion [5]. As research continues in alternative delivery systems, the future of oral drug delivery is "very rosy" and the market share will only increase. It makes the oral route the most preferred route of administration [36].

1.6.1 Conventional oral drug delivery systems and their limitations

For many decades treatment of an acute or chronic disease has been mostly accomplished by delivery of drugs to patients using conventional dosage forms like tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols, and injectables. Oral drug delivery has been known for decades as the most widely utilized and convenient route of drug administration compared to all other routes. The reasons that the oral route achieved such popularity may be in part attributed to its ease of administration, cost, as well as the traditional belief that by oral administration the drug is as well absorbed as the food stuffs that are ingested daily [33]. Conventional multidose therapies for long duration of action are not without problems. The problems are poor patient compliance; increased chances of missing the dose of a drug with short half-life for which frequent administration is necessary. A typical peak-valley plasma concentration–time profile is obtained which makes attainment of steady state condition difficult [33].

Therefore, to achieve as well as to maintain the intended drug concentration within the therapeutically effective range needed for treatment, it is often necessary to take this type of drug delivery systems several times a day. This results in significant fluctuations in

drug levels. The unavoidable fluctuation in the drug concentration may lead to under medication or over medication. As the drug concentration remains higher for longer duration there are increased chances of incidence of toxic or side effects that necessitates discontinuation of the treatment. However, making controlled release, moving down the gastrointestinal tract (GIT) would lead to higher bioavailability. Also some of the conventional dosage forms produce severe irritation, discomfort and other side effects when administered as immediate release dosage forms. Some drugs undergo gut metabolism and first pass metabolism in the liver producing hepatotoxic metabolites. For such drugs, immediate release formulations could lead to decreased and increased side effects.

Hence effective and safer use of existing drugs through controlled or targeted drug delivery systems, which releases drug in a controlled manner, is preferred.

1.6.2 Oral Controlled release formulations and their advantages

The focus of pharmaceutical research is being steadily shifted from the development of new chemical entities to the development of novel drug delivery systems of existing drug molecules to maximize their effectiveness in terms of therapeutic action and patent protection. Hence various drug delivery systems like controlled release, sustained release, prolonged release, extended release, depot and repository dosage forms have been designed. In recent years considerable attention has been focused on the development of controlled release drug delivery systems [33,36].

By definition controlled release drug delivery systems are defined as a method of oral drug delivery in which a therapeutic compound is packaged so that it is released slowly over an extended period of time ranging from hours to a week after administration of a single dose. This delivery method is advantageous over traditional oral delivery because the compound is less susceptible to gastric and hepatic degradation.

Over the past decade we have witnessed the wide spread and availability of a plethora of oral controlled release (CR) products in the market place. For example, by 1998, the U.S.FDA approved 90 oral CR products for marketing. From 1998 to 2003, in just five years, the FDA approved an additional 29 new drug applications that used CR technologies. Thus oral controlled release (OCR) systems constitute the largest

proportion (almost 76%) of the total drug delivery market and are expected to grow at 9% or more every year through 2007 [34]. The driving force behind this booming market can be divided into two main groups: patient related factors and market driven factors. Consequently, oral CR technologies are becoming more complex and encompassing multiple presentations.

The several advantages of a controlled drug delivery system over a conventional dosage form are improved patient convenience and compliance due to less frequent drug administration. Also there is effective therapeutic efficacy and better management of the disease, with reduction of adverse side effects and improvement in tolerability. Drug plasma levels are maintained at a constant level or within a narrow window with no alternative peak and trough profiles and with AUC of plasma concentration versus time curve comparable with total AUC from multiple dosing with immediate release dosage forms. The enhanced patient compliance is also due to reduction in dosing frequency in the number of dosage units to be administered. Reduction in total healthcare cost could probably be the important factor especially in poor or developing countries like India.

Ideal candidates for OCR formulation:

The ideal candidates for OCR formulation are drugs that possess a short half-life and drugs that have to be maintained within a narrow therapeutic index. Also drugs which are sufficiently absorbed by the small intestines, drugs which are toxic at high doses and which are highly susceptible to first pass metabolism are ideal for oral controlled release formulations [33].

1.6.3 Design of Controlled Drug Delivery Systems

The basic rationale of a controlled drug delivery system is to optimize the biopharmaceutic, pharmacokinetic and pharmacodynamic properties in such a way that its utility is maximized through reduction of side effects. Also there is cure or control of condition in the shortest possible time by using smallest quantity of drug administered by the most suitable route.

The performance of the drug presented as a controlled release system depends upon its

release from the formulation and movement within the body during passage to site of action. The release of formulation depends upon the fabrication of formulation and the physicochemical properties of the drug. Movement within the body depends upon pharmacokinetics of the drug.

The rate limiting step in the availability of a drug from controlled delivery system is the rate of release of drug from the dosage form which is much smaller then the intrinsic absorption rate for the drug [37].

1.6.4 Types of Controlled release Formulations

Oral controlled release delivery system is a drug- containing dosage form that releases the drug continuously in a predetermined pattern for a fixed period of time. The controlled release systems for oral use are mostly solids based on dissolution, diffusion or a combination of both mechanisms in the control of release rate of drug. Depending upon the manner of drug release these systems are classified as follows:

Continuous Release Systems:

These systems release the drug for a prolonged period of time along the entire length of GIT (especially upto the terminal region of small intestine) with normal transit of the dosage form [38]. The various systems under this category are:

- Diffusion controlled release systems –matrix & reservoir devices (coating/ microencapsulation)
- Dissolution controlled release systems – Matrix & Reservoir types
- Dissolution and diffusion controlled release systems -Hydrogels
- Ion –exchange resin –drug complexes
- pH dependent formulations
- Swelling systems
- Osmotic pressure controlled systems
- Hydrodynamic pressure controlled systems

Diffusion controlled systems:

In these types of systems, the rate controlling step is not the dissolution rate but the diffusion of dissolved drug through a polymer barrier. The drug release rate is never zero order since the diffusional path length increases with time as the insoluble matrix is gradually depleted of drug. The two types of diffusion controlled systems are – matrix & reservoir devices [39].

Reservoir Based Membrane Controlled Devices:

Drug release is controlled by a semi-permeable polymeric membrane. The polymer can be applied by coating or micro-encapsulation techniques. The diffusion through the membrane limits the release rate as shown in Fig. 1.7. Release is governed by Fick's first law of diffusion [39] which is as given:

$$J = -D\, dC_m/dx$$

Here the dissolved or dispersed drug is distributed uniformly in an inert polymer matrix and released by diffusion out of a polymer matrix [39]. The release rate depends on initial drug concentration as shown in Fig 1.8.The majority of oral drug delivery systems are matrix based.

Dissolution controlled systems:

Such systems are easiest to design. The drug present in such system may be the one with inherently slow dissolution rate or drugs that produce slow dissolving forms when it comes in contact with GI fluids and also drugs having a high aqueous solubility and dissolution rate .The last category of drugs present challenge in controlling their dissolution rate. Fig.1.9 diagrammatically represents the mechanism of dissolution controlled system. Release governed by Noyes-Whitney equation [40].

$$dc/dt = k_D\, A(Cs - C) = (D/h)\, A(Cs - C)$$

Matrix (or Monolith) Dissolution Controlled Systems

Matrix systems are also called as monoliths since the drug is homogeneously dispersed throughout a rate controlling medium. They are very common and employ waxes which control drug dissolution by controlling the rate of dissolution fluid penetration into the matrix or by itself getting dissolved at a slower rate. The drug release is often first-order from such matrices [33,37].

Reservoir devices

Here, the drug particles are coated or encapsulated by one of the several microencapsulation techniques with slowly dissolving materials like cellulose, PEGs etc. The dissolution rate of coat depends upon the solubility and thickness of the coating which may range from 1 to 200 microns.

Dissolution and Diffusion Controlled Release Systems:

Drug core is encased in a partially soluble membrane which permits entry of aqueous medium into the core and hence drug dissolution and allows diffusion of dissolved drug out of the system as shown in the Fig. 1.10. Here the rate controlling factor is fraction of soluble polymer in the coat [39,41].

Ion- exchange resin – Drug complexes:

Controlled delivery of ionizable acidic and basic drugs can be obtained by complexing them with insoluble nontoxic anion exchange and cation exchange resins respectively. The drug is released slowly by diffusion through the resin particle structure [42].

pH- Independent Formulations:

Such systems are designed to eliminate the influence of changing GI pH on dissolution and absorption of drugs by formulating them with sufficient amount of buffering agents that adjust the pH to the desired value as the dosage form passes along the GIT and permit drug dissolution and release at a constant rate independent of GI pH [43].

Osmotic pressure controlled systems:

The osmotic pump is similar to a reservoir device but contains an osmotic agent which acts to imbibe water from the surrounding medium via a semi-permeable membrane. Such a device is called the elementary osmotic pump wherein pressure is generated within the device which forces the active agent out of the device via an orifice. The rate controlling factors here are the orifice diameter, membrane area, membrane thickness & permeability, osmotic properties of the core and drug solubility [41, 44].

Hydrodynamic pressure controlled systems:

The hydrodynamic pressure generated by swelling of a hydrophilic gum can also be used to activate the delivery of drugs. The rate controlling factors here are fluid permeability and hydrodynamic pressure gradient [41, 45].

1.7. Release Controlling Excipients:

They are the inactive ingredients in the final dosage form that function primarily to extend the release of the embedded active drug substance. Several hydrophilic and hydrophobic polymers have been reported as carries/release retardant materials in the development of oral controlled release formulations of drugs. Selection of a suitable material depends on the dose size, desired release rate, and the physiochemical properties of the drug of interest [46]. Hydrophilic polymers have been paid considerable attention in the formulation of controlled release formulations for various drugs. Hydroxypropyl cellulose, carbopols, methylcellulose are some of the hydrophilic polymers which have been extensively used in the formulation of controlled release systems. Ethyl cellulose is one of the most widely used hydrophobic polymers in the formulation of controlled release systems [47, 48].

Examples of common controlled, modified, delayed and extended release controlled polymers are as given in Annexure 1.

Common polymers used for controlling the release:

Hydroxy Propyl Methyl Cellulose (HPMC)

It is a semi synthetic derivative of cellulose, has its popularity for the formulation of controlled release dosage forms as a swellable and hydrophilic polymer [49]. From a commercial point of view, HPMC is the most prominent carrier material in pharmaceutical applications. Its nontoxic property, ease of handling, ease of compression, ability to accommodate a large percent of drug, negligible influence of the processing variables on drug release rates and relatively simple tablet manufacturing technology make it an excellent carrier material [50]. In the GIT this polymer matrix undergoes surface wetting (rapid), surface swelling (slow process), surface erosion (ongoing), surface gel formation (ongoing), gradual inner polymer swelling and outer gel erosion. Effect of HPMC on the release profile and bioavailability of the drug from tablets prepared using HPMC was studied. The study shows that dissolution of the drug from drug-HPMC matrix was markedly delayed with increase in the concentration of HPMC in the tablet. The dissolution was markedly delayed as the viscosity of HPMC also increased whereas, complete release was observed from the tablet prepared without HPMC within one hour [51]. Another study in clinical trials showed that consumption of high viscosity HPMC significantly lowers cholesterol, postprandial glucose and insulin excursions [52].

Carbopols:

Carbopol polymers (carbomers) are synthetic, high molecular weight acrylic acid polymers cross-linked with polyalkenyl ethers or divinyl glycol. These polymers readily hydrate, absorb water, and swell quickly upto 1000 times their volume to form a gel when exposed to pH environment above 4 to 6. In addition to their hydrophilic nature and cross linked structure, their essential insolubility in water make these polymers potential candidates for use in controlled release formulations [44]. Among these, carbopol 934 and 971 are the most widely used pharmaceutical grade polymers for oral use. It was found that the carbopol matrices exhibited zero order release profiles at several concentrations studied [53,54].

The study was carried out to study the retardant effect of carbopol matrix. It was observed that the release was high with other polymer matrices like eudragit L-100 and

ethyl cellulose whereas, the release was retarded with carbopol matrix [53]. Another study was carried out to study the effect of polymer blends on release profiles of drug. The study showed that HPMC alone could retard the release profile but at higher polymer/drug ratio (more than 0.8:1) and at lower polymer/drug ratio (less than 0.7:1) the release was faster. When an appropriate blend of HPMC and carbopol was used, the release approached to zero order [54].

Ethyl Cellulose:

Ethyl cellulose is $C_{12}H_{23}O_6(C_{12}H_{22}O_5)_nC_{12}H_{23}O_5$ where *n* can vary to provide a wide variety of molecular weights. An ethyl ether of cellulose, is a long-chain polymer of β-anhydroglucose units joined together by acetal linkages. Release from matrix embedded systems is governed by drug diffusion from inert matrix. Ethylcellulose-coated beads and granules have also demonstrated the ability to absorb pressure and hence protect the coating from fracture during compression (works as cushioning agent). Drug release through ethyl cellulose-coated dosage forms can be controlled by diffusion through the film coating [41].

Hydroxy Propyl Cellulose

Hydroxypropyl cellulose (HPC) is non-ionic, water-soluble cellulose ether, formed by reaction of cellulose with propylene oxide. Low substituted HPCs (L-HPC) are normally used as excipients for good binding and disintegrating properties. It is soluble in water below 40°C, GI fluids, and many polar organic solvents. It is reported that L-HPC could be used as a matrix base for the formulation of controlled release tablets [45]. Its optimum use is in pH range between 6-8. The swellability of HPC depends on particle size, % loading of polymer & viscosity grade It is generally used in the concentration of 15- 35 % w/w as a retarding polymer [49].

Eudragit

It exists as a fully polymerized copolymer of methacrylic acid and an acrylic or methacrylic ester. Eudragit L & S are anionic polymers based on combination of

methacrylic acid and its esters. Eudragit RL & RS also contain a low content of quaternary ammonium groups. It is used in development of pH dependent systems.
It imparts good mechanical strength to tablets & control diffusion of drugs through pores and channels. Larger quantities (5–20%) of dry polymer are used to control the release of an active substance from a tablet matrix. Solid polymers may be used in direct-compression processes in quantities of 10–50% [39,55]. Type & quantity of polymethacralate determines the release pattern as given in Annexure 2.

1.8 Novel technological approaches for combining the drugs in single unit dosage form.

As discussed earlier, polychemotherapy is strongly recommended in the management of diabetes. This urges the need to explore various potential approaches to combine the molecules based upon their respective pharmacological rationales. These combinations can be all immediate release, one immediate release and other modified release or all modified release to name few of them. In the conventional design, it is possible to combine all immediate release but when either of the components is intended to be a modified release then conventional approach falls short to cater the requirement. In these cases, advanced technology/approach has to be employed to combine two molecules, where the release of both is essentially different. Following are among the various approaches those can be employed in these cases.

1.8.1 Tablet in tablet technology (Press coating):

It includes preparation of tablets by subjection to more than a single compression. The tablet-in-tablet consists of the inner tablet being the "core" and the outer portion being the "shell" (Fig.1.11). The significance of the technology lies in separating two different molecules those are incompatible with each other or the release of the respective molecule is intended to be different. In this technology, specially fabricated compression presses are used to place the preformed tablet precisely within the die for the second compression and the new fill material around the core tablet. Difference in the core and shell in terms of size, sequence of the release pattern and intended site of release in

gastro-intestinal tract are the important factors that govern the choice of the technology and decision of "core" and "shell" part [56,57].

1.8.2 Drug loading:

In this method, the solution of the soluble drug or a homogeneous suspension of the drug from the combination is coated on the core (Fig. 1.12). The core can be in the form of a tablet itself or pellets which may contain another molecule in the combination that can be further compressed into a tablet. The approach is drug-solubility dependant and suffers a loss of drug during manufacturing. This makes the approach commercially less popular [45, 48].

1.8.3 Multiple-layered tablet technology:

In this approach, tablets are prepared by compressing more than once which results in multiple-layered tablets (Fig.1.13). Layered tablets are prepared by the initial compression of a portion of fill material in a die and the addition of one or more portions of fill material to the same die, each additional fill being compressed to form a two or three layered tablet depending upon the number of separate fills. Usually each portion of fill material consists of different medicinal agent separated from the others for reasons of incompatibility, for providing drug release in two or more stages. Generally each portion of fill is colored differently to prepare a multiple-colored as well as a multiple layered tablet. In the specially crafted machines available for multilayer production the granulation receives a pre-compression stroke after the first and second fill, which lightly compacts the granulation and maintains a well defined surface of separation between each layer. These stratified tablets offer a number of advantages. Incompatible drugs can be formed into a single tablet by separating the layers containing them with a layer of inert material. It has permitted the formulation of time-delay medication and offers a wide variety of possibilities in developing color combinations which give the products identity and aesthetic appearance [56,57].

1.9 Objectives of the present research work

As the fourth leading cause of death, diabetes has reached epidemic proportions in many developed and developing countries. According to the most recent statistics, five percent of the world's population is suffering from diabetes and its prevalence is doubling every generation. The number of diabetic patients is set to continually rise at an alarming rate in India also, having the largest number of people with diabetes (35 million) and the fastest growing prevalence of this condition.

Type 2 diabetes mellitus (T2DM) is the most prevalent one and accounting for 90% of diabetic cases worldwide. It has been classically thought to be as a condition that can be managed initially with exercise and diet control, however, almost all patients require pharmacological treatment.

Currently, a variety of oral anti-hyperglycemic agents are available with clinicians for the management of T2DM, which needs individualized dosage regimen based on patient specific factors and disease state. As T2DM is the life long condition with no definite cure, so controlling the disease progression is the main aim of treatment. Use of a fixed dose combination of two or more drugs, with different mode of action, has become a rational approach for effective control of blood glucose level by the synergistic action.

In all, better glycemic control in diabetic conditions demand a specialized drug delivery system. It should be capable of delivering the drug(s) to the target site in desired concentration for a sufficient period of time in an effective, reliable, repeatable and safe manner. Revolutionary changes in pharmaceutical technology, during last few decades has made it possible to design drug delivery systems that can not only prolong the release of therapeutic agent over an extended period but also control the drug release rate with higher degree of predictability. Selection of appropriate controlled – release delivery platform technology depends on many parameters like nature of desired release profile, physicochemical properties of drug(s), nature of excipients, process variables, physical parameters of drug delivery system etc.

Metformin has a valuable role in diabetic treatment and currently is the most prescribed drug by clinicians in the treatment of T2DM because of its distinct advantages as discussed in early sections of this chapter.

However, gastrointestinal complaints like, anorexia, nausea, vomiting, abdominal discomfort, and diarrhea and hypoglycemic effect are common with Metformin due to its erratic and incomplete absorption leading to dose dumping when administered in the conventional multiple dosage form.

Therefore, it is intended to develop an oral controlled release delivery system for Metformin alone and in combination with gliclazide and glimiperide for improved therapeutic performance, which will not only help in better glycemic control but also offers various advantages like maximized drug therapeutic indices, reduced side effects and adverse effects, reduced dosing frequency, reduced cost of treatment, improved stability, better availability of drug, along with better patient compliance and improved quality of life. The developed delivery systems are planned to be evaluated, in vitro and in vivo both, for their performance and regulatory aspects of the drug delivery system.

The proposed research work aims to

1. Develop and validate selective and sensitive analytical methods for the estimation of metformin, gliclazide and glimiperide in bulk, pharmaceutical formulations (individual and in combinations), and biological matrices.
2. Establish the preformulation data, such as solubility profile, stability, partition coefficient, photo-stability, drug-excipient incompatibility etc., for the selected molecules.
3. Design and develop stable and reproducible extended release oral solid dosage form for metformin and its combinations with gliclazide and glimiperide and their evaluations.
4. In vivo assessment of the optimized formulations in healthy human volunteers.

Table 1.1: Categorization of Oral anti-diabetics

1. Sulfonylureas		
1st generation	**Dose**	**Short comings**
Chlorpropamide	100-500mg (o.d.)	Hypoglycemia, Hypersensitivity, weight gain and contraindicated in pregnancy are the limitations with sulfonylurea group of drugs.
Tolazamide	1000 mg (o.d. or in divided in two doses).	
Tolbutamide	250-2000mg divided in 2-3 doses.	
2nd generation		
Glimiperide.	1-4mg (o.d.)	
Glipizide	2.5-40mg Doses >15mg divided in two doses.	
Glyburide	1.25-20mg (o.d. or b.i.d.)	
2. Biguanides		
Metformin	500-2550mg/day	Transient dose related GI symptoms like diarrhea, nausea, vomiting, and anorexia.
3. Thiazolidinediones.		
Rosiglitazone	4-8mg (o.d. or b.i.d.)	Edema, weight gain and in rare cases Hepatotoxicity.
Pioglitazone	15-45mg (o.d.)	
4. Alpha-glucosidase inhibitors.		
Acarbose	25mg (t.i.d.)	Transient dose related GI symptoms like diarrhea, nausea, vomiting, and anorexia.
Miglitol	25mg (q.i.d.) for 1-2 weeks. Followed by 25 mg (b.i.d.) for 1-2 weeks.	
5. Meglitinides		
Repaglinide	0.5-4.0mg per meal	Hypoglycemia and weight gain.
Nateglinide	120mg before each meal	

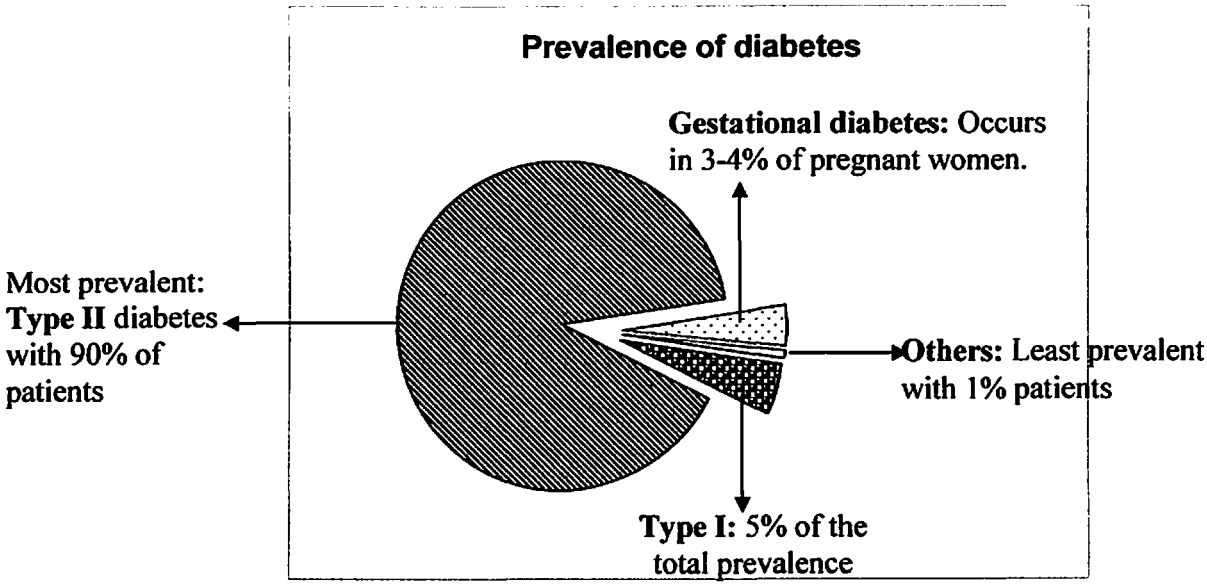


Fig.1.1: Graphical representation of prevalence of diabetic patients in year 2003 as per type of diabetes [1].

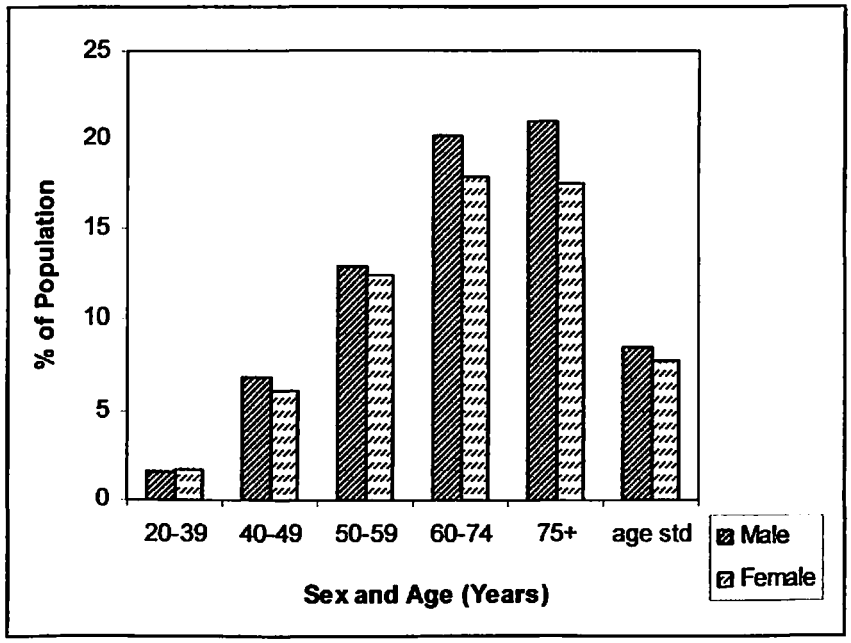


Fig 1.2: Sex & age wise comparison of diabetes prevalence in year 2003 [1].

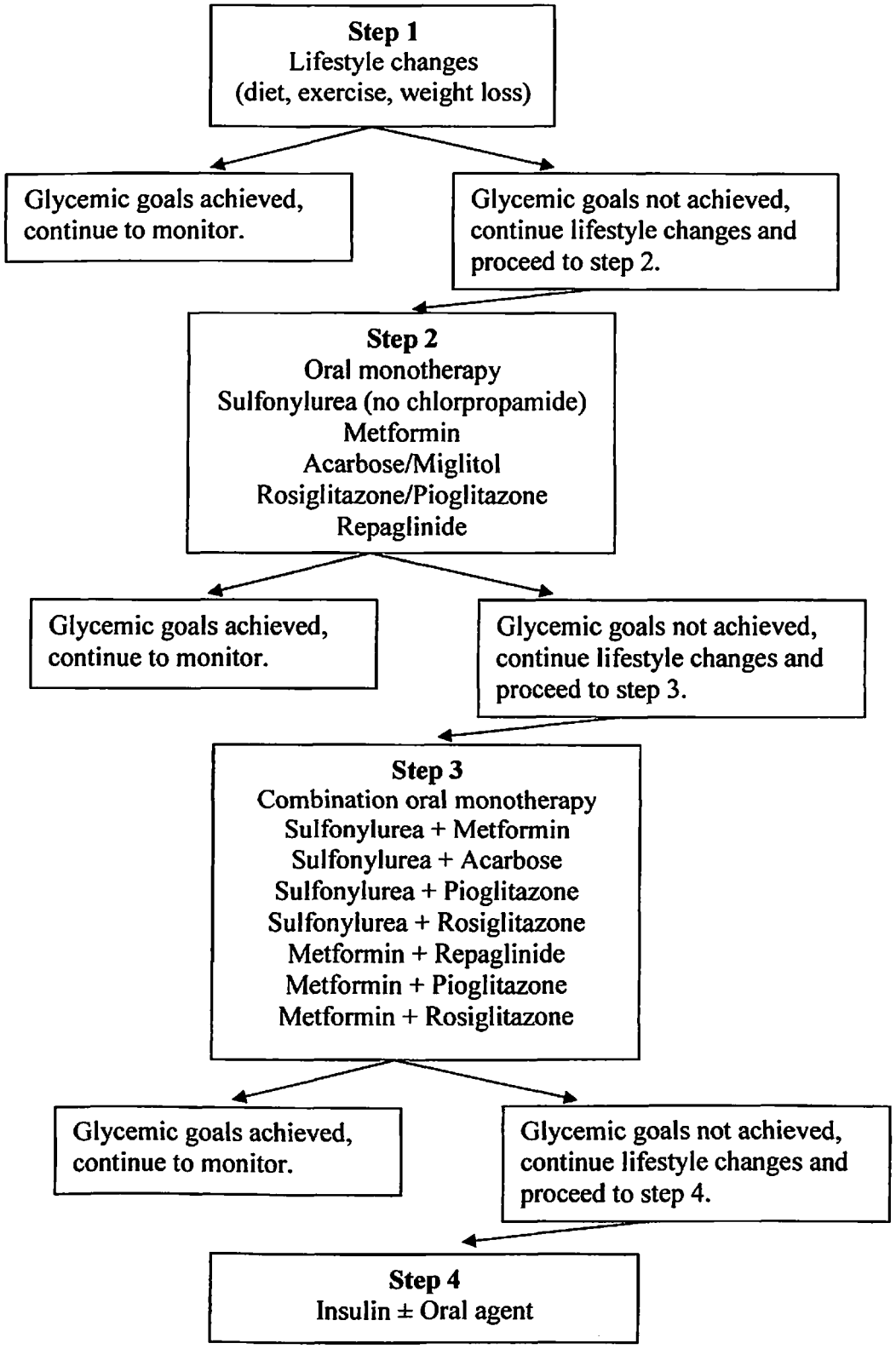


Fig. 1.3: Stepwise approach for diabetes treatment [15].

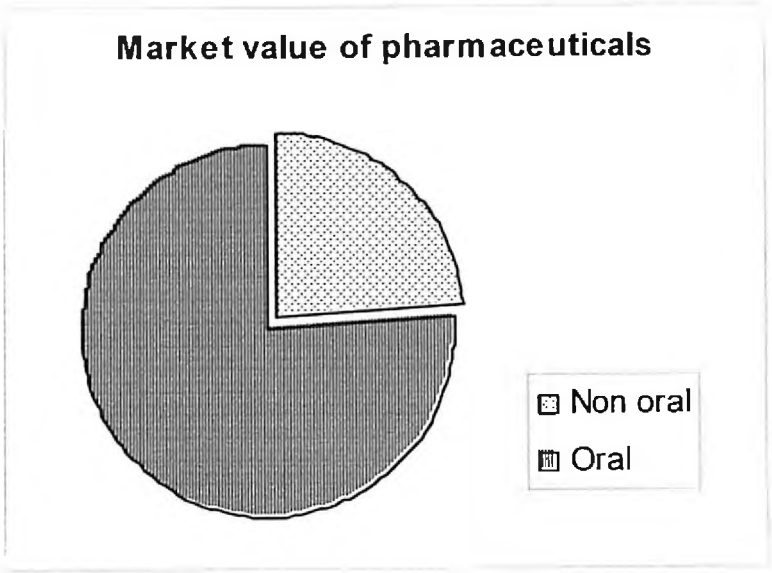


Fig. 1.4: Market value of pharmaceuticals in year 2006 [5].

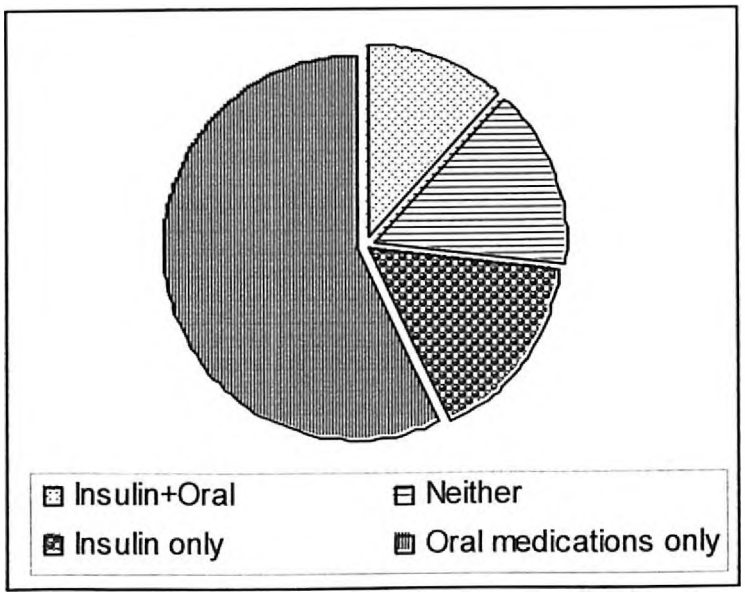


Fig.1.5: Treatment statistics for diagnosed diabetics in year 2006 [15].

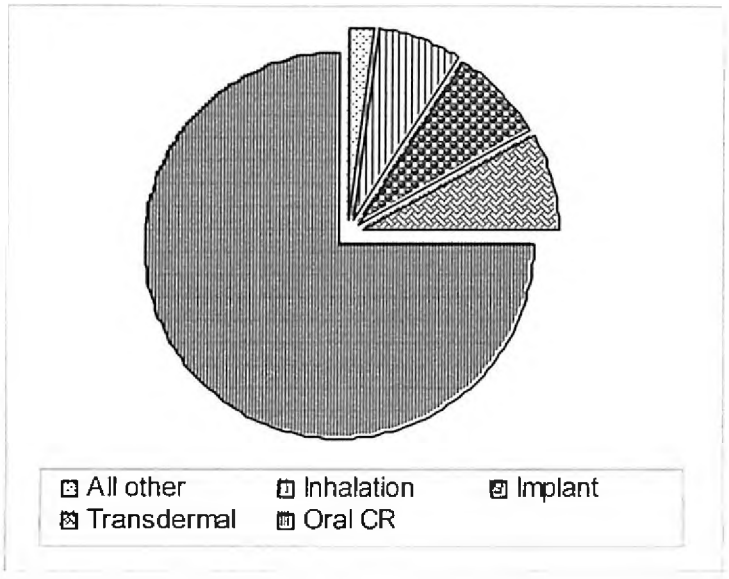


Fig. 1.6: Drug delivery based products in year 2006 [5].

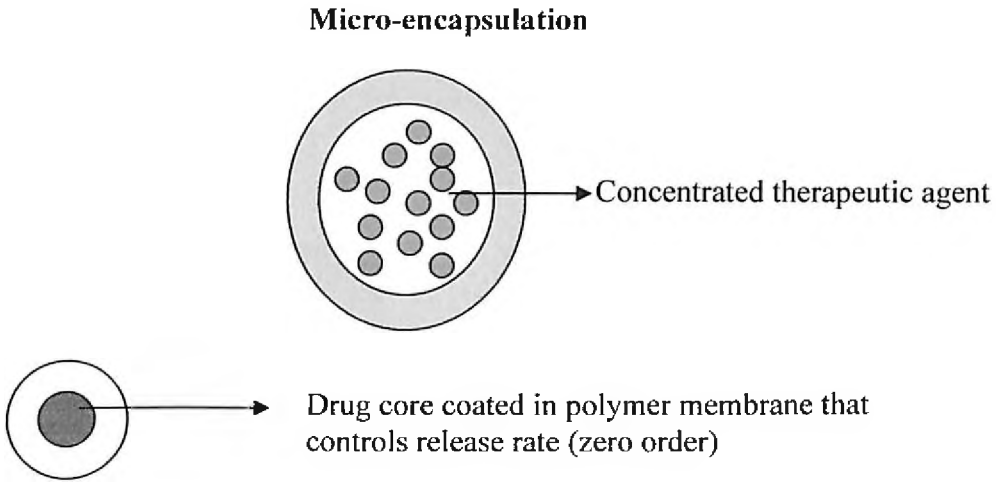


Fig 1.7: Drug release by diffusion across the insoluble membrane of reservoir system [39].

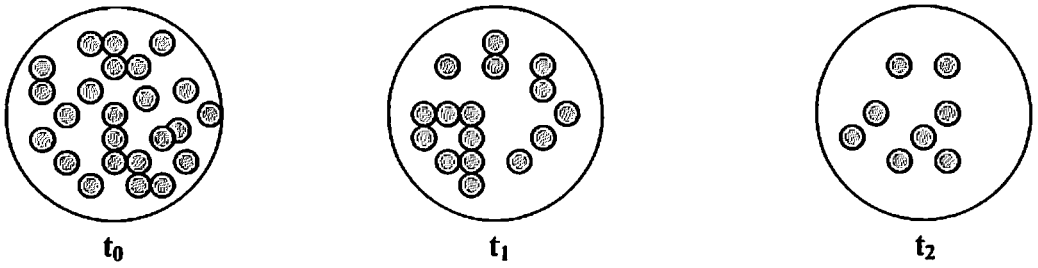


Fig 1.8: Drug release by diffusion across inert polymer matrix [39].

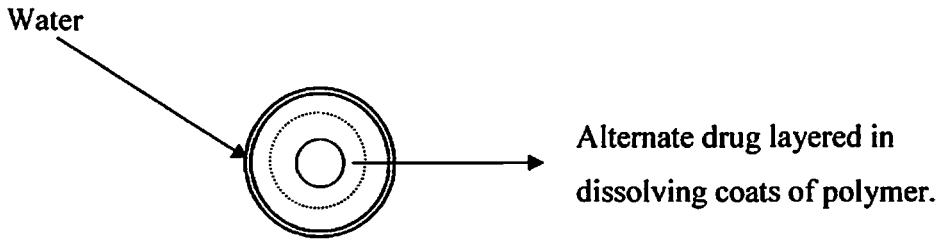


Fig 1.9: Diagrammatic representation of dissolution controlled systems [40].

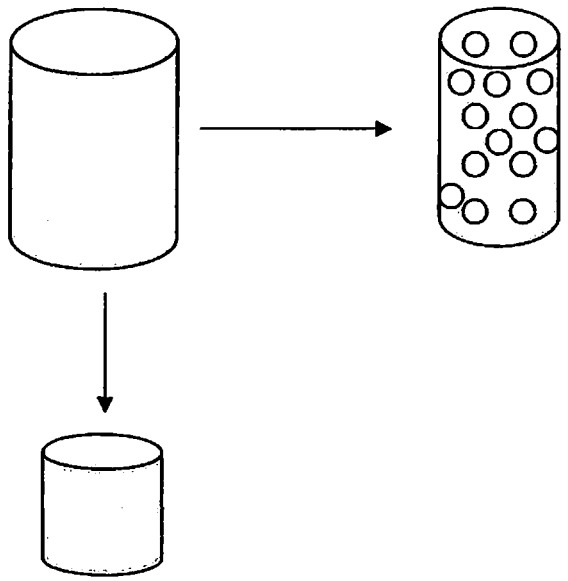


Fig.1.10: Diagrammatic representation of diffusion and dissolution controlled systems [41].

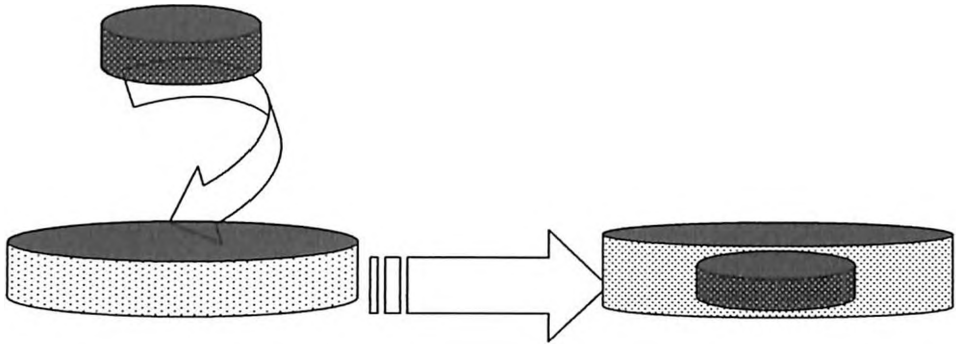


Fig 1.11: Diagrammatic representation of Press coating technology.

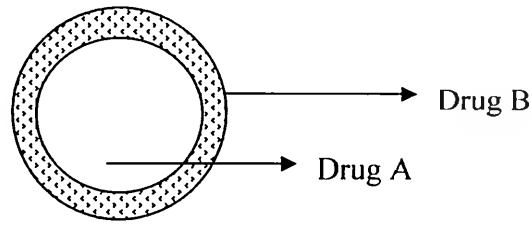


Fig 1.12: Diagrammatic representation of Drug loading technology.

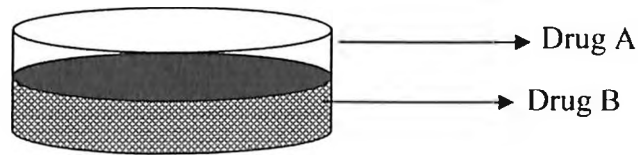


Fig 1.13: Diagrammatic representation of Multiple layered tablet technology.

Chapter 2

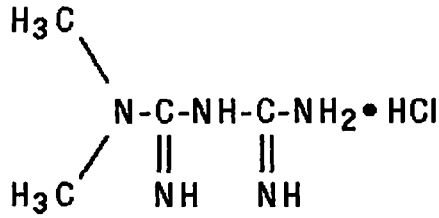
Drug Profile

2.1 Metformin Hydrochloride (MFH):

MFH is an oral antihyperglycemic agent that lowers blood glucose and is used in treating type 2 diabetes. It may be used alone or in combination with other anti-diabetic agents .It is now considered by most clinicians to be the first choice for type 2 diabetes patients who are insulin resistant and obese.

The chemical name of MFH is (N, N-dimethylcarbamimidoyl) aminoformamidine with empirical formula $C_4H_{11}N_5.HCl$ and molecular weight 165.63. Chemically, it belongs to the biguanides class of antidiabetic agents [58]. It is a Class III drug under the BCS classification system. MFH was approved in the 1950s in Europe and recently approved in the USA, 1995

Structural formula:



Appearance: MFH is a white to off white crystalline powder.

Ionization constant: MFH is a strong base with pKa_1: 2.8 and pKa_2: 11.5 [50]

Thermal analysis: MFH melts at 222°C – 226°C.

Solubility [58]:

In alcohol	1 in 100
In water	1 in 2

MFH is practically insoluble in acetone and methylene chloride.

pH: MFH is in the protonated form (monohydrochloride) and the pH of 1% aqueous solution is 6.68.

Partition Coefficient: Log $P_{(Octanol/water)}$: -2.06

Compendial status: MFH is official in IP, BP, EP and USP. Table 2.1 gives a comparison of different tests in the respective pharmacopoeial monograph of MFH [59-62].

Pharmacology:

MFH is an antihyperglycemic agent which improves glucose tolerance in patients with Type 2 diabetes, lowering both basal and postprandial plasma glucose. There is no blood glucose lowering effect in non-diabetic subjects. Its pharmacological mechanisms of action are different from other classes of oral antihyperglycemic agents. Augmentation of muscular glucose uptake and utilization, and reduction of increased hepatic glucose production through an antigluconeogenic action explain the blood-glucose lowering effect, but the contribution of each of these processes to the overall effect has not been defined. The intestinal glucose absorption may be slightly delayed.

Increased glucose utilization by the intestines and erythrocytes results in increased lactate formation. The formation of glucose from lactate and the lack of an insulinotropic effect explain the absence of clinical hypoglycemia during MFH treatment. Accordingly, MFH should be labeled antihyperglycemic rather than hypoglycemic [63,64]. The mechanism of action involves binding of the apolar biguanide hydrocarbon side –chain to membrane phospholipids, evoking a change in the electrostatic surface potential. Subsequently, various metabolic effects are elicited, depending on the target cell, tissue, organ, species, and metabolic regulation. In healthy subjects counter–regulatory mechanisms, such as increased gluconeogenesis from lactate mask the effect of the drug, and blood glucose remains unchanged [63]. MFH potentiates insulin action mainly by a postreceptor mechanism [64-66]. In this way, MFH negates insulin resistance. Insulin independent effects on muscular glucose uptake, FFA oxidation, and erythrocytes have also been demonstrated [64]. Apart from the glucose –lowering effect, MFH improves the blood lipoprotein profile not only in diabetes but also in non-diabetic subjects with hyperlipoproteinemia [64-66].

Pharmacokinetics:

MFH is absorbed over the whole range of the intestine; but the main part of the drug appears to be absorbed from the small intestine. It is incompletely absorbed. The absorption is slower than the elimination. Peak plasma concentrations of about 2mg/l are reached after 2 hours or later. [67-68].

Oral bioavailability of usual doses is 50-60% [67,69]. The difference between absorbed and available drug may reflect minor presystemic clearance of the drug or binding to the

intestinal wall [68,69]. Higher doses are proportionally less available probably due to decreased absorption [68]. Concomitant food intake may slightly impair absorption [70].

The distribution of MFH is rapid. The mean values for apparent volume of distribution of MFH range from 63 to 276 litres in different pharmacokinetic studies [67-69]. MFH accumulates in kidneys, salivary glands, and in the walls of esophagus, stomach and duodenum [62,71]. MFH is not bound to plasma proteins [67-69]. There is no placental transfer of MFH [72].

Intravenous single-dose studies in normal subjects demonstrate that MFH is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) or biliary excretion. A renal clearance value considerably exceeds creatinine clearance indicating that MFH is excreted by active tubular secretion. The mean plasma elimination half-life ranges from 1.5 to 4.5 hours [67,69]. Most of the drug is excreted within 8 hours after intravenous administration [67,68]. It is prolonged in patients with renal impairment and is correlated with creatinine clearance. Therefore there may be some prolongation of the half-life in the elderly because of impaired renal function.

Recent clinical trials:

- Controlled clinical studies with MFH and sulfonylureas have been conducted in the recent years. The analysis comprised 11 randomized trials of more than 6 week's duration of each therapy comprising totally 651 patients. The final results have confirmed the equivalent antihyperglycemic efficacy of MFH and sulfonylureas. The body weight of the patients treated with sulfonylurea was increased by 2.80Kg whereas body weight of patients treated with MFH was decreased by 1.20Kg [73]. The observation pertaining to body weight of the patient confirms that MFH does not increase body weight of the patient and can be specifically used for the obese type 2 diabetic patients.
- MFH is included in the open, randomized UK prospective diabetes study (UKPDS), which compares long-term effects of different therapies in patients with newly diagnosed type 2 diabetes mellitus. The study shows that in contrast to

insulin and sulfonylurea, MFH did not increase body weight and reduced fasting insulin levels [74].

- Two groups of obese type 2 diabetic patients are investigated in the randomized, double-blind, and parallel group design. One group is treated with MFH alone and the other group is treated with MFH and glyburide combination. This study shows that combination therapy gave synergistic effect in reducing glycemia as compared with monotherapy [75].

Mode of use:

Treatment with MFH for type 2 diabetes mellitus is usually initiated with 0.5 to 1.0g daily, followed by a gradual increase if necessary. No further effect on blood glucose can be expected from doses above 3g daily. The dose can be gradually decreased after good control on blood glucose level is achieved. Maximum permissible total daily dose of MFH is 850mg thrice a day. A single dose should not be in excess of 850mg, as this will increase the chances of gastrointestinal side effects [76].

Contraindications:

MFH is contraindicated in patients with renal disease or renal dysfunction, as the elimination of the drug is decreased [69]. MFH should be stopped for two days before major surgeries and followed by temporary insulin treatment. Once the patient is stable, MFH can be reinstated. It should be stopped at least two days before X-ray examinations with iodinated contrast materials such as intravenous urography and aortography, where a risk exists for temporary renal insufficiency. During long term therapy, liver function and serum B_{12} concentration should be monitored regularly [69].

Drug-drug interactions:

Cationic drugs like cimetidine increase the availability of MFH by competitively inhibiting its renal clearance. Therefore, whenever MFH is co-prescribed with cimetidine, its dose should be reduced [69].

Drugs with hyperglycemic potential (e.g. thiazides and corticosteroids) may partly offset the antihyperglycemic action of MFH. Therefore in these cases blood glucose level should be closely monitored [69].

Alcohol inhibits gluconeogenesis, thereby potentiates the antihyperglycemic action. Therefore patients treated with MFH should preferably avoid alcohol [63, 69].

MFH is not plasma protein bound and does not get metabolized in liver so interaction with highly protein bound drugs like gliclazide (having 80-90% plasma protein binding and get metabolized via liver) and glimiperide (having 99% plasma protein binding and get metabolized via liver) is not possible [77].

Adverse effects:

MFH may lead to metformin-associated lactic acidosis (MALA) due to alcoholism or impaired renal and hepatic dysfunction or cardiac failure. MALA is caused when the in vivo concentration of drug is very high. The high concentration of drug provokes increased lactate production [77].

MFH quite frequently (5 to 20%) causes gastrointestinal problems (nausea, vomiting, abdominal pain, and diarrhea).

During long term treatment with MFH, mal-absorption of vitamin B_{12} may occur leading to megaloblastic anemia [78].

Formulations:

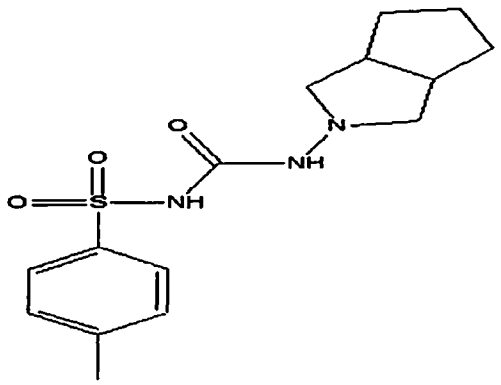
MFH is available for oral administration in tablet and syrup dosage form. The tablets contain either 500mg or 850 mg of active ingredient. Tablets of both the strengths are white colored and film coated.

MFH syrup is a new entry in the market. The 5ml syrup contains 500mg MFH.

2.2 Gliclazide (GLZ):

GLZ is an oral antidiabetic agent. Chemically it belongs to second generation sulfonylurea class. The chemical name of GLZ is (1-(hexahydrocyclopenta[c]pyroll-2(1H)-yl)-3[(4ethylphenyl) sulphonyl] urea [58]. The molecular weight of GLZ is 323.4 and empirical formula is $C_{15}H_{21}N_3O_3S$ [58].

Structural formula:



It is a white odorless crystalline powder. It has hypoglycemic and potentially useful hemobiological properties.

Ionization constant: GLZ is a weak acid with a pKa of 5.8 [58].

Thermal analysis: GLZ melts at approximately 180-182^{0}C.

Solubility: It is practically insoluble in water, freely soluble in dichloromethane, sparingly soluble in acetone and slightly soluble in ethanol 96%. [59-61].

Partition Coefficient: Log $P_{(Octanol/water)}$: 1.97.

Compendial status: GLZ is official in BP and EP. Table 2.2 gives a comparison of different tests in the respective pharmacopoeial monograph of GLZ [60,61].

Pharmacology

GLZ has pharmacological actions common to all sulfonylurea drugs. Their primary effect is to potentiate glucose-stimulated insulin release from functioning pancreatic islet β-cells [79,80]. Based on studies to measure potassium flux in isolated pancreatic islets, it has been demonstrated that the earliest ionic event in glucose-stimulated insulin secretion is a decrease in potassium efflux [81-83]. This is mediated by an increase in the intracellular ratio of ATP/ADP which inhibits K^+-ATP channels. The resultant depolarization opens voltage–sensitive calcium channels and the influx of calcium triggers insulin release leading to lowering blood glucose level [83]. GLZ works by potentiating this sequence of

events. In short term therapy with GLZ, there is a significant increase in circulating insulin concentrations, but with continued use there is usually reduction in insulin levels without deterioration of glycemic control. This is because of alterations in β-cell sensitivity and insulin resistance [84].

GLZ stimulates the most potent activator of liver phosphofructokinase at therapeutic concentration [85]. This results in inhibiting hepatic gluconeogenesis, which results in decreased blood glucose level [85].

Pharmacokinetics:

GLZ is absorbed in the gastrointestinal tract reaching peak serum concentrations within 4 hours to 6 hours. GLZ is well absorbed by the body, (approximately 80%) [86]. GLZ has a half life of 11-12 hours with the peak absorbance occurring at about 4-6 hours and food does not affect the rate or degree of absorption. A steady state level is reached after about 2 days of treatment.

GLZ binds primarily to plasma albumin (85-99%), allowing it to be distributed uniformly throughout the body. GLZ is distributed to the extra cellular fluid. The volume of distribution is about 17-25 liter [87]. ^{14}C-labeled tracer studies in rats have shown that GLZ, given orally or intravenously, tends to concentrate in the liver and kidneys and some was also found in the pancreas and adrenals but very little in the central nervous system. No studies have reported its presence in human breast milk.

Isotropic tracer studies have shown that GLZ is extensively metabolized in liver and less than 20% is excreted in the urine unchanged. The metabolites of GLZ lack hypoglycemic action except p-carboxylic acid metabolite.

The mean plasma half life is 10 hours. Single dose studies have demonstrated that maximal fall in blood glucose levels (23% of an 80mg dose; 30% of a 160mg dose) occur approximately five hours after drug administration [88,89]. The clearance of gliclazide has been found to be slightly reduced as a function of age. This reduction however, is not considered to be clinically significant. The elimination half-life of gliclazide is approximately 16 hours [87]. No clinically significant modifications in the pharmacokinetic parameters have been observed in elderly patients

The relationship between the dose administered and the area under the concentration curve as a function of time is linear for doses of GLZ up to 90 mg/day. At the highest

evaluated dose (135 mg/day), the AUC increases slightly more than proportionally to the dose [87].

Recent clinical trials:

- Three studies were performed to assess the efficacy of various sulfonylureas in the management of type 2 diabetes mellitus. In the first study patients received GLZ for 3 months. Good glycemic control was obtained in 65% of patients. In the second study, HbA1 level in type 2 diabetes mellitus patients was compared for 1 year with chlorpropamide, glipizide, gliquidone, glyburide and GLZ. The best results were obtained with GLZ and glyburide, leading to normal HbA1 levels in 74% and 80% of patients, respectively. In the third study, secondary failure study rates were assessed in Type 2 diabetic patients treated for 5 years with GLZ, gliburide or glipizide. GLZ has the lowest secondary failure rate (7%), which was significantly better than glipizide (~26% failures in 5 years). The failure rate with the treatment of glyburide (i.e. 18%) was comparable with GLZ [90].
- Sixty patients suffering with type 2 diabetes were assessed in a 3 month study in which they were randomly treated with GLZ or MFH. There was a comparable significant drop in blood sugar levels during 3 months in both, patients receiving MFH and those receiving GLZ but significant weight loss occurred only in patients treated with MFH. Fasting serum insulin levels decreased significantly in patients receiving MFH compared with the patients receiving GLZ [91].

Mode of use:

Treatment of GLZ is started with a dose of 40-80mg. If necessary, the dose can be increased to a maximum of 240mg. Rarely, the dose up to 320mg a day may be needed [92].

Contraindications [90,91,92]:

- GLZ is contraindicated for the patients with Type 1 diabetes mellitus.
- It is also contraindicated for the patients with impaired renal functioning and suffering from ketosis.

- GLZ is contraindicated, if the diabetic patient is undergoing surgery, after sever injury or suffering with infection.
- It is contraindicated for the patients having a history of allergic response to other sulfonylureas.
- GLZ is contraindicated in pregnant women, breast-feeding mothers, neonates, children, elderly patients and patients with hepatic diseases.

Drug-drug interactions:

Highly protein bound drugs like aspirin, NSAIDs, phenylbutazone, clofibrate, sulfonamides and coumarin anticoagulants displace GLZ from protein binding site, which increases the concentration of unbound GLZ in the blood. The increased concentration of unbound GLZ in blood potentiates the hypoglycemic action of GLZ [93].

Some drugs like cimetidine, imidazole antifungal agents and monoamine oxidase inhibitors inhibit hepatic microsomal enzymes, which leads to decreased metabolism of GLZ. This potentiates the hypoglycemic action of GLZ [93].

Some drugs like rifampicin, thiazide diuretics, barbiturates, phenytoin induce hepatic microsomal enzymes, thereby increasing metabolism of GLZ. Increased metabolism of GLZ reduces its hypoglycemic activity [93].

Adverse effects:

GLZ may cause severe hypoglycemia in the conditions like hepatic disease, malnutrition, anorexia or alcohol intoxication. Acute hypoglycemia may lead to severe brain damage or death [90,91].

The common adverse effects associated with GLZ are unusual weight gain, cold sweat, blurred vision, drowsiness and excessive hunger [90,91].

Other very rare adverse effects (found in about 2% of the patients treated with GLZ) are gastrointestinal disturbances and dermatological reactions.

Formultions:

GLZ is available for oral administration in tablet and capsule dosage forms.

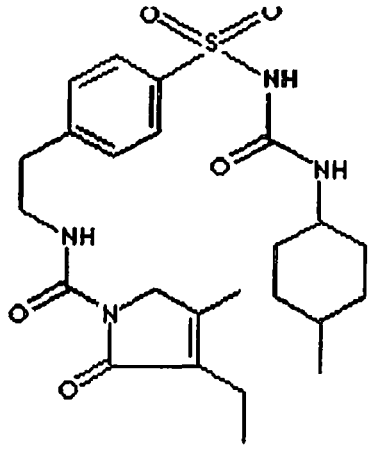
The tablets contain 40mg, 80 mg or 160mg of active ingredient. These tablets are white colored and uncoated.

The capsule contains 160mg of active ingredient, GLZ. The cap of capsule is pink colored and body is white colored.

2.3 Glimepiride (GPD):

GPD,1H-pyrolle-1-carboxamide,3-ethyl-2,5-dihydro-4-methyl-N-[2-[4-[[[[(4-methylcyclohexyl)amino]sulfonylurea]phenyl]ethyl]-2-oxo-trans. of molecular weight 490.62 with empirical formula $C_{24}H_{34}N_4O_5S$ is an oral hypoglycemic agent of the sulfonylurea class [60].

Structural formula:



It is a white to off white crystalline powder.

Thermal analysis: GPD melts at 207^{O}C .

Solubility: It is practically insoluble in water and is practically insoluble in acetone, ether, and chloroform. [59,60].

Partition Coefficient: Log $P_{(Octanol/water)}$: 2.59.

Compendial status: GPD is official only in USP. Table 2.3 gives a comparison of different tests in the respective pharmacopoeial monograph of GPD [62].

Pharmacology:

Being a typical sulfonylurea the mechanism of action of GPD in lowering blood glucose resembles to the mechanism of action of GLZ [94,95].

GPD binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane [96,97]. Membrane depolarization stimulates calcium ion influx through voltage-sensitive calcium channels. This increase in intracellular calcium ion concentration induces the secretion of insulin.

GPD is effective as initial drug therapy [81-83].

Pharmacokinetics:

After oral administration, GPD is completely (100%) absorbed from the GI tract. Studies with single oral doses in normal subjects and with multiple oral doses in patients with non insulin dependent diabetes mellitus (NIDDM) have shown significant absorption of GPD within 1 hour after administration and peak drug levels (C_{max}) at 2 to 3 hours. When GPD was given with meals, the mean T_{max} was slightly increased (12%) and the mean C_{max} and AUC were slightly decreased (8% and 9% respectively) [95].

The absolute bioavailability of GPD is complete [96]. Maximum serum concentrations are reached approximately 2.5 hours after oral intake and there is a linear relationship between dose and both maximum concentrations and area under the time/concentration curve [97].

After intravenous (IV) dosing in normal subjects, the volume of distribution was 8.8 L (113ml/kg) and the total body clearance was 47.8 ml/min. Protein binding was greater than 99.5% [85].

GPD is completely metabolized by oxidative biotransformation after either an intravenous or oral dose. The major metabolites are the cyclohexyl hydroxy methyl derivative (M1) and the carboxyl derivative (M2). Hepatic microsomal enzymes have been shown to be involved in the biotransformation of GPD to M1. M1 is further metabolized to M2 by one or several cytosolic enzymes. M1 possesses about 1/3 of the pharmacological activity as compared to its parent in an animal model; however, whether the glucose-lowering effect of M1 is clinically meaningful is not clear. M2 metabolite does not possess any pharmacological activity [96,97].

When ^{14}C-glimepiride was given orally, approximately 60% of the total radioactivity was recovered in the urine in 7 days and M1 (predominant) and M2 accounted for 80% to 90% of that recovered in the urine [96,97]. Approximately 40% of the total radioactivity was recovered in the feces and M1 & M2 (predominant) accounted for about 70% of that recovered in feces. No parent drug was recovered from urine or feces. After IV dosing in patients, no significant biliary excretion of GPD or its M1 metabolite has been observed.

Mode of use:

The usual starting dose of GPD as initial therapy is 1-2 mg once daily, administered with breakfast or the first main meal. Those patients who may be more sensitive to hypoglycemic drugs should be started at 1 mg once daily, and should be titrated carefully. The maximum starting dose of GPD should be not more than 2 mg [98-100].

The usual maintenance dose is 1 to 4 mg once daily. The maximum recommended maintenance dose is 8 mg once daily. After reaching a dose of 2 mg, dosage increases should be made in increments of no more than 2 mg at 1-2 week intervals based upon the patient's blood glucose response [98].

Contraindications:

GPD is contraindicated in patients with diabetic ketoacidosis or in patients with known hypersensitivity to GPD [100,101].

Drug-drug interactions:

Similar to GLZ, the hypoglycemic activity of GPD is potentiated by highly protein bound drugs like aspirin, NSAIDs, phenylbutazone, clofibrate, sulfonamides and coumarin anticoagulants [99,100].

GPD in presence of certain drugs tend to produce hyperglycemia and may lead to loss of control. These drugs include the thiazides and other diuretics, corticosteroids, phenothiazines, thyroid products, estrogens, oral contraceptives, phenytoin, nicotinic acid, sympathomimetics, and isoniazid [102].

Adverse effects:

GPD may cause severe hypoglycemia in the conditions like malnutrition, anorexia or alcohol intoxication [98].

The common adverse effects associated with GPD are vomiting, diarrhea and gastrointestinal pain [98].

Other very rare adverse effects (found in less than 1% of the patients treated with GPD) are dermatological reactions like pruritis, erythema and urticaria [98].

In isolated cases GPD was found to cause impairment of hepatic functions and jaundice [98].

Formulations:

GPD is available for oral administration in tablet dosage form.

The tablets contain 1, 2, 3 or 4mg of active ingredient, GPD. These tablets are oblong shaped with different colors for identification. Different colors of the tablets are given in Table 2.4.

Table 2.1: Comparison of tests in the respective pharmacopoeial monographs of MFH.

Tests	Pharmacopoeial monograph			
	IP 1996	**BP 2005**	**EP 2005**	**USP 2006**
Content	98.5% w/w to 101.0w/w	98.5% w/w to 101.0w/w	98.5% w/w to 101.0w/w	98.5% w/w to 101.0w/w
Appearance	White, crystalline powder.	White crystals.	White crystals.	Test is not official.
Solubility	Freely soluble in water, slightly soluble in ethanol, practically insoluble in acetone, chloroform, dichloromethane and ether.	Freely soluble in *water*, slightly soluble in *alcohol*, practically insoluble in *acetone*, and in methylene chloride	Freely soluble in *water*, slightly soluble in *alcohol*, practically insoluble in *acetone*, and in methylene chloride	Test is not official.
Identification	i. By infrared absorption spectroscopy. ii. Gives positive test for chlorides.	i. By infrared absorption spectroscopy. ii. Gives positive test for chlorides.	i. By infrared absorption spectroscopy. ii. Gives positive test for chlorides.	i. By infrared absorption spectroscopy. ii. Gives positive test for chlorides.
LOD (at 105°C)	0.5% w/w	0.5% w/w	0.5% w/w	0.5% w/w
Residue on ignition	NMT 0.1% w/w	NMT 0.1% w/w	NMT 0.1% w/w	NMT 0.1% w/w
Heavy metals	NMT 20ppm	Maximum 10ppm	Maximum 10ppm	NMT 0.001%
Related substances	Impurity A: NMT 0.02% Any other impurity: NMT 0.1%	Impurity A: NMT 0.02% Any other impurity: NMT 0.1%	Impurity A: NMT 0.02% Any other impurity: NMT 0.1%	Impurity A: NMT 0.02% Any other impurity: NMT 0.1% Total impurities: NMT 0.5%

Note: Impurity A: Cyanoguanidine

Table 2.2: Comparison of tests in the respective pharmacopoeial monographs of GLZ.

Tests	Pharmacopoeial monograph*	
	BP 2005	**EP 2005**
Content	99.0 w/w to 101.0 w/w	99.0 w/w to 101.0 w/w
Appearance	A white or almost white powder.	A white or almost white powder.
Solubility	Practically insoluble in water and is freely soluble in Methylene chloride, sparingly soluble in acetone, slightly soluble in alcohol.	Practically insoluble in water and is freely soluble in Methylene chloride, sparingly soluble in acetone, slightly soluble in alcohol.
Identification	By infrared absorption spectroscopy.	By infrared absorption spectroscopy.
LOD (at 105°C)	NMT 0.25% w/w	NMT 0.25% w/w
Residue on ignition	NMT 0.1% w/w	NMT 0.1% w/w
Heavy metals	NMT 10ppm	NMT 10ppm
Related substances	Impurity F: NMT 0.1% Impurity B: NMT 2ppm Any other impurity: NMT 0.1% Total impurities: NMT 0.2%	Impurity F: NMT 0.1% Impurity B: NMT 2ppm Any other impurity: NMT 0.1% Total impurities: NMT 0.2%

* Official only in BP and EP

Note:

Impurity B: 2-nitroso-octahydrocyclopenta [c] pyrrole.

Impurity F: 1-(hexahydrocyclopenta [c] pyrrole-2 (1H)-yl)-3-[(2-methylphenyl) sulphonyl] urea.

Table 2.3: Comparison of tests in the respective pharmacopoeial monographs of GPD.

Tests	Pharmacopoeial monograph (USP)*
Content	98.5% w/w to 102.0w/w
Identification	By infrared absorption spectroscopy.
LOD (at 105°C)	0.2% w/w
Residue on ignition	NMT 0.2% w/w
Heavy metals	NMT 0.001%
Related substances	Impurity A: NMT 0.8% Impurity B: NMT 0.4% Impurity C: NMT 0.1% Impurity D: NMT 0.2% Any other unspecified impurity: NMT 0.1% Total impurities: NMT 0.5% (excluding impurity A)

*Official only in USP

Note:

Impurity A: Glimiperide-cis-isomer

Impurity B: Sulfonamide

Impurity C: Urethane

Impurity D: Glimiperide-3-isomer

Table 2.4: Strength-wise colors of the GPD tablets available in the market

Strength of GPD tablet	Color of the tablet
1mg	Pink
2mg	Green
3mg	Pale yellow
4mg	Light blue

Chapter 3

Analytical and Bioanalytical Methods

Introduction

The pharmaceutical product development is an inherently complex process involving multiple components with various process parameters. The efficient development and validation of analytical methods are critical elements in the development of pharmaceutical products. To demonstrate the quality of the product, each stage of the product development process demands a series of analytical methods [103]. The product performance is assessed on the quality standards with aid of analytical methods developed for the specific needs of that particular development stage or for total use. It is therefore necessary to develop a simple and suitable method for accurate determination of one or more components of the product present in various development stages like preformulation, formulation development, stability studies, bioavailability studies etc [104,105].

Although one or more analytical method(s) for several drugs estimation are already reported in literature, such methods may not be always suitable for the specific requirements demanding in-house development and validation of analytical methods. The development of such an accurate, precise, sensitive as well as simple analytical method according to the specific requirements of the product and process for routine estimation can be achieved by use of sensitive analytical techniques like absorption spectroscopy (uv/visible), fluorescence spectroscopy or liquid chromatography [106,107].

The analytical method validation is essential in the pharmaceutical product development process to establish the confidence in the analytical methods used for testing the final product (drug content, release and stability testing), raw materials, in-process materials and excipients. The analytical method validation is the process of establishing the scientific evidence through well-planed studies that an analytical method is acceptable for its intended application.

The recent guidelines for methods development and validation for new non-compendial (In-house) test methods are provided by the FDA draft document, "Analytical Procedures and Methods Validation: Chemistry, Manufacturing, and Controls Documentation". This recent document applies to the method development and validation process for products included in investigational new drug (IND), new drug application (NDA) and abbreviated

new drug application (ANDA) submissions. Therefore, the regulatory agencies prospects for the analytical method development and validation are comprehensive [108,109].

The success in analytical method can be attributed to several important factors, which in turn contributes to regulatory compliance. In order to ensure compliance with quality and safety standards, the United States, Europe, Japan, India and other countries have published national or compendial (pharmacopoeial) guidelines that describe official test methods for many marketed drug products. In recent years, a great deal of effort has been put into the harmonization of pharmaceutical regulatory requirements in the United States, Europe, and Japan. As part of this initiative, the International Conference on Harmonization (ICH) has issued comprehensive guidelines for analytical method validation. The recent FDA methods validation draft guidance document as well as USP both refer to ICH guidelines [108,109]. Analytical guidance documents recently published by the ICH under following areas:

- Stability testing (Q1)
- Validation of analytical procedures (Q2)
- Impurities in drug substances and products (Q3)
- Specifications for new drug substances and products (Q6)

Though, analytical methods are available in official compendia or in literature, for in-house suitability, new simple analytical methods have been developed for routine analysis. In present work, each methods development and method validation was divided into following steps [110-112]

- Method development protocol
- Background information
- Laboratory method development
- Generation of test procedure
- Methods validation protocol
- Laboratory methods validation
- Validated test method generation
- Validation report

The validated studies employed for all three drugs viz. MFH, GPD and GLZ in this thesis includes the complete validation tests that summarize the results of the individual tests

including the specificity, accuracy, linearity, range, precision, and robustness testing including the forced degradation under different stress conditions for the quantitative impurity estimation.

- Specificity: ability to measure desired analyte in a complex mixture
- Accuracy: agreement between measured and real value
- Linearity: proportionality of measured value to concentration
- Precision: agreement between a series of measurements
- Range: concentration interval is precise, accurate, and linear
- Detection limit: lowest amount of analyte that can be detected
- Quantitation limit: lowest amount of analyte that can be measured
- Robustness: reproducibility under normal but variable conditions [111-113].

However, the analytical method validation is a continuous process with prime goal to ensure confidence in the analytical data generated throughout the product development and other processes.

Materials, Reagents and Instruments

Materials and Reagents

Metformin hydrochloride (MFH), reference standard (purity of 99.9%), the impurity (cyanoguanidine) and two batches of MFH tablets were prepared in Ipca Laboratories Limited, Mumbai with average tablet contents of 500 mg MFH. Gliclazide (GLZ), reference standard (purity of 100.01%) and impurity F, GLZ tablets were prepared in Ipca Laboratories Limited, Mumbai with average tablet contents of 60 mg GLZ. Glimipiride (GPD), reference standard (purity of 99.44%), sulphonamide impurity and GPD tablets were prepared in Ipca Laboratories Limited, Mumbai with average tablet contents of 2 mg GPD. Acetonitrile (ACN), triethylamine and ammonium dihydrogen phosphate used were of liquid chromatography grade (LC grade). All other chemicals and reagents used were of analytical grade unless indicated otherwise.

Instruments and Equipments

HPLC equipment of TSP make, with pumps of model P-2000, UV-visible detector (UV-1000) and AS-3000 auto-sampler, SN-4000 integrator was used. Chromatograms were analyzed using chromquest software.

3.1. Analytical Method: HPLC Method for Determination of MFH

3.1.1. Experimental

Chromatographic conditions

Chromatographic separation was performed on TSP high performance liquid chromatography system equipped with P-2000 pump model, UV-1000 detector, AS-3000 auto sampler and SN-4000 integrator was used. Chromquest software was used for chromatogram analysis. Betasil C8 column (125mm × 4.6 mm, with 5µ particle size) was used for the separation. The mobile phase consisted of a mixture of acetonitrile (ACN) and 1.7% ammonium dihydrogen phosphate (pH 3.0), adjusted with ortho-phosphoric acid) (20:80, v/v). The mobile phase was delivered at a flow rate of 1.0 ml/min with detection at 233 nm. The injection volume was 20 µl and analysis was performed at ambient temperature.

Standard solution

A stock solution containing 0.5mg/ml MFH was prepared by dissolving reference standard in mobile phase. Standard solutions were prepared by dilution of the stock solution with mobile phase to give solutions containing MFH in concentration range of 30 to 70µg/ml.

Method validation

The LC method was validated with respect to the following parameters: linearity, accuracy, precision, selectivity, stability indicating capability, and stability of reference standard solutions and tablet sample preparations.

Linearity

The linearity of the method was determined at five concentration levels (30µg/ml to 70µg/ml) over a range of 60% to 140% of intended test concentration (50µg/ml) (Table 3.1).

Selectivity

For detection of the related impurities, a mixed solution containing MFH with related substances was determined under the proposed chromatographic conditions.

The selectivity of the analytical method used for determination of MFH was assessed for positive and negative interference due to matrix components present in placebo mixture. Selectivity study was conducted in order to demonstrate that there is no interference in determination of MFH in presence of other excipients. This was achieved by injecting pure standard of MFH, controlled blend of placebo and placebo spiked with MFH.

Placebo blend equivalent to 500 mg of MFH in MFH tablet was dissolved and vortexed for 15 min with 500 ml of acetonitrile. Prepared solution was filtered through GF/C filter and diluted 20 times with mobile phase. Similarly placebo preparations were also processed after spiking pure MFH WS at 100% of the test level and analyzed by proposed method.

Forcedly degraded pure drug sample under different stress conditions (heat, light, hydrogen peroxide acid, and base) were prepared for further evaluation of the selectivity of the proposed LC method.

For preparing acid and base induced degradation product, 20 ml of 0.5 M HCl and 20 ml of 0.5 M NaOH were separately added to 10 ml of standard MFH solution (300 µg/ml) in a 100 ml volumetric flask and immersed in boiling water for 1 hour. The degraded samples were then neutralized (5 ml of sample neutralized with 0.5 M NaOH / 0.5 M HCl) and transferred into 100 ml volumetric flasks and brought to volume with mobile phase. The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible effect of light.

For preparing hydrogen peroxide induced degradation product, 5 ml of hydrogen peroxide (10.0% v/v) was added to 5 ml of standard MFH solution (100 µg/ml) in a 10

ml volumetric flask. The degraded samples were transferred into 100 ml volumetric flasks and brought to volume with mobile phase and prepared solutions were injected at 0 and 60 min.

For preparing dry heat degradation product, the standard solution of MFH in a 100 ml volumetric flask was exposed to 60°C for 7 days under dry heat condition in the dark and then cooled to room temperature. The degraded sample was dissolved and transferred into a 100 ml volumetric flask and brought to volume with mobile phase and prepared solutions were diluted to 50 µg/ml and injected.

The photochemical stability of the drug was also studied by exposing the standard MFH in a 100 ml volumetric flask in photo-stability chamber and then processed the same as indicated for dry heat degradation. The resulting solutions were used as the degraded sample solutions and determined under the described chromatographic conditions.

Accuracy

The accuracy studies was carried out by applying the developed method to mixtures of excipients to which known amount of MFH corresponding to 80, 100, and 120 % of label claim had been added. At each level of the amount, three determinations were performed (Table 3.2).

Precision

The precision of the assay method was investigated by performing five replicate analyses of standard sample at intended test concentration of MFH (50µg/ml) on the same day and on three separate days and evaluated by calculating relative standard deviation (R.S.D.) of the peak area of the analyte. The method precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine replicate determinations. The R.S.D. value of the assay results, expressed as a percentage of the label claim, was used to arrive the precision level.

Analysis of MFH tablet

To determine the content of MFH in each three batches of MFH ER tablets (label claim: 500 mg per tablet), the contents of 20 tablets were accurately weighed, their mean weight determined and they were finely powdered. A quantity of powder equivalent to 500 mg MFH was weighted and transferred to a 100 ml volumetric flask which was filtered and diluted with mobile phase. The resulting solution was used as the sample solution for assay. Finally, 20 µl diluted solution was injected in to column and chromatogram was recorded. The analysis was repeated in triplicate. The possibility of excipients interference in the analysis was studied.

3.1.2 Results and discussion

Method development

Besides quantification of MFH, determination of possible degradation products and impurities is of importance during the development of a pharmaceutical dosage form. To analyze MFH together with its impurities and possible degradation products reversed phase LC in combination with ultraviolet (UV) detection was developed and optimized. The absorption maximum of MFH was observed at the wavelength of 233 nm. Not any excipients and intermediates displayed absorption maximum at 233 nm. It should be noted that excipients in pharmaceutical dosage form showed negligible absorption at this wavelength. The attention was mainly focused on the optimization of the rest of the chromatographic conditions such as mobile phase, the polarity and sorts of chromatographic column in order to detect with isocratic elution.

The final described chromatographic conditions achieved satisfactory resolution, reasonable retention and symmetric peak shapes for MFH and cyanoguanidine under which the retention time was 8.09 min for MFH and 3.39 min for cyanoguanidine respectively.

Method validation

Linearity

The results of the linearity study showed that an excellent correlation ($r = 0.99981$) existed between peak area and concentration of the drug within the concentration range,

30µg/ml to 70µg/ml for MFH. Table 3.1 and Fig.3.1 indicate linearity of the proposed method of quantification for MFH.

Selectivity

A solution containing MFH and cyanoguanidine was determined under the proposed chromatographic conditions. The representative chromatograms of MFH and cynoguanidine are shown in Fig.3.2 and 3.3 respectively, indicating the satisfactory resolution between MFH and known related impurity.

Injected solutions of placebo preparation showed no peak eluting near vicinity of MFH retention time indicating the specificity of the proposed method (Fig.3.4). Moreover, placebo blend spiked with MFH at 100% level showed mean recovery of 101.03% indicating there is no interference in the estimation of MFH. The tablet excipients were also determined and found no interferences with the determination. The above results showed that the developed LC method was selective for the determination of MFH in drug substance and pharmaceutical preparations.

For the further evaluation of the selectivity of the LC method, the forcedly degraded sample solutions prepared by subjecting the pure drug to various stress conditions such as heat, light, hydrogen peroxide, acid and base were determined under the proposed chromatographic conditions. The obtained chromatograms for the separation of MFH from its degraded products in forcedly degradation study are shown in Fig.3.5. MFH exhibited a symmetric peak shape and could be well resolved from its degradation products. During the alkali hydrolysis, assay of MFH was reduced remarkably where all the degradant formed may not have UV absorbance but peak was pure. The tablet excipients were also determined and found no interferences with the determination. The above results showed that the developed LC method was selective for the determination of MFH in drug substance and pharmaceutical preparations.

Accuracy

The accuracy was then calculated as the percentage of the drug recovered from the formulation matrix. Mean recovery for MFH from the formulation was 101.82% (96.81%

to 104.91%) (n = 9), indicating the good accuracy of the developed method for the determination of MFH in the tablets. Table 3.2 shows the data for accuracy study.

Precision

About the system precision of the developed LC method, for the determination of standard sample of MFH, the injection repeatability was found to be 1.2 % and sample repeatability was found to be 0.5 %. While, the intra-day precision ranged from 0.3 to 0.6%, inter-day precision ranged from 0.5 to 1.1%, respectively. About the method precision, the obtained R.S.D. value for the determination of MFH in tablet was 0.46 % (n = 9). The results for the system precision and method precision indicated the good precision of the developed method.

Solution stability

In order to demonstrate the stability of both standard solutions and tablet sample solutions during analysis, both solutions were analyzed over a period of 24 hours at room temperature. The results showed that for both the solutions, the retention time and peak area of MFH remained almost unchanged (R.S.D. less than 0.11 and 0.47%) and no significant degradation was observed within the indicated period, suggesting that both the solutions were stable for at least 24 hours, which was sufficient for establishing the analytical processing stability.

Ruggedness

Ruggedness of analytical method is the reproducibility of test results obtained by the analysis of same test sample under variety of normal test conditions i.e. different instruments, analysts, days etc. Ruggedness of stability indicating method of MFH was carried out by two different analysts on two different instruments on two different days and reported as % R.S.D. The % R.S.D. of the assay was found to be 0.5 % indicating the ruggedness of the method for estimation of MFH.

Table 3.3 presents the validation summary data of analytical method for quantification of MFH.

Method application

The validated LC method was successfully applied for the assay of MFH in drug substance and tablet formulations for three batches. Assay results for three batches of MFH tablet formulation, expressed as the percentage of the label claim, were found to be 100.48%, 100.97 and 99.53% (n = 3), respectively, showing that the content of MFH in the tablet formulation conformed to the content requirements (90–110% of the label claim). The above results demonstrated that the developed LC method achieved rapid and accurate determination of MFH and can be used for the determination of MFH in drug substance and pharmaceutical formulations (Table 3.4).

3.1.3 Conclusions

The developed LC method is simple and selective for simultaneous determination of MFH, its related impurity - cyanoguanidine and other possibly impurities present in drug substance and pharmaceutical formulations. The response of the method was found to be linear in the range of 30-70 µg/ml, and it proved to be precise and accurate. The stress testing showed that all degradation products were well separated from the MFH, confirming its stability indicating capability. This stability indicating LC–UV method was found to be suitable for the pharmaceutical quality control of MFH and its formulated products in ordinary laboratories.

3.2. Analytical Method: HPLC Method for Determination of GLZ

3.2.1. Experimental

Chromatographic conditions

Chromatographic separation was performed on TSP high performance liquid chromatography system equipped with P-2000 pump model, UV-1000 detector, AS-3000 auto sampler and SN-4000 integrator was used. And chromquest software was used for chromatogram analysis. The superspher 60-RP8 column (250 mm × 4 mm, 4µm, 100 Å) was used for the separation. The mobile phase consisted of a mixture of acetonitrile (ACN), 0.1% triethylamine and 0.1% trifluoroacetic acid mixture (45:55, v/v) and was delivered at a flow rate of 0.9 ml/min with detection at 230 nm. The injection volume was 20 µl and analysis was performed at ambient temperature.

Standard solution

A stock solution containing 0.2 mg/ml GLZ was prepared by dissolving reference standard of 40 mg in 200 ml volumetric flask and dissolved in 10 ml of acetonitrile and diluted up to the mark with mobile phase. Standard solutions were prepared by dilution of the stock solution with mobile phase to give solutions containing GLZ in concentration range of 120µg/ml -280µg/ml.

Method validation

The LC method was validated with respect to the following parameters: linearity, accuracy, precision, selectivity, stability indicating capability, and stability of reference standard solutions and tablet sample preparations.

Linearity

The linearity of the method was determined at five concentration levels (120µg/ml to 280µg/ml) over a range of 60% to 140% of intended test concentration (200µg/ml) (Table 3.5).

Selectivity

The selectivity study of analytical method used for determination of GLZ was assessed for positive and negative interference due to matrix components present in placebo mixture. Selectivity study was conducted in order to demonstrate that there is no interference in determination of GLZ in presence of other excipients. This was achieved by injecting pure standard of GLZ, controlled blend of placebo and placebo spiked with GLZ. For detection of the related impurities, a mixed solution containing GLZ with related substances was determined under the proposed chromatographic conditions.

Placebo blend equivalent to 60 mg of GLZ in GLZ tablet was dissolved and vortexed for 15 min with 100 ml of acetonitrile. Prepared solution was filtered through GF/C filter and diluted 3 times with mobile phase. Similarly placebo preparations were also processed after spiking pure GLZ WS at 100% of the test level and analyzed by proposed method.

Forcedly degraded pure drug sample under different stress conditions (heat, light, hydrogen peroxide acid, and base) were prepared for further evaluation of the selectivity of the proposed LC method.

For preparing acid and base induced degradation product, 20 ml of 0.5 M HCl and 20 ml of 0.5 M NaOH were separately added to 10 ml of 1.2 mg/ml of standard GLZ solution in a 100 ml volumetric flask and immersed in boiling water at 60°C for 1 hour. The degraded samples were then neutralized (5 ml of sample neutralized with 0.5 M NaOH / 0.5 M HCl) and transferred into 10 ml volumetric flasks and brought to volume with mobile phase. The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible effect of light.

For preparing hydrogen peroxide induced degradation product, 5 ml of hydrogen peroxide (10.0 % v/v) was added to 5 ml (0.4 mg/ml) standard GLZ solution in a 10 ml volumetric flask. The degraded samples were transferred into 100 ml volumetric flasks and brought to volume with mobile phase. The prepared solutions were injected at 0 and 30 min and immediately analyzed by the proposed method.

For preparing dry heat degradation product, the working standard of GLZ in a 100 ml volumetric flask was exposed to 60° C for 7 days under dry heat condition in the dark and then cooled to room temperature. The degraded sample was dissolved and transferred into a 100 ml volumetric flask and brought to volume with mobile phase and prepared solutions were diluted to 0.2 mg/ml and injected.

The photochemical stability of the drug was also studied by exposing the standard GLZ in a photo-stability chamber and then processed the same as indicated for dry heat degradation. The resulting solutions were used as the degraded sample solutions and determined under the described chromatographic conditions.

Accuracy

The accuracy studies was carried out by applying the developed method to mixtures of excipients to which known amount of GLZ corresponding to 80, 100, and 120 % of label claim had been added. At each level of the amount, three determinations were performed. Table 3.6 presents the data for accuracy of the method of quantification for GLZ.

Precision

The precision of the assay method was investigated by performing five replicate analyses of standard sample at intended test concentration of GLZ, i.e., 200µg/ml on the same day and on three separate days and evaluated by relative standard deviation (R.S.D.) of the peak area of the active ingredient. The method precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine replicate determinations. The R.S.D. value of the assay results, expressed as a percentage of the label claim, was used to evaluate the precision.

Analysis of the GLZ Tablet

To determine the content of GLZ in each three batches of GLZ ER tablets (label claim: 60 mg per tablet), the contents of 20 tablets were accurately weighed, their mean weight determined and they were finely powdered. A quantity of powder equivalent to 60 mg GLZ was weighed and transferred to a 200 ml volumetric flask and volume was made with acetonitrile. The prepared solution was kept for 1 hour and filtered through GF/C filter and filtrate was diluted 10 times with mobile phase. The resulting solution was used for assay. Finally, 20 µl diluted solution was injected in to column and chromatogram was recorded. The analysis was repeated in triplicate and the possibility of excipients interference in the analysis was studied.

3.2.2 Results and discussion

Method development

The analytical method is official in BP 2007 for GLZ IR tablet with the mobile phase same as that used for GLZ ER tablet. Triethylamine was used in the mobile phase as an ion-pairing agent for better resolution and to reduce the asymmetry of the peak whereas trifluoroacetic acid was used to attain the pH 2.0 and form a buffer. The concentration of both the reagents is very small (0.1% v/v each) therefore no precipitation was observed during preparation of mobile phase.

Besides quantification of GLZ, determination of possible degradation products and impurities is of importance during the development of a pharmaceutical dosage form. To analyze GLZ together with its impurities and possible degradation products, the reversed

phase LC in combination with ultraviolet (UV) detection was developed and optimized. The absorption maximum in the UV-spectrum of GLZ was observed at the wavelength of 235 nm. Not any excipients and intermediates displayed absorption maximum at 235 nm. It should be noted that excipients in pharmaceutical dosage form showed negligible absorption at this wavelength. The attention was mainly focused on the optimization of the rest chromatographic conditions such as mobile phase, the polarity and size of chromatographic column in order to detect with isocratic elution.

The final described chromatographic conditions achieved satisfactory resolution, reasonable retention and symmetric peak shapes for GLZ and impurity F under which the retention time was 13.84 min for GLZ and 12.78 min for, impurity F respectively.

Method validation

Linearity

The results of linearity showed that an excellent correlation (r = 0.9997) existed between peak area and concentration of the drug within the concentration range 120μg/ml to 280μg/ml. Table 3.5 and Fig. 3.6 indicate the linearity of the method of quantification for GLZ.

Selectivity

A mixed solution containing GLZ and impurity F was determined under the proposed chromatographic conditions. The representative chromatograms of GLZ and impurity F are shown in Fig.3.7 and 3.8 respectively, indicating the satisfactory resolution (not less than 1.5 in line with BP requirement) between GLZ and known related impurity F.

Injected solutions of placebo preparation showed no peak eluting in near vicinity of GLZ retention time indicating the specificity of the proposed method (Fig.3.9). Moreover, placebo blend spiked with GLZ at 100% level showed mean recovery of 99.98% indicating there is no interference in the estimation of GLZ. The tablet excipients were also determined and found no interferences with the determination. The above results showed that the developed LC method was selective for the determination of GLZ in drug substance and pharmaceutical preparations.

For further evaluation of the selectivity of the LC method, the forcedly degraded sample solutions prepared by subjecting the pure drug to various stress conditions such as heat, light, hydrogen peroxide, acid and base were determined under the proposed chromatographic conditions. The obtained LC chromatograms for the separation of GLZ from its degraded products in forcedly degradation study are shown in Fig. 3.10.

From forced degradation studies, it can be confirmed that there is no degradation product interfered in the retention time of GLZ. In both, acid and base hydrolytic treatments showed complete degradation of drug. In acid hydrolysis, a known secondary peak (RRT 0.28) was found to increase w. r. t. time, where as in alkali hydrolysis, the secondary peak was observed to increase at RRT 0.92 which is attributed to impurity F. Treatments such as oxidation, UV, heat showed no major degradation peak but showed slight increase in response of unknown impurities. From these studies, it is observed and confirmed that on exposure to UV and heat, no other formulation components or potential degradation products of unknown identities interferes with the estimation of GLZ indicating the selectivity and specificity of the method.

Accuracy

The accuracy was calculated as the percentage of the drug recovered from the formulation matrix. Mean recovery for GLZ from the formulation was 99.98% (99.03% to 101.73%) (n = 9), indicating the good accuracy of the developed method for the determination of GLZ in the tablets. Table 3.6 presents the data for accuracy of the method of quantification for GLZ.

Precision

About the system precision of the developed LC method, for the determination of three standard samples of GLZ, the injection repeatability was found to be 0.60 % and sample repeatability was found to be 0.27 %. While, the intra-day precision ranged from 0.6 to 1.45%, inter-day precision ranged from 0.7 to 0.98 %, respectively. About the method precision, the obtained R.S.D. value for the determination of GLZ in tablet was 0.52 % (n = 9). The results for the system precision and method precision indicated the good precision of the developed method.

Solution stability

In order to demonstrate the stability of both standard solutions and tablet sample solutions during analysis, both solutions were analyzed over a period of 24 hour at room temperature. The results showed that for both the solutions, the retention time and peak area of GLZ remained almost unchanged (R.S.D. less than 0.23% and 0.64%) and no significant degradation was observed within the indicated period, suggesting that both the solutions were stable for at least 24 hours, which was sufficient for establishing the analytical processing stability.

Ruggedness

Ruggedness of stability indicating method of GLZ was carried out by two different analysts on two different instruments on two different days and reported as % R.S.D. The % R.S.D. of the assay was found to be 1.10 indicating the ruggedness of the method for estimation of GLZ.

Table 3.7 presents the validation summary data of the analytical method for quantification of GLZ.

Method application

The validated LC method was successfully applied for the assay of GLZ in drug substance and tablet formulations for three batches. Assay results for three batches of GLZ ER tablet formulation, expressed as the percentage of the label claim, were found to be 99.64%, 100.49% and 99.83% (n = 3), respectively, showing that the content of GLZ in the tablet formulation conformed to the content requirements (90–110% of the label claim). The above results demonstrated that the LC method achieved rapid and accurate determination of GLZ and can be used for the determination of GLZ in drug substance and pharmaceutical formulations (Table 3.8).

3.2.3 Conclusions

The developed LC method is simple and selective for simultaneous determination of GLZ, its related impurity - impurity F- and other possible impurities present in drug substance and pharmaceutical formulations. The response of the method was found to be

linear in the range of 120 – 280 µg/ml, and it proved to be precise and accurate. The stress testing showed that all degradation products were well separated from the GLZ, confirming its stability indicating capability. This stability indicating LC–UV method was found suitable for the pharmaceutical quality control of GLZ and its formulated products in ordinary laboratories.

3.3. Analytical Method: HPLC Method for Determination of GPD.

3.3.1. Experimental

Chromatographic conditions

Chromatographic separation was performed on TSP high performance liquid chromatography system equipped with P-2000 pump model, UV-1000 detector, AS-3000 Auto sampler and SN-4000 integrator was used. Chromquest software was used for chromatogram analysis. The RP18 symmetry column (150 mm × 3.9 mm, 5µm, 100 Å) was used for the separation. The mobile phase consisted of a mixture of acetonitrile (ACN) and 0.05 % acetic acid (45:55, v/v) and was delivered at a flow rate of 2.0 ml/min with detection at 220 nm. The injection volume was 20 µl and analysis was performed at ambient temperature.

Standard solution

A stock solution containing 0.5 mg/ml GPD was prepared by dissolving reference standard of 25 mg in 50 ml volumetric flask and dissolved in 10 ml of tetrahydrofuran (THF) and diluted up to the mark with acetonitrile, which further diluted 10 times in mobile phase. Standard solutions were prepared by dilution of the stock solution with mobile phase to give solutions containing GPD in concentration range of 30µg/ml to 70µg/ml.

Method validation

The LC method was validated with respect to the following parameters: linearity, accuracy, precision, selectivity, stability indicating capability, and stability of reference standard solutions and tablet sample preparations.

Linearity

The linearity of the method was determined at five concentration levels (30µg/ml to 70µg/ml) over a range of 60% to 140% of intended test concentration (50µg/ml) (Table 3.9).

Selectivity

For detection of the related impurities, a mixed solution containing GPD with related substances was determined under the proposed chromatographic conditions.

The selectivity study of analytical method used for determination of GPD was assessed for positive and negative interference due to matrix components present in placebo mixture. Selectivity study was conducted in order to demonstrate that there is no interference in determination of GPD in presence of other excipients. This was achieved by injecting pure standard of GPD, controlled blend of placebo and placebo spiked with GPD.

Placebo blend equivalent to 2 mg of GPD in GPD tablet was dissolved and vortexed for 15 min with 20 ml of acetonitrile. Prepared solution was filtered through GF/C filter and diluted 2 times with mobile phase. Similarly placebo preparations were also processed after spiking pure GPD WS at 100% of the test level and analyzed by proposed method.

Forcedly degraded pure drug sample under different stress conditions (heat, light, hydrogen peroxide acid, and base) were prepared for further evaluation of the selectivity of the proposed LC method.

For preparing acid and base induced degradation product, 20 ml of 0.5 M HCl and 20 ml of 0.5 M NaOH were separately added to 10 ml standard solution of GPD (0.5 mg/ml) in a 100 ml volumetric flask and immersed in boiling water at 60°C for 1 hour. The degraded samples were then neutralized (3 ml of sample neutralized with 0.5 M NaOH / 0.5 M HCl) and transferred into 10 ml volumetric flasks and brought to volume with mobile phase. The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible effect of light.

For preparing hydrogen peroxide induced degradation product, 10 ml hydrogen peroxide (10% v/v) was added to 10 ml of 0.1mg/ml of standard GPD solution in a 10 ml

volumetric flask. The degraded samples were transferred into 100 ml volumetric flasks and brought to volume with mobile phase to prepare the solutions.

For preparing dry heat degradation product, the working standard of GPD in a 100 ml volumetric flask was exposed to 60° C for 7 days under dry heat condition in the dark and then cooled to room temperature. The degraded sample was dissolved and transferred into a 100 ml volumetric flask and brought to volume with mobile phase and prepared solutions were diluted to 0.05 mg/ml and injected.

The photochemical stability of the drug was also studied by exposing the standard GPD in photo-stability chamber and then processed the same as indicated for dry heat degradation. The resulting solutions were used as the degraded sample solutions and determined under the described chromatographic conditions.

Accuracy

The accuracy studies was carried out by applying the developed method to mixtures of excipients to which known amount of GPD corresponding to 80, 100, and 120 % of label claim had been added. At each level of the amount, three determinations were performed (Table 3.10).

Precision

The precision of the assay method was investigated by performing five replicate analyses of standard sample at intended test concentration of GPD (50μg/ml) on the same day and on three separate days and evaluated by relative standard deviation (R.S.D.) of the peak area of the analyte. The method precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine replicate determinations. The R.S.D. value of the assay results, expressed as a percentage of the label claim, was used to evaluate the method precision (Table 3.11).

Analysis of GPD tablet

To determine the content of GPD in each three batches of GPD tablets (label claim: 2 mg per tablets), the contents of 20 tablets were accurately weighed, their mean weight determined and they were finely powdered. A quantity of powder equivalent to 12.5 mg

GPD was weighted and transferred to a 25 ml volumetric flask which was treated with 5ml of THF and 20 ml of acetonitrile was added. The prepared solution was sonicated for 2 min and 5 ml of filtered solution was diluted 10 times with mobile phase. The resulting solution was used as the sample solution for assay. Finally, 20 µl diluted solution was injected in to column and chromatogram was recorded. The analysis was repeated in triplicate and the possibility of excipients interference in the analysis was studied.

3.3.2 Results and discussion

Method development

Besides quantification of GPD, determination of possible degradation products and impurities is of importance during the development of a pharmaceutical dosage form. To analyze GPD together with its impurities and possible degradation products, the reversed phase LC in combination with ultraviolet (UV) detection was developed and optimized. The absorption maximum was observed in the UV-spectrum of GPD at the wavelength of 220 nm. No excipients and intermediates displayed absorption maximum at 220 nm. It should be noted that excipients in pharmaceutical dosage form showed negligible absorption at this wavelength. Our attention was mainly focused on the optimization of the rest chromatographic conditions such as mobile phase, the polarity and sorts of chromatographic column in order to detect with isocratic elution.

The final described chromatographic conditions achieved satisfactory resolution, reasonable retention and symmetric peak shapes for GPD and sulfonamide under which the retention time was 6.61 min for GPD and 5.60 min for, sulfonamide respectively.

Method validation

Linearity

The results of the linearity study showed that an excellent correlation (r = 0.9999) existed between peak area and concentration of the drug within the concentration range 30-70µg/ml. Table 3.9 and Fig. 3.11 indicate the linearity of the quantification method for GPD.

Selectivity

A mixed solution containing GPD and sulfonamide was determined under the proposed chromatographic conditions. The representative chromatograms of GPD and sulfonamide impurity are shown in Fig.3.12 and 3.13 respectively, indicating the satisfactory resolution between GPD and sulfonamide impurity.

Injected solutions of placebo preparation showed no peak eluting in near vicinity of GPD retention time indicating the specificity of the proposed method. Moreover, placebo blend spiked with GPD at 100% level showed mean recovery of 100.01% indicating there is no interference in the estimation of GPD (Fig. 3.14). The tablet excipients were also determined and found no interferences with the determination. The above results showed that the developed LC method was selective for the determination of GPD in drug substance and pharmaceutical preparations.

For the further evaluation of the selectivity of the LC method, the forcedly degraded sample solutions prepared by subjecting the pure drug to various stress conditions such as heat, light, hydrogen peroxide, acid and base were determined under the proposed chromatographic conditions. The obtained LC chromatograms for the separation of GPD from its degraded products in forcedly degradation study are shown in Fig.3.15. There was no other degradant peak formed during acid hydrolysis study abut very small response observed at the retention time of Sulphonamide. Peak area of GPD was decreased over the time which may be attributed to the precipitation of the sample solution after addition of acid. It was also observed that the amount of precipitate was gradually increased on hydrolysis and the precipitate was unevenly distributed in sample matrix.

Alkali hydrolysis study showed degradation peak at 20 min and well separated unknown impurities were also formed. With the increase in the time of hydrolysis, the well separated unknown impurities merged into each other. The study of peak purity could prove that the peak due to GPD remains pure though there is decrease in peak area response over the period of hydrolysis.

Oxidation method of degradation showed decrease in peak area response of GPD but no degradants were formed. No major degradation was observed when the active was exposed to heat, light & UV light. When exposed to UV light for 72 hrs, drug product

showed slight degradation. The peak area response of GPD was considerably decreased and some unknown degradants were formed at very low detection level. No major degradants were observed in the drug product when exposed to heat and light.

The peak purity of GPD peak was found to be 0.997 – 1.000 in every sample of forced degradation which is comparable to GPD standard peak. It showed that there is no interference due to any other impurities or degradants at the retention time of GPD.

The tablet excipients were also determined and found no interferences with the determination. The above results showed that the developed LC method was selective for the determination of GPD in drug substance and pharmaceutical preparations.

Accuracy

The accuracy was then calculated as the percentage of the drug recovered from the formulation matrix. Mean recovery for GPD from the formulation was 100.46% (97.25% to 103.31%) (n = 9), indicating the good accuracy of the developed method for the determination of GPD in the tablets. Table 3.10 presents the data indicating accuracy of the quantification method for GPD.

Precision

About the system precision of the developed LC method, for the determination of three standard samples of GPD, the injection repeatability was found to be 0.19 % and sample repeatability was found to be 0.1 %. While, the intra-day precision ranged from 0.3 to 0.6%, between-day precision ranged from 0.5 to 1.1%, respectively. About the method precision, the obtained R.S.D. value for the determination of GPD in tablet was 0.46 % (n = 9). The results for the system precision and method precision indicated the good precision of the developed method.

Solution stability

In order to demonstrate the stability of both standard solutions and tablet sample solutions during analysis, both solutions were analyzed over a period of 24 hours at room temperature. The results showed that for both the solutions, the retention time and peak area of GPD remained almost unchanged (R.S.D. less than 0.19 and 0.52%) and no

significant degradation was observed within the indicated period, suggesting that both the solutions were stable for at least 24 hours, which was sufficient for establishing the analytical processing stability.

Ruggedness

Ruggedness of stability indicating method of GPD was carried out by two different analysts on two different instruments on two different days and reported as % R.S.D. The % R.S.D. of the assay was found to be 1.15 % indicating the ruggedness of the method for estimation of GPD.

Table 3.11 represents validation summary data of the quantification method for analysis of GPD.

Method application

The validated LC method was successfully applied for the assay of GPD in tablet formulations for three batches. Assay results for three batches of GPD Tablet formulations, expressed as the percentage of the label claim, were found to be 100.59%, 100.16% and 100.21% (n = 3), respectively, showing that the content of GPD in the tablet formulation conformed to the content requirements (90–110% of the label claim). The above results demonstrated that the developed LC method achieved rapid and accurate determination of GPD and can be used for the determination of GPD in drug substance and pharmaceutical formulations (Table 3.12).

3.3.3 Conclusions

The developed LC method is simple and selective for simultaneous determination of GPD, its related impurity - sulfonamide and other possibly impurities present in drug substance and pharmaceutical formulations. The response of the method was found to be linear in the range of 30 – 70 µg/ml, and it proved to be precise and accurate. The stress testing showed that all degradation products were well separated from the GPD, confirming its stability indicating capability. This stability indicating LC–UV method was found suitable for the pharmaceutical quality control of GPD and its formulated products in ordinary laboratories.

3.4. Analytical methodology for simultaneous estimation of combination products.

A quick method was developed for simultaneous determination of combination product as an in-process test at all stages of product development. e.g. dry blend, granules, lubricated granules, pre-compressed tablets etc. The objective of this exercise was to speed up the development work and avoid undue storage of the product at each stage. However, previously described analytical methodology was used for complete testing of the products and monitoring the stability studies. The summary of the method and its part validation is given below:

3.4.1. Simultaneous estimation of MFH ER and GLZ ER tablet.

Chromatographic separation was performed on TSP High performance liquid chromatography system equipped with P-2000 pump model, UV-1000 detector, AS-3000 auto sampler and SN-4000 integrator. Chromquest software was used for chromatogram analysis. The Nucleosil 100- 10SA column (4.60 mm × 25cm, 5µm) was used for the separation. The mobile phase consisted of a mixture of 1.7% of $NH_4H_2PO_4$ (pH adjusted to 3.0 with ortho-phosphoric acid) and acetonitrile (70:30, v/v) and was delivered at a flow rate of 1.5 ml/min with detection at 233 nm. The injection volume was 20 µl and analysis was performed at ambient temperature.

Validation summary of analytical method for simultaneous estimation of MFH ER and GLZ ER bi-layered tablet.

The analytical method for simultaneous determination of MFH and GLZ from MFH ER + GLZ ER bi-layered tablet was validated to determine the range of linearity, specificity (in terms of placebo interference), accuracy and precision of the method.

The response of detector was found to be linear over 0.6µg/ml to 12µg/ml for GLZ with correlation coefficient of 0.9998 and for MFH, the linearity was observed between 5µg/ml to 100µg/ml with correlation coefficient of 0.9999. The results of accuracy study indicated that the accuracy in terms of % recovery was found to be 100.65% for GLZ and 100.76% for MFH. The RSD value of precision study for MFH was 0.74% and for GLZ, it was 0.48%. As no peak from placebo was eluted at the RT of MFH and GLZ, it indicated that placebo interference is nil and the method is very specific to determine

MFH and GLZ simultaneously in MFH ER +GLZ ER bi-layered tablet. The validation summary of the method is presented in Table 3.13.

3.4.2. Simultaneous estimation of MFH ER and GPD tablet

Chromatographic separation was performed on TSP High performance liquid chromatography system equipped with P-2000 pump model, UV-1000 detector, AS-3000 auto sampler and SN-4000 integrator. Chromquest software was used for chromatogram analysis. The Nucleosil (100-5 SA, 250cm X 4.60mm, 5µ) was used for the separation. The mobile phase consisted of a mixture of buffer and acetonitrile in 70:30, v/v proportion. The buffer was prepared by dissolving 17gm of ammonium dihydrogen orthophosphate in 1000ml of water. pH of the buffer was adjusted to 3.0 with diluted orthophosphoric acid. The mobile phase was delivered at a flow rate of 1.0 ml/min with detection at 228 nm. The injection volume was 20 µl and analysis was performed at ambient temperature.

Validation summary of analytical method for simultaneous estimation of MFH ER and GPD IR bi-layered tablet.

The analytical method for simultaneous determination of MFH ER and GPD IR from MFH ER + GPD IR bi-layered tablet was validated to determine the range of linearity, specificity (in terms of placebo interference), accuracy and precision of the method.

The response of detector was found to be linear over 2.5µg/ml to 50µg/ml for GPD with correlation coefficient of 0.9999 and for MFH, the linearity was observed between 25µg/ml to 500µg/ml with correlation coefficient of 1.0000. The range of linearity is much lower in case of simultaneous estimation for each drug than in individual estimation because at this concentration, the mean response was observed. The results of accuracy study indicated that the accuracy in terms of % recovery was found to be 99.91% for GPD and 99.46% for MFH. The RSD value of precision study for MFH was 0.29% and for GPD, it was 0.52%. As no peak from placebo was eluted at the RT of MFH and GPD, it indicates that placebo interference is nil and the method is very specific to determine MFH and GPD simultaneously in MFH ER +GPD IR bi-layered tablet. The validation summary of the method is presented in Table 3.14.

3.5 Bioanalytical Methods:

The bioavailability studies were carried out at Contract Research Organization (CRO), Lambda Therapeutic Research Pvt. Ltd, Ahmedabad. The study was monitored by the researcher himself. All Standard Operating Procedures (SOPs) of CRO were followed for clinical and bioanalytical studies.

3.5.1 HPLC method for the estimation of MFH in human blood serum.

Summary of bioanalytical method:

The concentrations of MFH in human plasma were determined using a precise and accurate HPLC procedure. The samples were analyzed on HPLC of Shimadzu make by using Kromasil C-18 column and isocratic mobile phase system. The mobile phase consisted of 11% acetonitrile and 89% buffer of pH 6.0. pH 6.0 buffer was prepared by using 75% of 5mM 1-octane sulfonic acid sodium salt solution and 25% of 50 mM di-potassium hydrogen phosphate solution. MFH was monitored by HPLC using the UV detector at 234nm. The retention time for MFH was about 8.5 minutes. The Shimadzu CLASS-VP software was used for evaluation of chromatograms.

Validation summary of bioanalytical method.

The bioanalytical method for estimation of MFH in human plasma was validated to determine the range of linearity, sensitivity, accuracy and precision of the method at CRO.

The peak area ratio for an 8-point calibration curve was found to be linear from 25.11 ng/ml to 1207.952 ng/ml. The method was found to be sensitive enough to determine the lowest concentration of MFH, i.e., 25.11 ng/ml of MFH. Accuracy of the method was established by determining within-batch accuracy and between-batch accuracy. The precision was also measured within-batch and between-batch. The validation parameters of bioanalytical method for MFH are summarized in Table 3.15. Based upon the data of validation the method was found to be sensitive enough to determine very low concentration of MFH, precise and accurate.

3.5.2: HPLC method for the estimation of GLZ in human blood serum.

Summary of bioanalytical method:

The concentrations of GLZ in human plasma were determined using a precise and accurate HPLC procedure. The samples were analyzed on HPLC of Shimadzu make by using Kromasil C-18 column and isocratic mobile phase system. The mobile phase consisted of 50% acetonitrile and 50% potassium dihydrogen phosphate buffer of pH 4.0. GLZ was monitored by HPLC using the UV detector at 230nm. The retention time for GLZ was about 8.8 minutes. The Shimadzu CLASS-VP software was used for evaluation of chromatograms. Based upon the data of validation the method was found to be sensitive enough to determine very low concentration of GLZ, precise and accurate.

Validation summary of bioanalytical method.

The bioanalytical method for estimation of GLZ in human plasma was validated to determine the range of linearity, sensitivity, accuracy and precision of the method at CRO.

The peak area ratio for an 8-point calibration curve was found to be linear from 0.102µg/ml to 8.020µg/ml. The sensitivity of the method was established by determining limit of quantitation (LOQ). The LOQ of this method was found to be 0.102 µg/ml. Within-batch and between-batch accuracy was determined to establish accuracy of the method. The validation parameters of bioanalytical method for GLZ are summarized in Table 3.16.

3.6 Conclusion:

All the analytical methods were well validated and are suitable for estimation of MFH, GLZ and GPD individually or in simultaneous in formulations (both individual or combination formulations), various quality samples or dissolution samples.

Bioanalytical methods were also validated and found to be suitable for plasma sample analysis for pharmacokinetics and bioavailability studies.

Table 3.1: Linearity of the proposed method for MFH.

Concentration (µg/ml)	Mean peak area	% RSD
30	1187346	0.41
40	1598405	0.37
50	2022136	0.55
60	2429300	0.29
70	2891093	0.14

Table 3.2: Accuracy of the proposed method for quantification of MFH.

Conc. Level	Amount of placebo (mg)	Amount of MFH added (mg)	Amount of MFH recovered (mg)	% Recovery
80 %	1810	26.80	31.13	100.16
	1820	38.50	37.88	98.39
	1840	38.90	37.66	96.81
Mean	1823	34.73	35.56 ± 3.84	98.45
100 %	820	51.20	52.15	101.86
	810	50.20	50.94	101.47
	910	51.60	52.40	101.55
Mean	847	51.00	51.83 ± 0.78	101.63
120 %	320	58.90	60.16	102.14
	400	59.50	62.42	104.91
	360	60.20	61.36	101.93
Mean	360	59.53	61.31 ± 1.13	102.99

Table 3.3: Validation summary of the proposed method for quantification of MFH.

Performance parameter	Results	Acceptance limit
A. Selectivity	No interference observed	Interference NMT 1.0 %
B. Accuracy	% Recovery = 101.8	% Recovery (98 - 102)
C. Precision	% RSD = 0.56	% RSD NMT 2.0
D. Linearity	R = 0.9981	R > 0.99
E. Range of linearity	30µg/ml to 70µg/ml	30µg/ml to 70µg/ml
F. Ruggedness	% RSD = 0.5	% RSD NMT 2.0

Table 3.4: Table showing method application for MFH ER tablets.

Batch no.	1	2	3
Assay (%)	99.80	100.30	100.10
	100.98	101.76	99.17
	100.66	100.87	99.34
Mean (%)	100.48	100.97	99.53
SD	± 0.61	± 0.74	± 0.49

Table 3.5: Linearity of the proposed method for GLZ.

Concentration (µg/ml)	Mean peak area	% RSD
120	5205226	0.21
160	6992651	0.33
200	8879559	0.19
240	10344939	0.27
280	11756399	0.15

Table 3.6: Accuracy of the proposed method for quantification of GLZ.

Conc. Level	Amount of placebo (mg)	Amount of GLZ added (mg)	Amount of GLZ recovered (mg)	% Recovery
80 %	1862	641.60	644.52	100.46
	1862	641.80	642.30	100.08
	1864	639.60	641.84	100.35
Mean	1863	641.00	642.89 ± 1.43	100.29
100 %	1700	798.60	799.56	100.12
	1703	800.20	798.24	99.76
	1704	800.40	797.09	99.59
Mean	1702	799.73	798.30 ± 1.24	99.82
120 %	1536	971.20	987.96	101.73
	1542	956.40	947.13	99.03
	1544	955.20	946.56	99.10
Mean	1541	960.93	960.55 ± 23.74	99.96

Table 3.7: Validation summary of the proposed method for quantification of GLZ.

Performance parameter	Results	Acceptance limit
A. Selectivity	No interference observed	Interference NMT 1.0 %
B. Accuracy	% Recovery = 99.98	% Recovery (98 - 102)
C. Precision	% RSD = 0.27	% RSD NMT 2.0
D. Linearity	R = 0.9979	R > 0.99
E. Range of linearity	120µg/ml to 280µg/ml	120µg/ml to 280µg/ml
F. Ruggedness	% RSD = 1.10	% RSD NMT 2.0

Table 3.8: Table showing method application for GLZ ER tablets.

Batch no.	1	2	3
Assay (%)	100.12	100.83	99.36
	99.71	101.01	100.63
	99.10	99.65	99.52
Mean (%)	99.64	100.49	99.83
SD	± 0.51	± 0.74	± 0.69

Table 3.9: Linearity of the proposed method for GPD.

Concentration (µg/ml)	Mean peak area	% RSD
30	346138	0.40
40	466347	0.27
50	587604	0.34
60	702964	0.18
70	823333	0.37

Table 3.10: Accuracy of the proposed method for quantification of GPD.

Conc. Level	Amount of placebo (mg)	Amount of GPD added (mg)	Amount of GPD recovered (mg)	% Recovery
80 %	1052	10.10	9.92	98.22
	1053	10.30	10.46	101.55
	1053	10.40	10.48	100.77
Mean	1053	10.27	10.29 ± 0.32	100.19
100 %	1051	12.70	13.12	103.31
	1050	12.50	12.53	100.24
	1051	12.60	12.85	101.98
Mean	1051	12.60	12.83 ± 0.30	101.85
120 %	1048	15.40	15.87	103.05
	1048	15.20	14.99	98.62
	1048	15.30	14.88	97.25
Mean	1048	15.30	15.25 ± 0.54	99.65

Table 3.11: Validation summary of the proposed method for quantification of GPD.

Performance parameter	Results	Acceptance limit
A. Selectivity	No interference observed	Interference NMT 1.0 %
B. Accuracy	% Recovery = 100.46	% Recovery (98 - 102)
C. Precision	% RSD = 0.10	% RSD NMT 2.0
D. Linearity	R = 0.9999	R > 0.99
E. Range of linearity	30µg/ml to 70µg/ml	30µg/ml to 70µg/ml
F. Ruggedness	% RSD = 0.98	% RSD NMT 2.0

Table 3.12: Table showing method application for GLZ ER tablets.

Batch no.	1	2	3
Assay (%)	101.77	99.67	100.59
	100.68	101.37	99.55
	99.32	99.44	100.49
Mean (%)	100.59	100.16	100.21
SD	1.23	1.05	0.57

Table 3.13: Validation summary of analytical method for simultaneous determination of MFH and GLZ in MFH ER and GLZ ER bi-layered tablet.

Parameter	Results	Acceptance limit
Specificity (Placebo interference)	Nil	NMT 1.0%
Accuracy		
MFH	100.76%	98.0% to 102.0%
GLZ	100.65%	
Precision		
MFH	0.74%	RSD: NMT 0.2%
GLZ	0.48%	
Linearity		
MFH	0.9999	Correlation coefficient should be ≥ 0.99
GLZ	0.9998	
Range of linearity		
MFH	5µg/ml-100µg/ml	5µg/ml-100µg/ml
GLZ	0.6µg/ml-12µg/ml	0.6µg/ml-12µg/ml
Ruggedness		
MFH	1.47	% RSD for two analysts should be NMT 2.00%
GLZ	0.57	

Table 3.14: Validation summary of analytical method for simultaneous determination of MFH and GPD in MFH ER and GPD IR bi-layered tablet.

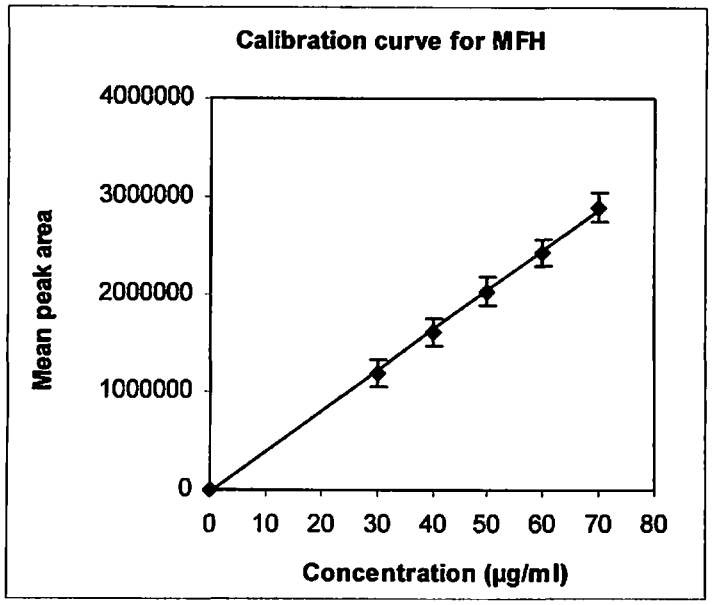
Parameter	Results	Acceptance limit
Specificity (Placebo interference)	Nil	NMT 1.0%
Accuracy		
MFH	99.46%	98.0% to 102.0%
GPD	99.91%	
Precision		
MFH	0.29	RSD: NMT 2.0%
GPD	0.52	
Linearity		
MFH	1.0000	Correlation coefficient should be ≥ 0.99
GPD	0.9999	
Range of linearity		
MFH	25µg/ml to 500µg/ml	25µg/ml to 500µg/ml
GPD	2.5µg/ml to 50µg/ml	2.5µg/ml to 50µg/ml
Ruggedness		
MFH	0.85%	% RSD for two analysts should be NMT 2.00%
GPD	0.91%	

Table 3.15: Validation parameters of bioanalytical method for MFH.

Validation parameters		**Results**	
		Drug (MFH)	Int. Std.
Linearity	Range (ng/ml)	25.110 to 1207.95	NA
Sensitivity	Limit of Quantitation (ng/ml)	25.110	NA
Accuracy	Within batch	90.40 to 114.10%	NA
	Between batch	102.80 to 112.10%	NA
	By different analyst on different instrument	109.70 to 114.70%	NA
Precision	Within batch	1.1 to 5.5%	NA
	Between batch	2.90 to 9.00%	NA
	By different analyst on different instrument	2.00 to 6.10%	NA
Recovery		102.45% ±1.40	32.77%
Solution stability	Short term stock solution (6.0 hours)	98.30%	98.40%
	Long term stock solution (After 9 days)	100.60% (within 2°C to 8°C	99.60% (below -20°C)
	Auto sampler/Wet extract	102.10% ± 1.37	NA
	Freeze thaw (3 cycles)	102.50% and 101.20%	NA
	Bench top (6 hours)	102.50% and 101.60%	NA
System suitability	RT, Area (Column ID 326) LC-01	0.80%, 1.00%	0.80%, 0.70%
	RT, Area (Column ID 314) LC-02	1.60%, 1.30%	1.60%, 1.30%

Table 3.16: Validation parameters of bioanalytical method for GLZ.

Validation parameters		Results	
		Drug (GLZ)	Int.Std.
Linearity	Range (µg/ml)	0.102 to 8.020	NA
Sensitivity	Limit of Quantitation (µg/ml)	0.102	NA
Accuracy	Within batch	90.90 to 109.00%	NA
	Between batch	92.30 to 102.60%	NA
	By different analyst on different instrument	91.10 to 103.40%	NA
Precision	Within batch	1.3 to 9.1%	NA
	Between batch	2.70 to 11.40%	NA
	By different analyst on different instrument	2.90 to 16.90%	NA
Recovery		101.31% ± 1.59	14.44%
Solution stability	Short term stock solution (6.0 hours)	102.60%	103.50%
	Long term stock solution (After 9 days)	102.70% (within 2°C to 8°C	98.50% (below -20°C)
	Auto sampler/Wet extract	98.81% ±1.64	NA
	Freeze thaw (3 cycles)	98.70% and 104.70%	NA
	Bench top (6 hours)	100.70% and 99.60%	NA
System suitability	RT, Area (Column ID 407) LC-01	0.0%, 0.60%	0.10%, 0.10%
	RT, Area (Column ID 402) LC-02	0.10%, 0.70%	0.20%, 0.40%



Peak area is a mean of 6 replicates.

Fig 3.1: Calibration curve for MFH indicating linearity of the proposed method.

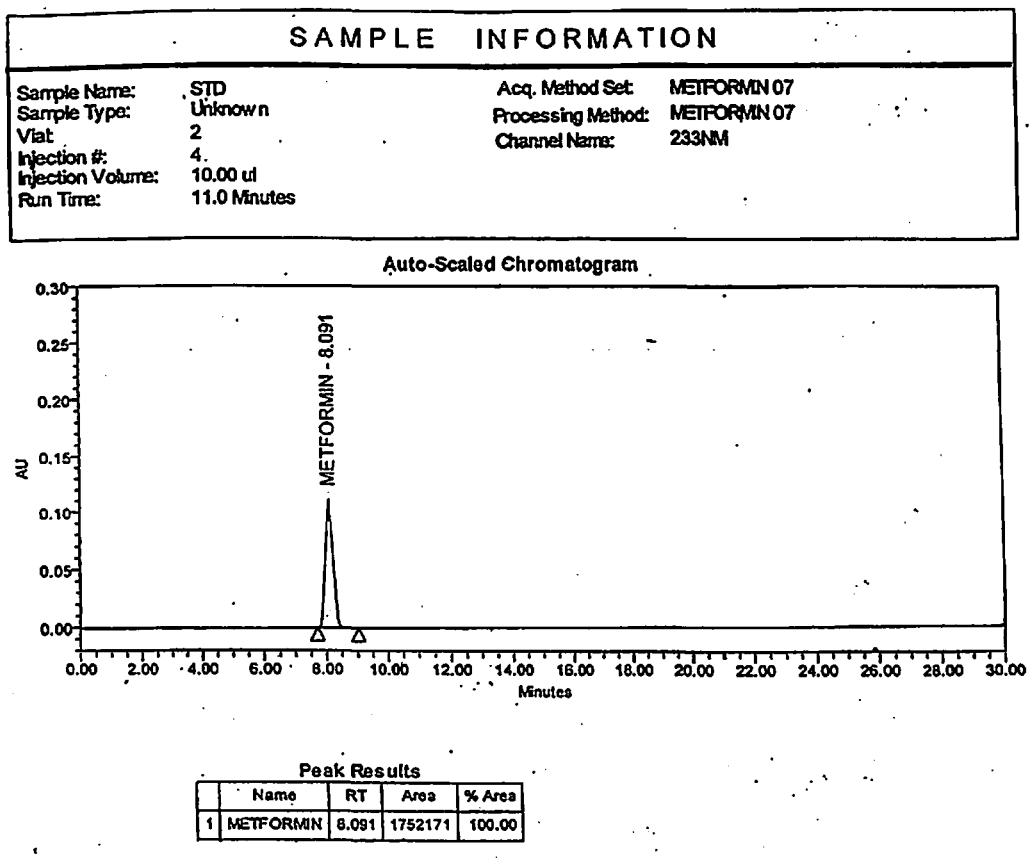
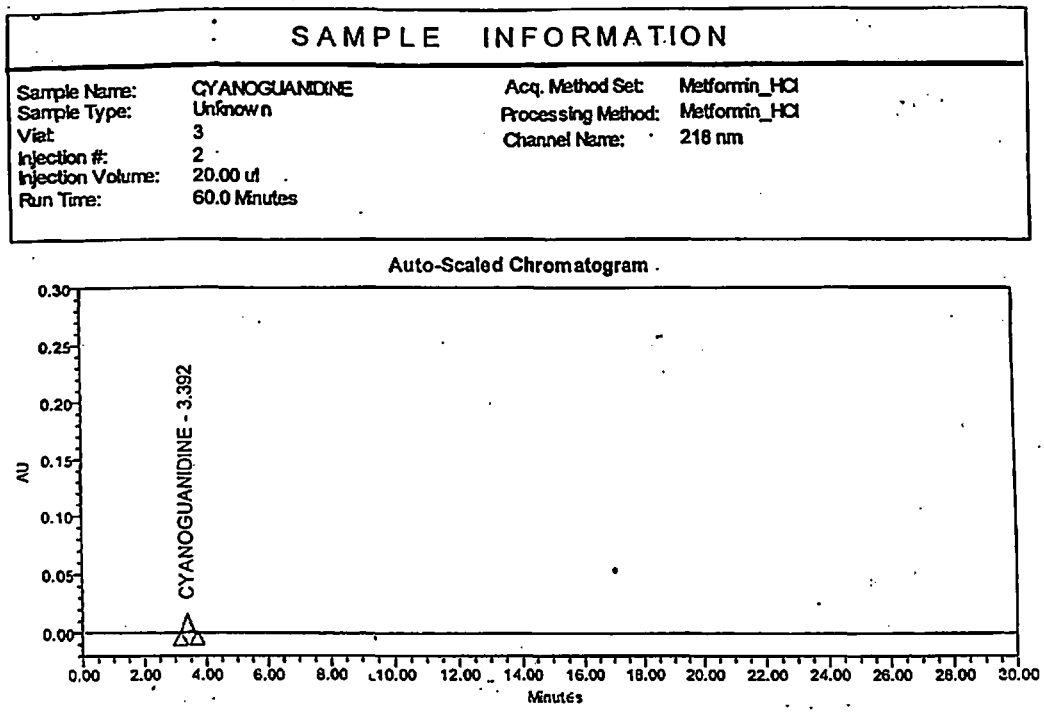


Fig.3.2: Chromatogram of MFH indicating specificity of the proposed method.



Peak Results

	Name	RT	Area	% Area
1	CYANOGUANIDINE	3.392	137085	100.00
2	MELAMINE	6.244		
3	METFORMIN	13.275		

Fig.3.3: Chromatogram of cyanoguanidine indicating specificity of the proposed method.

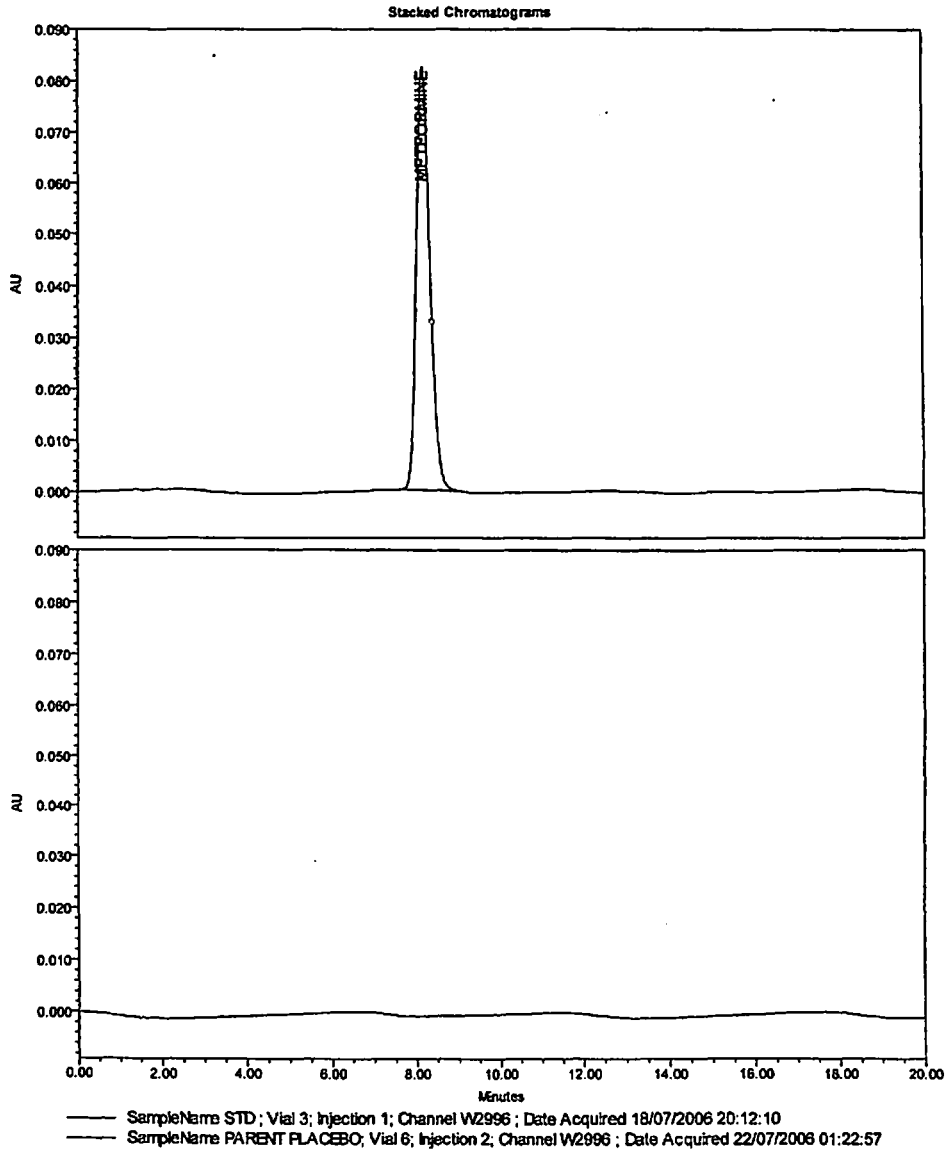


Fig.3.4: Chromatogram of MFH indicating selectivity of the proposed method.

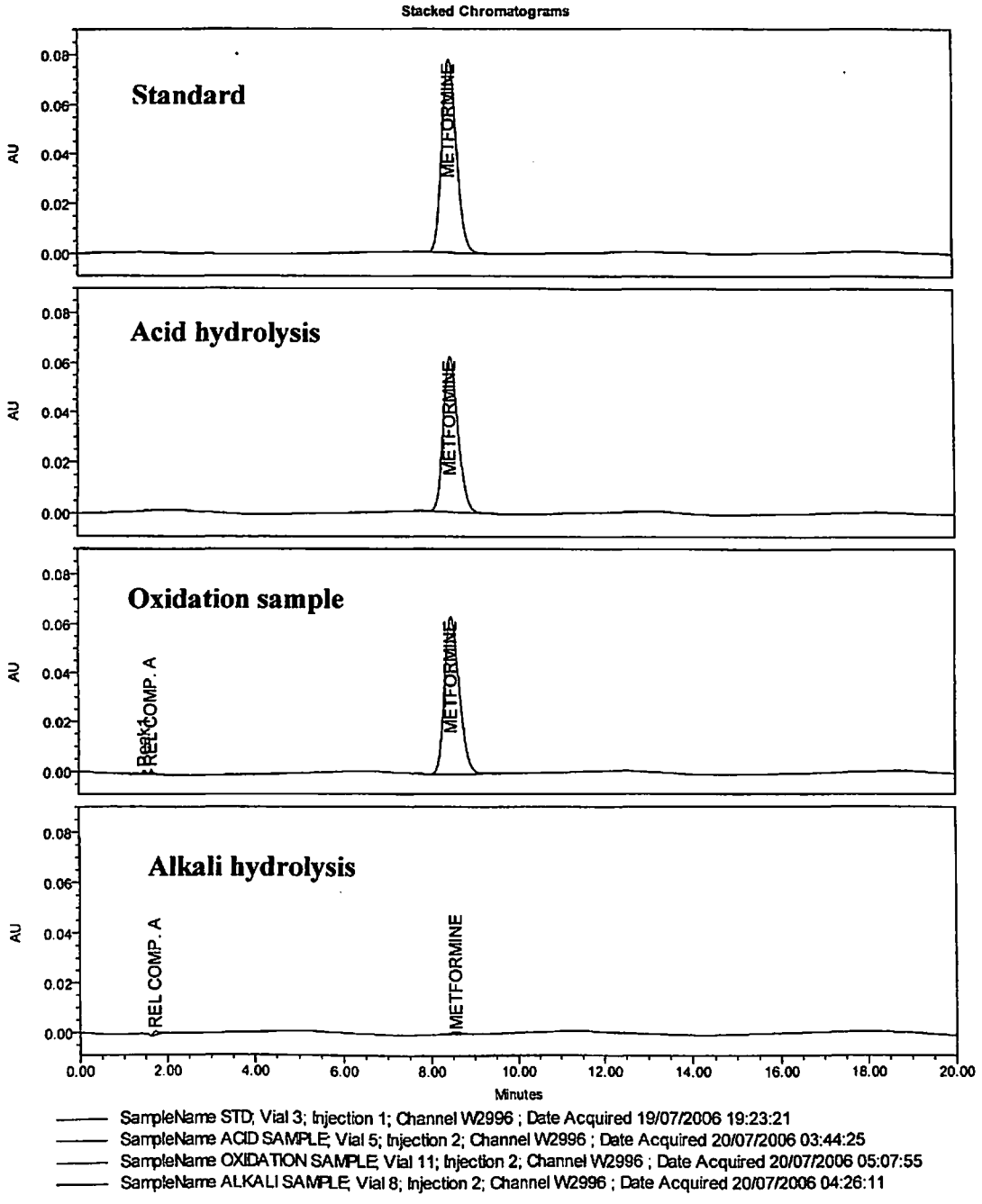
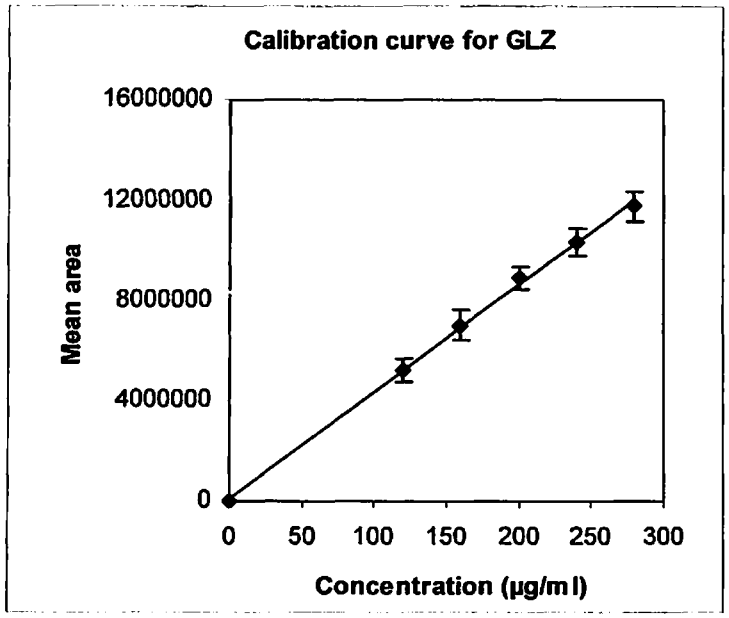


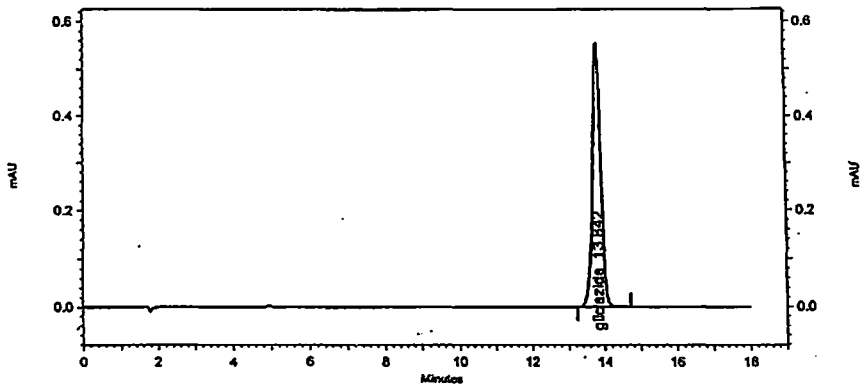
Fig.3.5: Chromatogram of MFH indicating specificity in forced degradation.



Peak area is a mean of 6 replicates.

Fig 3.6: Calibration curve for GLZ indicating linearity of the proposed method.

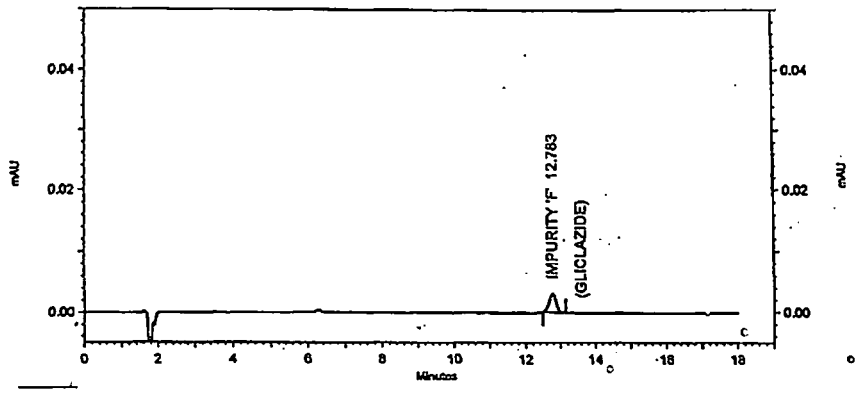
File Name : D:\Public\Data\7augliclazide004.DAT
Sample Name : GLICLAZIDE STD
Vial no : B03
Inj. Volume. : 20µl



Name	Retention Time	Area	Area Percent
gliclazide	13.842	8751655	100.000
Totals		8751655	100.000

Fig.3.7: Chromatogram of GLZ indicating specificity of the proposed method.

File Name : D:\Public\Data\7augliclazide002.DAT
Sample Name : IMPURITY 'F'
Vial no : B02
Inj. Volume. : 20µl



Name	Retention Time	Area	Area Percent
IMPURITY 'F' GLICLAZIDE	12.783	42112	100.000
Totals		42112	100.000

Fig.3.8: Chromatogram of impurity F indicating specificity of the proposed method.

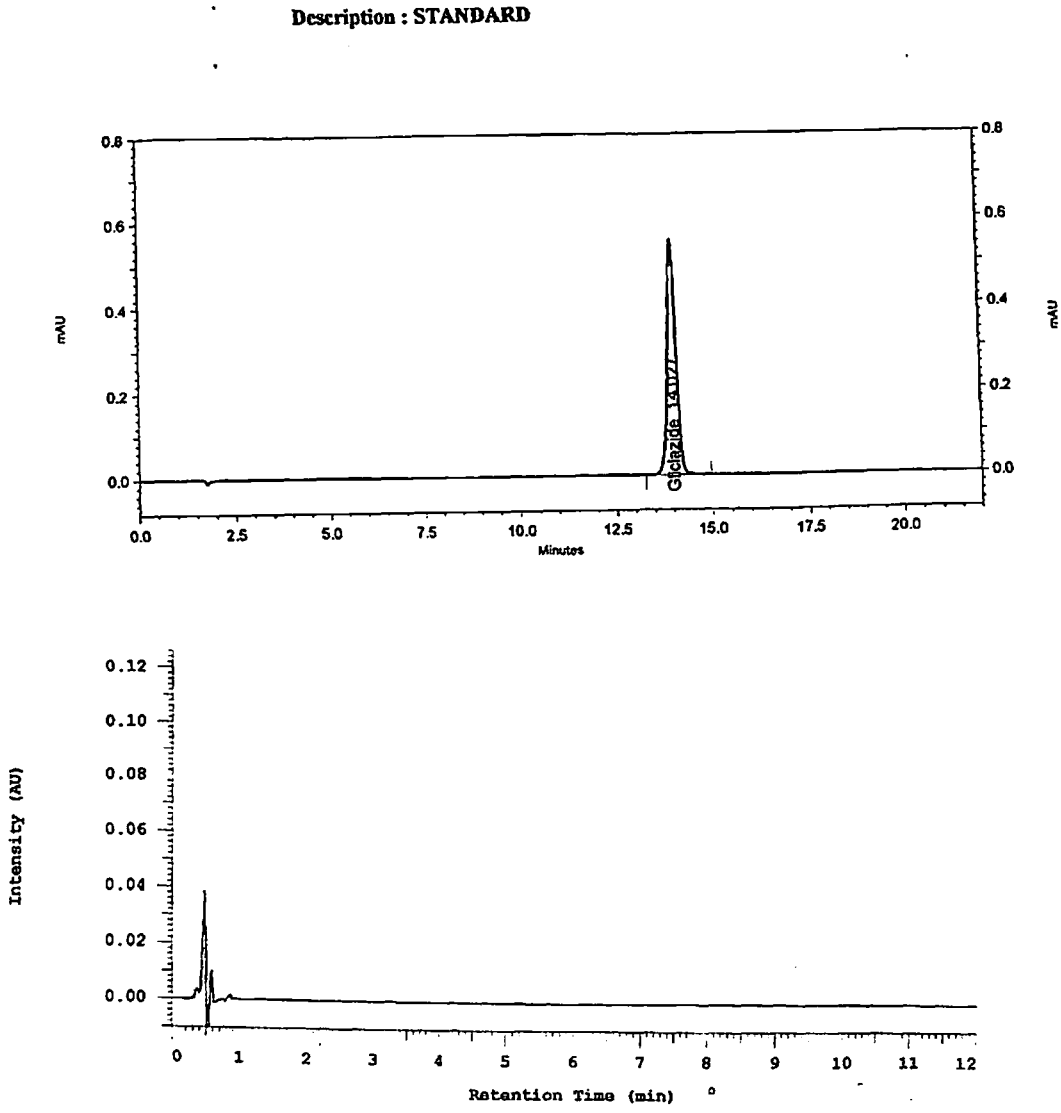
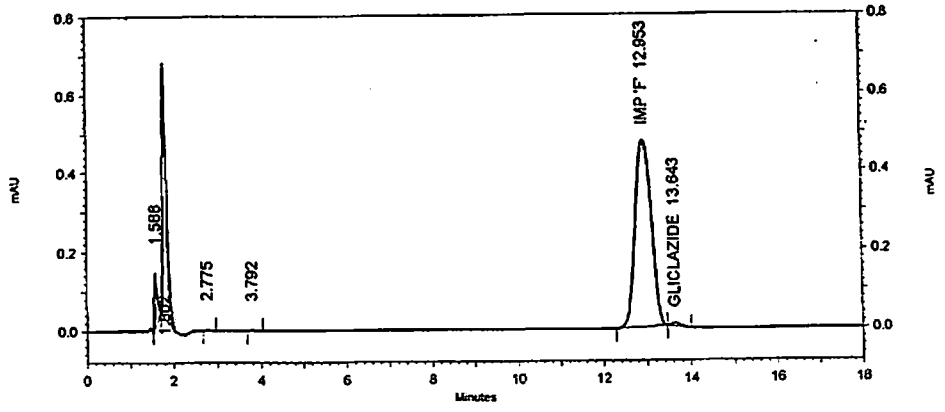
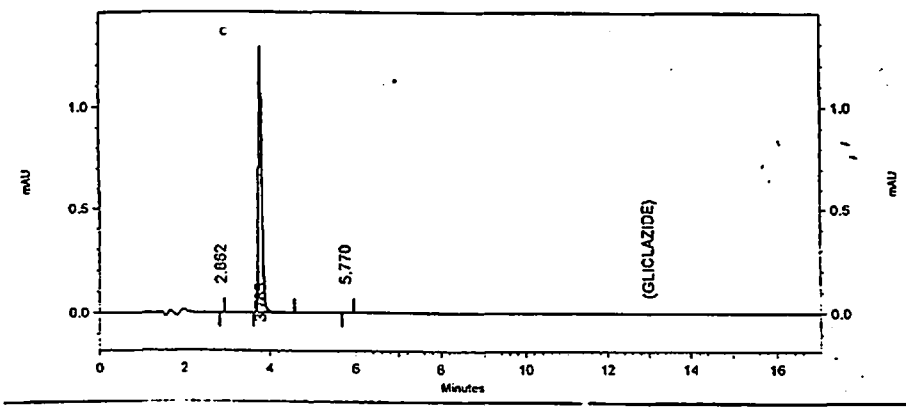


Fig.3.9: Chromatogram of GLZ indicating selectivity of the proposed method.

Alkali hydrolysis



Acid hydrolysis



Oxidation samples

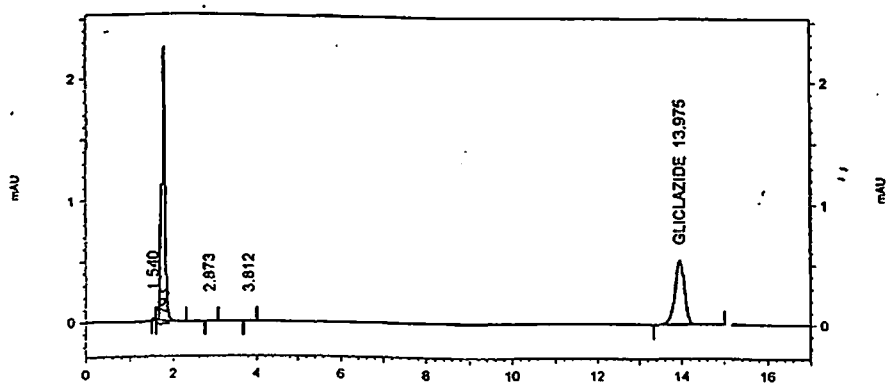
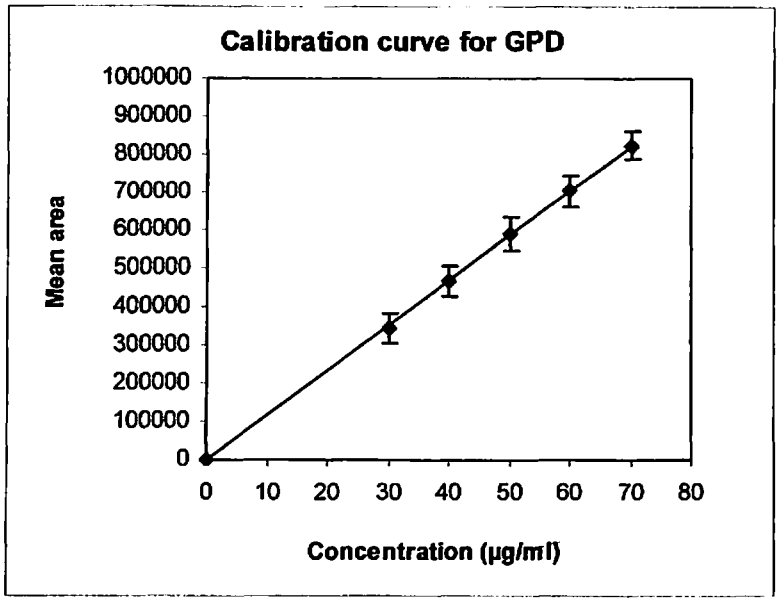


Fig.3.10: Chromatogram of GLZ indicating specificity in forced degradation.



Peak area is a mean of 6 replicates.

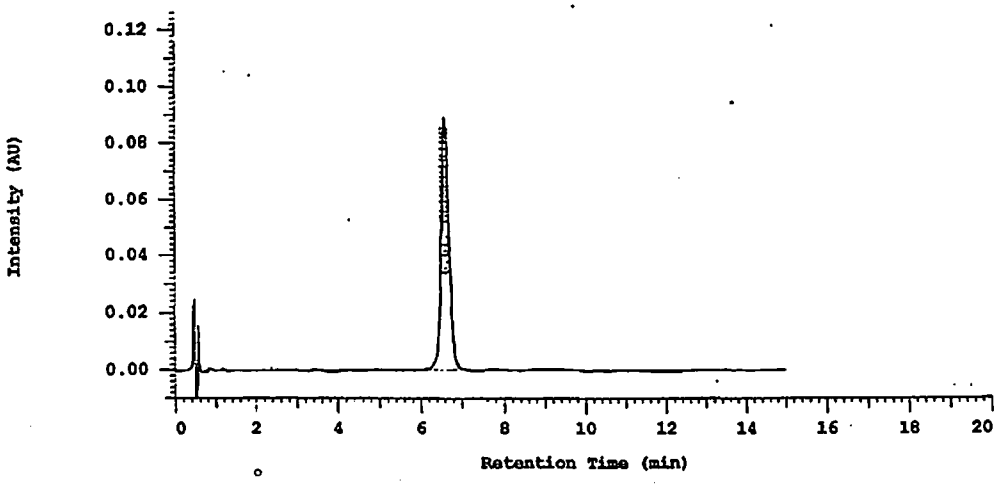
Fig 3.11: Calibration curve for GPD indicating linearity of the proposed method.

Sample Name: STD

Vial Number: 12

Vial Type: UNK

Volume: 20.0 ul

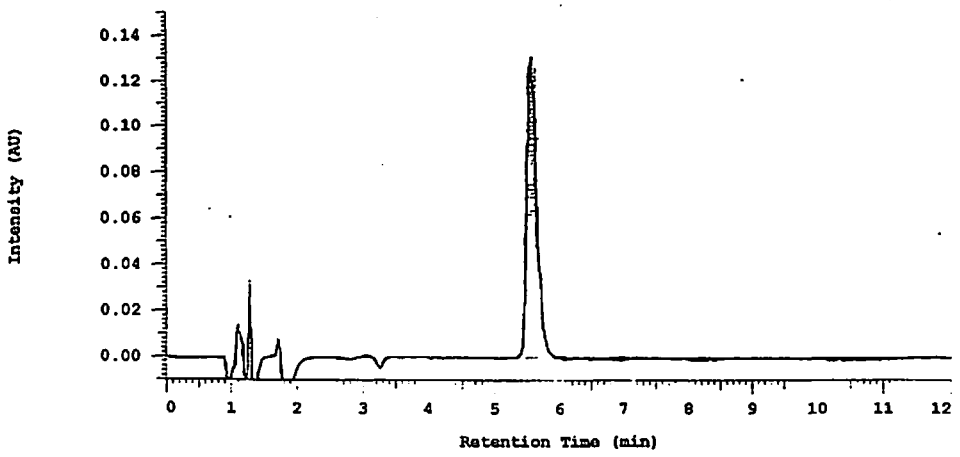


No.	Name	RT	Area	Area %
1	GLIMEPIRIDE	6.61	590183	100.000
			590183	100.000

Peak rejection level: 0

Fig.3.12: Chromatogram of GPD indicating specificity of the proposed method.

Processing Method: GLIMEPRIDE RS
System(acquisition): Lachrom-2
Sample Name: SULPHONAMIDE 20 PPM
Vial Number: 7
Volume: 20.0 µl



No.	RT	Name	Area	Area %
1	5.60	sulphonamide	671396	100.000
			671396	100.000

Peak rejection level: 0

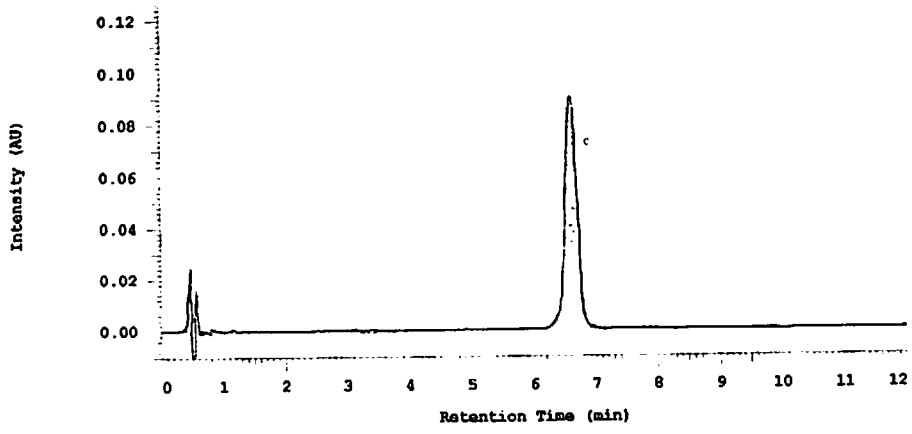
Fig.3.13: Chromatogram of sulphonamide indicating specificity of the proposed method.

Sample Name: STD

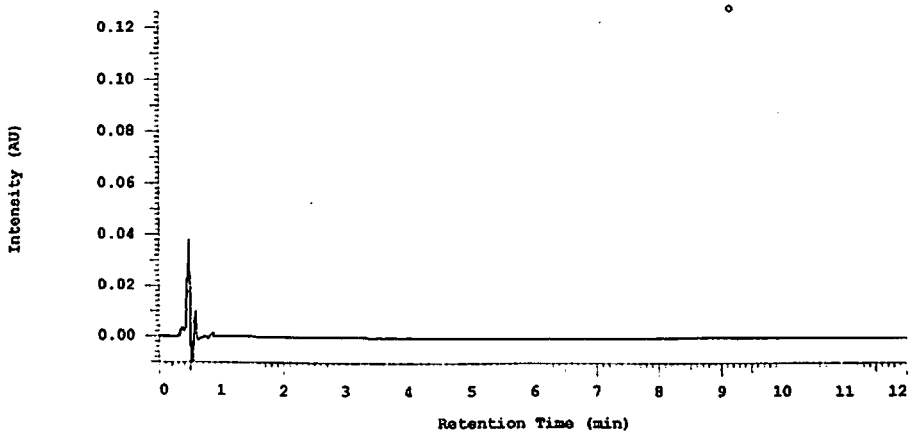
Vial Number: 12

Vial Type: UNK

Volume: 20.0 ul



No.	Name	RT	Area	Area %
1	GLIMEPIRIDE	6.61	590183	100.000

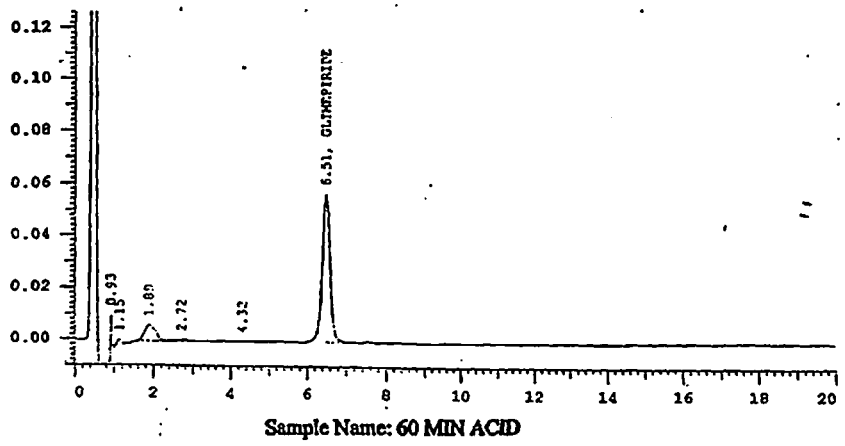


No.	Name	RT	Area	Area %
			0	0.000

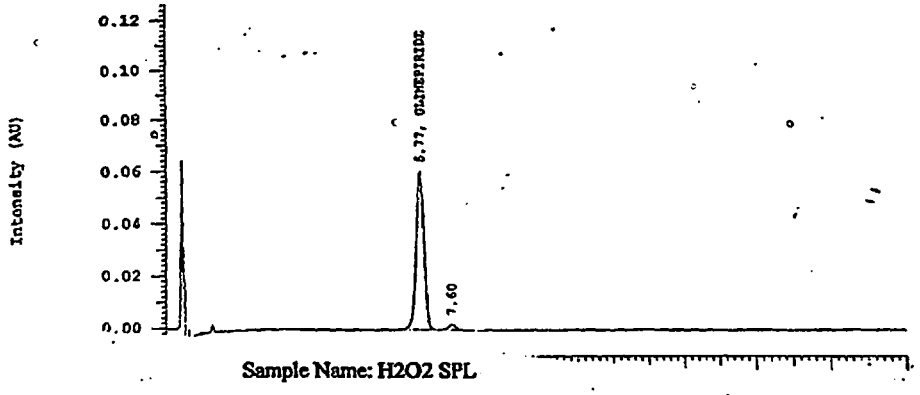
Peak rejection level: 0

Fig.3.14: Chromatogram of GPD indicating selectivity of the proposed method.

Alkali hydrolysis



Acid hydrolysis



Oxidation samples

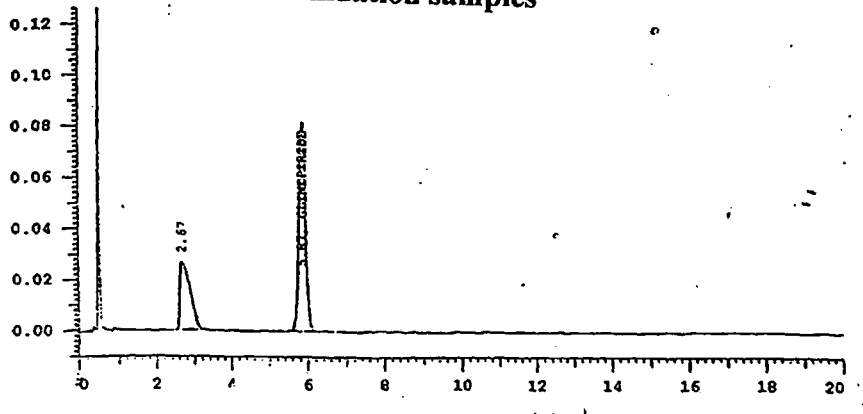


Fig.3.15: Chromatograms of GPD indicating specificity in forced degradation.

Chapter 4

Preformulation Studies

4. Preformulation: A case of "learning" before "doing".

Preformulation studies are important part of any dosage form development process. These studies focus on those physicochemical properties of the drugs that could affect drug's performance, stability and development of suitable dosage form. Historically, preformulation had its birth in the very early sixties. Starting with the early sixties more and more stability indicating evaluation methods were being introduced. These stability indicating assay methods brought many instances to the surface where a formulation was found to be unstable after a significant amount of time (6 to 12 months) [114]. It was apparent that work had to be done prior to the point when formulation was initiated, and that was the beginning of the branch of the pharmaceutical sciences, which, today is known as "Preformulation". Preformulation is considered as an interface between the drug substance and the drug product [114-116].

Preformulation studies is an integral part of any development program because it gives an adequate understanding of the properties of drug substance and minimizes problems in later stages of drug development, reduce drug development costs, and decrease time to take the product to market [117-119]. Thorough preformulation work is the foundation of developing robust formulation. The goals of preformulation studies are to choose the correct form of drug substance, evaluates its physical properties, and generate a thorough understanding of the material's stability under various conditions, leading to the optimal drug delivery system. Hence the requirement of preformulation is not only acknowledged by most companies but a prerequisite for the IND (investigative new drug) application. The necessity of preformulation is not only confined to innovator companies but also generic companies are recognizing its importance. From the generic developer's point of view, preformulation is more a task of finding the formula that most resembles that of the innovator, and ascertaining that it, physically and stability wise, matches that of the inventor.

The stage in the research and development process at which preformulation begins can greatly affect the odds of a new compound becoming a commercially viable drug product. In general, sooner the preformulation data is available, the earlier decisions can be made about the nature of the physical-chemical properties and how these might impact on the development potential of the new drug candidate e.g. when the preformulation scientist works closely with discovery scientists, preformulation data along with biological data can be used to select from a group of compounds, the best compound for future development [120]. This is one reason that many

companies have adopted the team concept of product development. Ideally, then all groups "work together" in a team, and facilitate not only meaningful approaches but also accelerate them to reduce the time from conception to marketing.

In today's industrial environment it is imperative to move the drug into human clinical trials as soon as possible in order to determine if the candidate is a potential marketable drug. The bulk of preformulation work occurs after a new chemical entity and its appropriate salt form have been selected for testing in humans. Typical studies would include a pH-stability and solubility profile, studies for polymorphs, partitioning, dissolution behavior, crystal size and shape and compatibility with excipients to be used [121]. Accelerated conditions (heat, light and humidity) are used to promote degradation of the drug compound being evaluated. In order to identify and quantitate the mechanism of degradation, the degradation products must be identified and separable in the chromatographic procedure. This information is critical to the formulation scientist in order to stabilize the drug molecule in the dosage form.

Following is the preformulation study of the drug substances selected for the development of novel combination and novel drug delivery systems for diabetes. Though, some information on drug's physicochemical properties is known, characters important for research work, are studied in detail in the following experiments.

4.1 Experimental

Materials:

The active materials metformin hydrochloride (MFH), gliclazide (GLZ) and glimiperide (GPD) used for formulation development studies were obtained from M/s. Ipca Laboratories Ltd, India. Other inactive excipients used in the study were procured from the approved vendors of M/s. Ipca Labs Ltd, India, as per the Standard Operating Procedure (SOP). All other chemicals and reagents used were of pharmaceutical or analytical grade.

Instruments and Equipments:

Accelerated stability studies were carried out in walk-in stability chambers of Newtronic Equipment Company Pvt. Ltd. (Model no.: NEC-2280R) at Ipca Labs Ltd., Mumbai. Photo-stability was carried out in the photo-stability chamber of Newtronic Equipment Company Pvt. Ltd. (Model No.: NEC 103RSP) at Ipca Labs Ltd, Mumbai. The drug content and impurity

profiling was done by High Performance Liquid Chromatography (HPLC) as mentioned in Chapter 3. DSC analysis was done by using make of Mettler, Model no. Toledo FP-90 and FP-85 with FP-99a software. Particle size analysis was done by using particle size analyzer of Malvern and model no.: Mastersizer 2000.

4.1.1 Characterization of the drug substance:

Chemical characterization of the drug substances (MFH, GLZ, and GPD) were carried out by using various compendial methods. Identification, assay or percent purity analysis was carried out according to IP, BP and/or USP. The IR spectra obtained were compared with the respective standard spectra of all the three drug substances.

Physical characterization of the drug substances was carried out by particle size analysis and hygroscopicity.

Particle size analysis:

Particle size analysis for all the three drug substances was carried out to check the powder characteristics, using Malvern particle size analyzer. The particle size data obtained is tabulated in Table 4.1 and graphically presented in Fig.4.1 for MFH; Fig. 4.2 for GLZ and Fig.4.3 for GPD. The analysis was done on 3 different lots of the drug substances to derive suitable particle size range for design and development of the dosage form. The selected particle size range for the development work is given in Table 4.2. The particle size is expressed in terms of D_{10}, D_{50} and D_{90}. D_{10}, D_{50} and D_{90} stand for the particle size of the 10, 50 and 90 percentile particles of the test sample respectively.

Hygroscopicity:

In order to study the moisture pick-up by drug substance, MFH (10 gm each) was exposed to different set of humidity conditions (i.e., 25% ±5%, 50% ±5%, 75% ±5% and 90% ±5%) in different packs i.e., open petri dish, primary pack (packed and sealed in double polyethylene bags) and secondary pack (packed and sealed in double polyethylene bags in miniature fiber drums of 0.5Kg capacity). The temperature was kept constant at controlled room temperature i.e., 25°C ± 2°C for all sets of experiments. Exposure time of sample was 7 hours for the sample in humidity of 90% ±5% and 12 hours for the sample in other humidity conditions. The samples

were withdrawn at defined intervals (i.e., 6 and 12hours). The samples at humidity of 90% ±5% were withdrawn after 3 hours, 6 hours and 7hours. The samples were evaluated for physical appearance and LOD (Loss on Drying). HPLC analysis was also carried out to monitor the content of MFH and degradant impurities. The results obtained are tabulated in Table 4.3, 4.4 and 4.5.

The objective of this study for a short period of 12 hours was to establish the environmental conditions required for manufacturing of the batches during which, drug (in pure form) will never be directly exposed to humidity conditions in manufacturing area for more than 12 hours pending for further processing.

As GLZ and GPD were reported to be non-hygroscopic, detailed hygroscopicity study was not carried out for GLZ and GPD

4.1.2 Drug – Excipient compatibility study (stability study in solid admixture)

Stability of drug substances was conducted in presence of common pharmaceutical excipients and compared with stability of respective drugs in pure form. The common pharmaceutical excipients used in the drug-excipient compatibility study were lactose monohydrate, microcrystalline cellulose (MCC), maize starch, Polyvinyl Pyrollidone K30 and Polyvinyl Pyrollidone K90 (PVP K30 and PVP K90), Hydroxy Propyl Methyl Cellulose K4M (HPMC K4M), Hydroxy Propyl Methyl Cellulose K15M (HPMC K15M), Hydroxy Propyl Methyl Cellulose K100M (HPMC K100M), carbopol, Sodium Lauryl Sulfate (SLS), colloidal silicon dioxide (aerosil 200), Sodium Starch Glycollate (SSG) , purified talc and magnesium stearate. The drug: excipient ratio was selected based upon the strength of the particular drug substance in the formulation. The respective ratios are presented in Table 4.6 to 4.8.

In order to prepare the solid admixture, first the drug substance and excipients both were sifted separately through 100 # mesh and mixed thoroughly. The mixture thus prepared was sifted again through 100 # mesh to ensure uniform blending. Drug alone (used as a control) or in combination with excipients were filled and plugged in 5ml clear, neutral glass vials and kept at ambient as well as accelerated storage conditions. The storage conditions were selected for the studies as per ICH guidelines for stability study. The selected conditions were controlled room temperature (CRT: 25±2°C and 60 ±5% RH), 40±2°C and 75 ±5% RH, 30±2°C and 65 ±5% RH, 55°C±2°C and refrigerated condition (FT: 5±2°C). The samples, in at least duplicate, were

withdrawn at predetermined time intervals (0, 30 and 60 days). The samples were analyzed after suitable dilution for drug content and impurity profile by HPLC and DSC. DSC pattern of drug substance alone and in combination with selected excipients were also studied at initial time point and after 2 months accelerated stability study (Fig. 4.4 to 4.12).

4.1.3 pH Stability study:

pH stability of the drug substances (MFH, GLZ and GPD) was studied in solutions of varying pH values. The drug substances were dissolved separately in solutions of different pH (1.0, 2.0, 3.0, 7.0, 8.0, 10.0 and 12.0). The pH was adjusted by adding varying proportion of 0.1N NaOH and 0.1N HCl in distilled water at controlled room temperature (25°C ±2°C). The prepared samples were stored at 37±2°C in 100ml volumetric flasks on a water bath shaker. The samples in triplicate were withdrawn at the end of 24hours and analyzed by HPLC for drug content and degradant impurities content. The findings of the study are presented in Table 4.9 to 4.11.

4.1.4 Effect of environmental conditions on stability

In order to study the effect of environmental conditions, the drug substances were exposed to the extremes of temperature, light and oxidizing conditions as indicated below.

Thermal stability:

In order to study the effect of temperature on MFH, GLZ and GPD, the samples (10 gm each) were packed separately in glass vials and heated at 150°C for 24 hours in the oven. The samples were withdrawn after 6, 12 and 24 hours for HPLC analysis. The study was carried out for 24 hours as 150°C is a drastic temperature condition and at no stage of product development, drug substance will get exposed to such drastic temperature conditions. The results obtained are tabulated in Table 4.12 to 4.14.

Photo-stability:

In order to study the stability under photolytic condition, about 10gm of sample (MFH, GLZ and GPD) was directly exposed to UV light source at 254 nm for 7 days. The sample was checked for physical appearance everyday and chemical analysis was done by HPLC after 7days. The results obtained are tabulated in Table 4.15 to 4.17.

Stability in strong oxidizing conditions:

In an experiment designed to study the impact of oxidizing agent on MFH, GLZ and GPD; a 5% solution was prepared in 30% hydrogen peroxide. The solution was heated to 80°C for 72 hours with continuous stirring. The samples were withdrawn after 24, 48 and 72 hours and analyzed by HPLC for drug content and percentage degradant impurities. The results obtained in the experiment are tabulated in Table 4.18 to 4.20.

4.1.5 Establishment of the solubility characteristics

pH Solubility profile

The pH solubility profile of MFH, GLZ and GPD was established over the range of varying pH solutions (pH 1.2, 2.0, 4.5, 6.8 and 7.2). The selection of the pH was done in relevance to the in vivo physiological pH conditions. These solubility experiments were conducted over the period of 24 hours, as the product is a solid dosage form. The samples were withdrawn and analyzed by HPLC. The solubility values of different media are provided in Table 4.21 and are graphically represented in Fig.4.13 to Fig.4.15.

4.2 Results and Discussions:

4.2.1 Characterization of the drug substance:

The procured drugs, MFH, GLZ and GPD passed the various test for identification and analysis as per BP 2005 and USP 2006. The IR spectra of the drugs were found comparable to their respective standards.

Particle size analysis:

Table 4.1 shows the observed particle size of drug substances in the form of D_{90}, D_{50} and D_{10} values. The value of D_{90} showed that maximum particles (90 percentile) were of size $\leq$ 100.90μm for MFH, 65.78μm for GLZ and 18.29μm for GPD. The value of D_{50} showed that half of the particles (50 percentile) were of particle size $\leq$ 56.8μm for MFH, 24.70μm for GLZ and 5.08μm for GPD. The D_{10} value showed that very less particles (10 percentile) were $\leq$ 17.03μm for MFH, 5.24μm for GLZ and 1.40μm for GPD. Based on the results of particle size analysis of three lots of drug substances, the range of particle size was defined for the development work.

This range is presented in Table 4.2. The particle size distribution of MFH, GLZ and GPD is graphically represented in Fig. 4.1, 4.2 and 4.3 respectively.

Hygroscopicity:

The physical appearance of MFH was unchanged in color and texture when exposed in Petri dish to 25%, 50% and 75% humidity for 12 hours. But at 90% humidity, drug substance liquefied within 3 hours, LOD changed significantly from 0.10% (initial LOD) to 14.76% which further changed to 33.19% when exposed for 7 hours. But increased humidity does not affect the quality attributes of MFH with respect to MFH content, cyanoguanidine content and total impurities.

MFH when exposed in primary pack and secondary pack to the similar set of conditions of humidity, it was observed that there was no change in physical appearance of MFH. The material did not show any appreciable change in LOD. The quality attributes of MFH like MFH content, cyanoguanidine content and total impurities did not show any significant change. This study suggests that at low humidity condition there was no absorption of moisture, however, at 95% RH, moisture absorption was high when it was exposed directly to the humidity condition. MFH did not absorb moisture even at high humidity (95%) when the study was carried out in primary and secondary packing.

4.2.2 Drug – Excipient compatibility study (stability study in solid admixture)

It was observed that none of the excipients were found to have any deleterious effect on stability of studied drug substances even at accelerated stability conditions (40°C/75%RH). The ratio of % assay calculated as "Initial: 2 months" was close to "1" for all the drug-excipient samples which confirms that the difference in the assay values after 2 months accelerated stability study was insignificant.

Among the polymers studied, all grades of HPMC polymers showed least effect on stability of all three drug substances even at 40°C/75%RH. All the three studied drug substances were found to be stable with other commonly used diluents (i.e., lactose monohydrate, MCC and maize starch), lubricants (i.e., aerosil 200, talc and magnesium stearate), binders (PVP K30 and PVP K 90) and disintegrant (SSG).

As shown in DSC scans (Fig. 4.4 to 4.12), the shape and position of the peaks were same after 2 months accelerated stability study (40°C/75% RH) as observed at initial time point for all the

drug substances (alone and in combination with the studied excipients) confirming the stability of the drug substances at studied condition.

4.2.3 pH Stability study:

The content of MFH did not alter significantly in strong acidic pH (1.0 to 3.0) and the cyanoguanidine impurity was also very low (0.02% ± 0.004). The peak purity, single largest unknown impurity and total impurities were not significantly altered. In strong alkaline conditions (pH 12.0), there was a drop in content of MFH (96.53% ± 1.10) and increase in cyanoguanidine impurity (0.91% ± 0.007). Remarkable increase in the content of other unknown impurities was also observed, which attributed to increase in total impurities.

The content of GLZ was decreased in both strong acidic and strong alkaline conditions. The content of GLZ was very low at acidic pH 1.0 (29.90% ± 1.12). At strong alkaline pH of 12.0 the content of GLZ was found to be 92.85% ± 1.09. Impurity F was identified as a main degradation product. At pH 1.0, the content of impurity F was found approx. 10 times higher than its content at pH 12.0. Thus the degradation was found to be maximum in acidic condition as compared with alkaline pH.

GPD was found to be unstable both in strong alkaline and strong acidic medium. The degradation was maximum in strong acidic pH 1.0, which was confirmed by reduced content of GPD at pH 1.0 (38.20% ± 1.14). The main degradation product was identified as sulfonamide impurity which was maximum in case of acidic pH 1.0.

4.2.4 Evaluation of stability in various environmental conditions

Thermal stability:

The thermal stability study carried out on MFH reveled that on heating MFH for 6 hours at 150°C, its appearance changed to brownish crystalline powder and also lost its luster. Also the cyanoguanidine content increased as compared with initial values. The content of MFH decreases from 99.70% ± 1.24 to 95.12% ± 1.14. It shows that about 4 to 5% MFH degrades when observed for 24 hours. However, at no stage of product development, MFH would be exposed to such drastic temperature conditions.

After heating GLZ at 150°C for 24 hours, the content of GLZ was decreased by ~2 % (97.28% ± 1.37) and the impurity F content was increased to 0.3% ± 0.007 as compared with initial value

(0.05% ± 0.005) indicating significant degradation of GLZ at extremely high temperature conditions (i.e., 150°C for 24 hours). But GLZ would never be exposed to such a drastic condition through out the development work.

GPD was found to be very stable in thermal stability study. Even after 24 hours at 150°C content of GPD was not altered (99.30% ± 1.40). The sulfonamide impurity (0.06% ± 0.005) and total impurities (0.16% ± 0.007) were also remained almost same as compared to their respective initial values. (sulfonamide impurity=0.04% ± 0.003 and total impurities=0.10% ± 0.008)

Photo-stability:

It is clear from Table 4.15, 4.16 and 4.14 that all the three drug substances showed good stability in photo-stability study. Physical appearance of any of the drug substance was not changed from 1st day till 7th day. The content and impurity profile of all the three drug substances were unaltered at the end of 7th day.

Stability in strong oxidizing conditions:

The sample of MFH in strong oxidizing solution of 30% hydrogen peroxide after 24 hours showed significant decrease in MFH content to 81.83% ± 1.12 and increase in cyanoguanidine content (3.02% ± 0.06) and other unknown impurities. The sample was further tested at the end of 72 hours, which showed further decrease in MFH content (66.47% ± 1.27) and increase in cyanoguanidine content (9.46% ± 0.05) along with increase in unknown impurities.

GLZ showed sharp decrease in its content to 53.20% ± 1.14 in strong oxidizing solution of 30% hydrogen peroxide within 24 hours but the content of impurity F was increased only to 1.0% ± 0.05. Sharp decrease in GLZ content can be attributed to increase in unknown impurities to 34.60% ± 1.06. When the sample was further analyzed after 72 hours GLZ content showed further decrease to 30.80% ± 1.10.

GPD is relatively stable in strong oxidizing solutions as compared with MFH and GLZ. It showed very low decrease in GPD content after 24 hours to 98.20% ± 1.08 but after 72 hours it showed remarkable decrease in GPD content to 39.60% ± 1.01 with increase in sulfonamide impurity to 40.50% ± 0.99.

4.2.5 Establishment of the solubility characteristics

pH Solubility profile

It was observed from solubility studies that MFH is more soluble in alkaline pH conditions (pH 6.8) and solubility decreased relatively towards acidic condition.

GLZ showed more solubility at higher pH values in alkaline pH 7.2. The solubility of GLZ was very less in acidic pH of 1.2, 2.0 and 4.5. GLZ exists more in ionic form in alkaline pH that attributes to its higher solubility in alkaline medium.

The solubility profile of GPD followed pH-dependant pattern. GPD was more soluble in higher pH values at alkaline side. Especially its solubility was found maximum at pH 7.2 phosphate buffer. It was observed that GPD precipitated in acidic pH of 1.2, 2.0 and 4.5 after 3 hours. Precipitation of GPD in acidic pH from 1.2 to pH 4.5 attributes to its poor solubility in acidic pH.

4.3 Conclusions:

MFH, GLZ and GPD passed various test for identification and analysis as per BP 2005 and USP 2005.

The range of the particle size for all the three drug substances was selected to maintain reproducibility in their respective dissolution profiles and bioavailability.

From the hygroscopicity study it can be concluded that MFH is hygroscopic in nature and above 75% humidity MFH absorbs moisture if it is directly exposed to humidity conditions. Therefore MFH has to be stored at least in primary pack (packed and sealed in double polyethylene bags) if it has to be stored at the humidity of 75% or more.

The insignificant difference in the assay value of the drug-excipient samples after accelerated stability study conformed that all the studied excipients are compatible to the respective drug substances. None of the excipients found to have deleterious effect on the stability of MFH, GLZ and GPD. Therefore it can be concluded that any of the commonly used pharmaceutical excipient can be used for formulation development of the studied drug substances.

MFH was found to be most stable in acidic pH of 1.0 and least stable in alkaline pH of 12.0. Although in the acidic pH range (1.0 to 3.0) the quality attributes of MFH were not much altered; in alkaline pH range (8.0 to 12.0) remarkable degradation was observed which was evident from decreased MFH content from 99.51% ± 1.20 to 96.53% ± 1.10 and increase in cyanoguanidine

content from 0.10% ± 0.004 to 0.91% ± 0.007 at pH 12.0. The probable reason for degradation can be dealkylation of metformin to cyanoguanidine in alkaline pH.

Both GLZ and GPD show highly pH dependant stability. Both GLZ and GPD are unstable in strong acidic as well as strong alkaline pH. But the degradation of GLZ was found to be maximum in acidic condition. GPD also showed remarkable degradation in acidic condition but it was less as compared with GLZ. In strong alkaline pH; both GLZ and GPD showed less degradation as compared with their degradation in acidic conditions. The behavior can be explained by acid hydrolysis of drug substances (GLZ and GPD) in acidic pH.

Based upon thermal stability study, it can be concluded that MFH degrades when exposed to very high temperature conditions for prolonged time period. It was confirmed by increase in the percentage of cyanoguanidine impurity as well as total impurities. But at no stage of product development MFH would be exposed to such drastic temperature conditions therefore thermal degradation of MFH can be considered as insignificant for the concern research work.

GPD was found to be quite stable in thermal stability study. Even after 24 hours at 150°C there was a slight decrease in GPD content and increase in sulfonamide impurity. At the end of 24 hours there was hardly any degradation taken place in high temperature conditions.

GLZ was also found to be stable in high temperature conditions as compared with MFH. But the degradation was more as compared with GPD. The GLZ content was decreased by 2 to 3% when observed for 24 hours.

Based upon the photo-stability study, it can be concluded that all the three drug substances in the present study, (MFH, GLZ and GPD) do not undergo any type of degradation when exposed to UV or fluorescent light. Therefore there is no need to take any precaution pertaining to light exposure while handling these drug substances.

Based upon the experiment carried out to determine stability in oxidizing conditions, it can be concluded that all three drug substances, (i.e. MFH, GLZ and GPD) undergo degradation in strong oxidizing condition at high temperature. GPD was found to have better stability in strong oxidizing conditions as compared with remaining two drug substances (i.e., MFH and GLZ) because it undergoes slow degradation (only on prolonged exposure) in oxidizing conditions as compared to GLZ and MFH.

All the three drug substances studied for pH solubility profile showed pH dependant solubility profile and were found to be more soluble in higher pH values ranging from 6.8 and 7.2. The

behavior can be explained on the bases of the pKa values of these drug substances. The pKa value of MFH is 11.5, GLZ is 5.8 and GPD is 6.1 therefore all the drug substances are in ionized form in alkaline pH which attributes to their pH dependant solubility.

4.4 Selection and/or development of Dissolution methodology for in vitro release studies of extended release tablets:

Dissolution testing is a test currently used to demonstrate the performance of all solid oral dosage forms in which absorption of the drug from gastro-intestinal tract is necessary for the product to exert a therapeutic effect. The development of a dissolution procedure involves selecting the dissolution media, apparatus and agitation rate appropriate to the product.

The solubility of the active ingredient (s) is one of the key aspects in the screening of possible dissolution media. Another important selection criterion for a dissolution medium is t_{max} of the drug substance as it can give the most accurate in vitro - in vivo correlation. The dissolution characteristics of the formulation are to be evaluated over the physiological pH range of 1.2 to 7.5.

In case of MFH ER tablet, phosphate buffer of pH 6.8 was used because,

- The solubility study carried out over the different pH range in section 4.1.5 showed that MFH has maximum solubility in phosphate buffer of pH 6.8.
- The absorption of MFH takes place in entire range of intestine (preferably in small intestine).

The existing conventional MFH tablets are tested for dissolution rate profile by pharmacopoeial method from USP monograph of "Metformin hydrochloride tablets" given below:

Medium: pH 6.8 phosphate buffer; 1000ml

Apparatus: (2) Paddle, 50 rpm

Time: 30 minutes

Tolerance: Not less than 80% (Q) of the labeled amount of metformin hydrochloride is dissolved in 30 minutes.

The selection of this medium is confirmed by checking the dissolution rate profile of MFH ER tablet in different dissolution media and is discussed in detail in Chapter 5.

In case of GLZ ER tablet, buffer of pH 7.5 was used because,

- The solubility study carried out over the different pH range in section 4.1.5 showed that GLZ has solubility only at alkaline pH.
- GLZ is absorbed principally through the entire range of intestine.

The existing conventional GLZ tablets are tested for dissolution rate profile by pharmacopoeial method from BP monograph of "Gliclazide tablets" given below:

Medium: Phosphate buffer pH 7.4; 900ml

Apparatus: (2) Paddle, 100 rpm

Time: 45 minutes

Tolerance: Not less than 70% of the labeled amount of Gliclazide is dissolved in 45 minutes.

The selection of this medium is confirmed by checking dissolution profile of GLZ ER tablet in different dissolution media and is discussed in detail in Chapter 5.

In case of GPD IR tablet, buffer of pH 7.2 was used because GPD has solubility only in alkaline medium and undergoes precipitation in acidic pH as observed in section 4.1.5.

The basket method is routinely used for capsule formulations, while paddle method is used mostly for tablets dosage forms. Therefore paddle method was used in all the three formulations.

The agitation rate is such decided that the disintegrated tablet in the dissolution medium gets dispersed properly in the dissolution medium. The detailed study to decide the agitation speed for all the three formulations is discussed in Chapter 5.

The selection of dissolution media for combination products was done by selecting a medium in which both the components of the combination have good solubility. As discussed earlier, all the three drug substances showed good solubility in alkaline media (MFH in pH 6.8 buffer, GLZ in pH 7.5 buffer and GPD in pH 7.2 buffer). Therefore, for a bi-layer MFH ER + GLZ ER tablet, buffer of pH 7.5 was used and for a bi-layer MFH ER + GPD IR tablet, buffer of pH 7.2 was used.

Table 4.1: Results: Particle size analysis of the drug substances.

Drug substance	Lot no.	Particle size (μm)#		
		D_{90}	D_{50}	D_{10}
MFH	4005MTR	100.90 ±20.1	56.8±12.4	17.03±3.2
	4006MTR	89.80 ±15.1	46.5±10.5	15.83±2.8
	4007MTR	119.90 ±10.1	66.3±9.9	21.44±1.7
GLZ	4007GLR	65.78± 15.5	34.70±8.3	5.24±2.1
	4009GLR	51.11± 10.1	40.05±5.3	9.4±3.8
	4010GLR	72.80± 13.4	28.60±5.9	7.56±1.42
GPD	4005GDR	18.29±5.6	5.08±1.8	1.40±0.9
	4008GDR	15.17±4.8	6.38±2.1	2.21±1.1
	4011GDR	12.64±3.7	7.21±1.4	1.88±0.7

#: The particle size mentioned in the table are the average values obtained after triplicate readings with standard deviation.

Table 4.2: Range of particle size of drug substances selected for the development work.

Drug substance	Range of particle size (μm)		
	D_{90}	D_{50}	D_{10}
MFH	75 to 130	30 to 75	NMT* 30
GLZ	45to 80	20 to 45	NMT* 20
GPD	NMT* 20	NMT* 10	NMT* 5

* **NMT**= Not more than

Table 4.3: Results: Hygroscopicity study by direct exposure at 25°C (in petri dishes) for MFH.

Humidity (%)	Exposure period (hours)	Physical evaluation			Content (%)[$]		
		Appearance		LOD (%)	MFH	Cyanogunidine	Total impurities
		Color	Nature				
25	6	White	Crystalline	0.12	99.74 ± 1.02	0.10 ± 0.02	0.160 ± 0.02
	12	White	Crystalline	0.10	99.75 ± 0.94	0.071 ± 0.01	0.179 ± 0.03
50	6	White	Crystalline	0.12	99.76 ± 1.11	0.073 ± 0.04	0.167 ± 0.02
	12	White	Crystalline	0.10	99.66 ± 0.81	0.158 ± 0.01	0.182 ± 0.05
75	6	White	Crystalline	0.31	99.70 ± 1.24	0.098 ± 0.03	0.202 ± 0.03
	12	White	Crystalline	0.11	99.71 ± 1.44	0.088 ± 0.02	0.202 ± 0.02
95	3	White	Liquefied	14.76	99.76 ± 0.99	0.119 ± 0.02	0.121 ± 0.06
	6	White	Liquefied	25.25	99.77 ± 1.41	0.104 ± 0.04	0.126 ± 0.01
	7	White	Liquefied	33.19	99.74 ± 0.78	0.116 ± 0.01	0.144 ± 0.08

$: Mean of triplicate analysis with standard deviation.

Table 4.4: Results: Hygroscopicity study by exposure in primary pack at 25°C for MFH.

Humidity (%)	Exposure period (hours)	Physical evaluation			Content (%)$		
		Appearance		LOD (%)	MFH	Cyanoguanidine	Total impurities
		Color	Nature				
25	6	White	crystalline	0.10	99.76 ± 1.05	0.138 ± 0.04	0.102 ± 0.03
	12	White	crystalline	0.06	99.69 ± 1.14	0.071 ± 0.02	0.239 ± 0.07
50	6	White	crystalline	0.08	99.75 ± 1.37	0.120 ± 0.05	0.130 ± 0.06
	12	White	crystalline	0.15	99.70 ± 1.30	0.109 ± 0.03	0.191 ± 0.04
75	6	White	crystalline	0.17	99.68 ± 1.09	0.095 ± 0.04	0.225 ± 0.06
	12	White	crystalline	0.13	99.70 ± 1.90	0.103 ± 0.07	0.197 ± 0.09
95	3	White	crystalline	0.07	99.70 ± 0.99	0.095 ± 0.03	0.205 ± 0.10
	6	White	crystalline	0.08	99.79 ± 1.23	0.098 ± 0.08	0.112 ± 0.06
	7	White	crystalline	0.11	99.75 ± 1.42	0.114 ± 0.06	0.136 ± 0.04

Primary pack: Packed and sealed in double polythene bags

$: Mean of triplicate analysis with standard deviation.

Table 4.5: Results: Hygroscopicity study by exposure in secondary pack at 25°C for MFH.

Humidity (%)	Exposure period (hours)	Physical evaluation			Content. (%)[$]		
		Appearance		LOD (%)	MFH	Cyanoguanidine	Total impurities
		Color	Nature				
25	6	White	crystalline	0.09	99.71 ± 1.22	0.153 ± 0.06	0.130 ± 0.02
	12	White	crystalline	0.16	99.78 ± 1.19	0.098 ± 0.05	0.122 ± 0.06
50	6	White	crystalline	0.07	99.65 ± 1.08	0.120 ± 0.05	0.230 ± 0.05
	12	White	crystalline	0.13	99.69 ± 1.15	0.108 ± 0.08	0.202 ± 0.06
75	6	White	crystalline	0.10	99.67 ± 1.07	0.100 ± 0.03	0.230 ± 0.07
	12	White	crystalline	0.16	99.69 ± 1.31	0.102 ± 0.08	0.208 ± 0.04
95	3	White	crystalline	0.20	99.80 ± 1.06	0.095 ± 0.07	0.105 ± 0.08
	6	White	crystalline	0.10	99.79 ± 1.91	0.097 ± 0.09	0.113 ± 0.07
	7	White	crystalline	0.09	99.75 ± 1.65	0.115 ± 0.05	0.135 ± 0.08

Secondary pack: Packed and sealed in double polyethylene bags in miniature fiber drums of capacity 0.5 Kg.

$: Mean of triplicate analysis with standard deviation.

Table 4.6: Drug – Excipient compatibility study report for MFH at accelerated stability condition.

Drug/ Drug + Excipient	Ratio	Assay (%)$		% Impurities$			
				Cyanoguanidine		Total	
		Initial	2 Months	Initial	2 Months	Initial	2 Months
MFH alone	NA*	99.76 ± 1.02	100.01 ± 1.29	Nil	Nil	0.04 ± 0.004	0.05 ± 0.003
MFH + Lactose monohydrate	5:1	99.85 ± 1.01	99.86 ± 1.09	Nil	0.05 ± 0.002	0.06 ± 0.003	0.08 ± 0.005
MFH+ Maize starch	5:1	99.72 ± 1.08	101.30 ± 1.70	Nil	0.02 ± 0.001	0.06 ± 0.007	0.11 ± 0.007
MFH + MCC	5:1	99.95 ± 1.12	99.20 ± 1.08	Nil	0.04 ± 0.005	0.04 ± 0.005	0.12 ± 0.004
MFH + SSG	1:0.1	99.72 ± 1.10	100.08 ± 1.91	Nil	0.02 ± 0.004	0.09 ± 0.004	0.09 ± 0.005
MFH+ HPMC K15M	5:1	100.61 ± 1.09	99.62 ± 1.09	Nil	0.01 ± 0.006	0.09 ± 0.006	0.14 ± 0.008
MFH + HPMC K4M	5:1	99.81 ± 1.03	99.32 ± 1.31	Nil	Nil	0.10 ± 0.007	0.15 ± 0.005
MFH + HPMC K100M	5:1	99.91 ± 1.05	100.10 ± 1.24	Nil	Nil	0.10 ± 0.003	0.10 ± 0.004
MFH+ Carbopol 971	5:1	100.10 ± 1.31	99.21 ± 1.17	Nil	0.02 ± 0.007	0.09 ± 0.005	0.09 ± 0.007
MFH + PVP K30	1:0.1	99.27 ± 1.05	99.25 ± 1.23	Nil	0.04 ± 0.006	0.12 ± 0.005	0.18 ± 0.006
MFH + PVP K90	1:0.1	99.53 ± 1.23	100.04 ± 1.51	Nil	Nil	0.10 ± 0.006	0.12 ± 0.003
MFH + SLS	1:0.1	101.02 ± 1.37	99.14 ± 1.48	Nil	0.03 ± 0.004	0.14 ± 0.008	0.15 ± 0.005
MFH+ Aerosil-200	1:0.1	100.24 ± 1.22	101.37 ± 1.32	Nil	0.02 ± 0.008	0.13 ± 0.004	0.14 ± 0.004
MFH+ Purified Talc	1:0.1	100.54 ± 1.38	100.52 ± 1.29	Nil	0.01 ± 0.009	0.09 ± 0.003	0.11 ± 0.007
MFH+ Magnesium stearate	1:0.1	101.08 ± 1.31	99.22 ± 1.28	Nil	Nil	0.09 ± 0.004	0.12 ± 0.003

***NA:** Not applicable $: Mean of triplicate analysis with standard deviation.

Limits as per DMF: Cyanoguanidine: NMT 0.1% Total impurities: NMT 0.3%

Table 4.7: Drug – Excipient compatibility study report for GLZ at accelerated stability condition.

Drug/ Drug + Excipient	Ratio	Assay (%) $		%Impurities $			
				Impurity F		Total	
		Initial	2 Months	Initial	2 Months	Initial	2 Months
GLZ alone	NA	100.32 ± 1.28	99.53 ± 1.08	0.02 ± 0.004	0.05 ± 0.006	0.11 ± 0.006	0.18 ± 0.005
GLZ + Lactose monohydrate	1:5	99.82 ± 1.31	99.72 ± 1.18	0.04 ± 0.006	0.04 ± 0.004	0.10 ± 0.004	0.16 ± 0.004
GLZ+ Maize starch	1:5	101.10 ± 1.74	100.28 ± 0.97	0.04 ± 0.005	0.05 ± 0.008	0.12 ± 0.003	0.17 ± 0.007
GLZ + MCC	1:5	99.80 ± 1.13	101.27 ± 1.67	0.01 ± 0.008	0.03 ± 0.006	0.15 ± 0.004	0.16 ± 0.005
GLZ + SSG	1:0.5	100.82 ± 1.18	99.38 ± 1.29	0.02 ± 0.003	0.04 ± 0.005	0.16 ± 0.005	0.17 ± 0.004
GLZ+ HPMC K15M	1:5	99.64 ± 1.44	101.77 ± 1.26	0.03 ± 0.006	0.05 ± 0.009	0.14 ± 0.007	0.18 ± 0.005
GLZ + HPMC K4M	1:5	99.71 ± 1.57	99.38 ± 1.43	0.04 ± 0.007	0.06 ± 0.007	0.12 ± 0.005	0.16 ± 0.006
GLZ + HPMC K100M	1:5	101.09 ± 1.63	100.24 ± 1.35	0.01 ± 0.005	0.02 ± 0.006	0.14 ± 0.004	0.15 ± 0.004
GLZ+ Carbopol 971	1:5	100.53 ± 1.71	99.81 ± 1.62	0.04 ± 0.008	0.06 ± 0.004	0.16 ± 0.007	0.18 ± 0.007
GLZ + PVP K30	1:0.5	99.61 ± 1.09	99.31 ± 1.29	0.05 ± 0.006	0.06 ± 0.006	0.10 ± 0.004	0.12 ± 0.006
GLZ + PVP K90	1:0.5	99.74 ± 1.11	100.46 ± 1.34	0.02 ± 0.004	0.05 ± 0.007	0.14 ± 0.004	0.18 ± 0.005
GLZ + SLS	1:0.5	100.28 ± 1.48	99.80 ± 1.09	0.03 ± 0.005	0.03 ± 0.005	0.16 ± 0.007	0.19 ± 0.007
GLZ+ Aerosil-200	1:0.5	101.04 ± 1.39	101.24 ± 0.97	0.01 ± 0.006	0.05 ± 0.008	0.11 ± 0.008	0.15 ± 0.008
GLZ+ Purified Talc	1:0.5	99.82 ± 1.01	99.29 ± 1.08	0.02 ± 0.004	0.04 ± 0.006	0.14 ± 0.007	0.17 ± 0.007
GLZ+ Magnesium stearate	1:0.5	100.21 ± 1.81	100.35 ± 1.33	0.05 ± 0.008	0.06 ± 0.007	0.15 ± 0.005	0.06 ± 0.005

***NA:** Not applicable $: Mean of triplicate analysis with standard deviation.

Limits as per DMF: Impurity F: NMT 0.1% Total impurities: NMT 0.5%

Table 4.8: Drug – Excipient compatibility study report for GPD at accelerated stability condition.

Drug/ Drug + Excipient	Ratio	Assay (%)$		% Impurities$			
				Sulfonamide		Total	
		Initial	2 Months	Initial	2 Months	Initial	2 Months
GPD alone	NA*	100.95 ± 1.08	99.20 ± 1.21	0.05 ± 0.002	0.08 ± 0.004	0.12 ± 0.008	0.18 ± 0.005
GPD + Lactose monohydrate	1:20	99.51 ± 0.98	99.27 ± 1.15	0.08 ± 0.004	0.09 ± 0.005	0.14 ± 0.007	0.16 ± 0.008
GPD+ Maize starch	1:20	99.34 ± 1.28	99.38 ± 1.21	0.04 ± 0.008	0.08 ±0.003	0.14 ± 0.005	0.15 ± 0.007
GPD + MCC	1:20	99.49 ± 1.17	99.09 ± 1.09	0.09 ± 0.004	0.09 ± 0.004	0.18 ± 0.008	0.19 ± 0.006
GPD + SSG	1:10	101.47 ± 1.09	100.88 ± 1.38	0.05 ± 0.003	0.07 ± 0.007	0.15 ± 0.007	0.17 ± 0.005
GPD+ HPMC K15M	1:10	100.57 ± 1.46	100.38 ± 1.82	0.01 ± 0.004	0.06 ± 0.008	0.18 ± 0.005	0.19 ± 0.004
GPD + HPMC K4M	1:10	99.30 ± 1.08	99.43 ± 1.41	0.08 ± 0.006	0.09 ± 0.006	0.15 ± 0.004	0.19 ± 0.008
GPD + HPMC K100M	1:10	101.24 ± 1.52	99.58 ± 1.32	0.07 ± 0.005	0.07 ± 0.004	0.12 ± 0.008	0.18 ± 0.006
GPD+ Carbopol 971	1:10	100.59 ± 1.29	101.24 ± 1.11	0.02 ± 0.009	0.06 ± 0.005	0.12 ± 0.007	0.18 ± 0.005
GPD + PVP K30	1:10	99.81 ± 1.34	99.82 ± 1.14	0.04 ± 0.004	0.08 ± 0.006	0.15 ± 0.004	0.17 ± 0.008
GPD + PVP K90	1:10	99.38 ± 1.36	100.27 ± 1.25	0.08 ± 0.008	0.09 ± 0.007	0.12 ± 0.005	0.16 ± 0.004
GPD + SLS	1:10	100.52 ± 1.62	99.82 ± 1.34	0.04 ± 0.004	0.08 ± 0.006	0.14 ± 0.007	0.15 ± 0.005
GPD+ Aerosil-200	1:5	99.37 ± 1.07	100.75 ± 1.21	0.03 ± 0.005	0.06 ± 0.004	0.18 ± 0.004	0.19 ± 0.006
GPD+ Purified Talc	1:5	99.54 ± 1.51	101.21 ± 0.94	0.06 ± 0.007	0.07 ± 0.005	0.15 ± 0.008	0.17 ± 0.005
GPD+ Magnesium stearate	1:5	100.18 ± 1.10	100.22 ± 1.04	0.05 ± 0.003	0.05 ± 0.008	0.16 ± 0.009	0.19 ± 0.007

***NA:** Not applicable $: Mean of triplicate analysis with standard deviation.

Limits as per DMF: Sulfonamide: NMT 0.1% Total impurities: NMT 0.5%

Table 4.9: Results: pH stability profile of MFH at the end of 24 hours.

pH	% Content $		
	MFH	Cyanoguanidine	Total Impurities
1.0	99.63 ± 1.27	0.02 ± 0.004	0.05 ± 0.006
2.0	99.64 ± 1.08	0.02 ± 0.006	0.05 ± 0.004
3.0	99.71 ± 1.18	0.03 ± 0.003	0.04 ± 0.007
7.0	99.70 ± 1.09	0.05 ± 0.004	0.04 ± 0.008
8.0	99.74 ± 0.09	0.08 ± 0.007	0.10 ± 0.005
10.0	99.05 ± 1.02	0.31 ± 0.005	0.82 ± 0.008
12.0	96.53 ± 1.10	0.91 ± 0.007	2.39 ± 0.09

$: Mean of triplicate analysis with standard deviation.

Table 4.10: Results: pH stability profile of GLZ at the end of 24 hours.

pH	% Content $		
	GLZ	Impurity F	Total Impurities
1.0	29.90 ± 1.12	69.67 ± 1.91	70.12 ± 1.31
2.0	49.70 ± 1.08	50.39 ± 1.28	51.31 ± 1.08
3.0	69.02 ± 1.09	31.08 ± 1.34	32.09 ± 1.15
7.0	99.58 ± 1.20	0.09 ± 0.005	0.16 ± 0.008
8.0	98.72 ± 1.09	0.08 ± 0.006	0.12 ± 0.007
10.0	97.38 ± 1.31	1.27 ± 0.05	1.60 ± 0.08
12.0	92.85 ± 1.09	3.04 ± 0.06	6.59 ± 0.09

$: Mean of triplicate analysis with standard deviation.

Table 4.11: Results: pH stability profile of GPD at the end of 24 hours.

pH	% Content $		
	GPD	Sulfonamide	Total impurities
1.0	38.20 ± 1.14	43.20 ± 1.25	60.52 ± 1.01
2.0	49.37 ± 1.25	33.50 ± 1.31	49.34 ± 0.92
3.0	68.27 ± 1.09	21.82 ± 1.16	29.37 ± 0.84
7.0	99.10 ± 1.24	0.05 ± 0.004	0.19 ± 0.04
8.0	98.61 ± 1.08	1.06 ± 0.05	1.71 ± 0.08
10.0	90.27 ± 1.34	9.30 ± 0.08	10.32 ± 0.85
12.0	74.70 ± 1.42	23.5 ± 0.98	24.83 ± 1.08

$: Mean of triplicate analysis with standard deviation.

Table 4.12: Results: Thermal stability of MFH at 150°C.

Duration (hours)	Appearance		% Content $		
	Color	Nature	MFH	Cyanoguanidine	Total impurities
Initial	White	Crystalline powder	99.70 ± 1.24	0.05 ± 0.008	0.071 ± 0.005
6	Brownish	Crystalline powder	99.36 ± 1.31	0.01 ± 0.005	0.081 ± 0.004
12	Brown	Crystalline powder	96.22 ± 1.08	0.10 ± 0.004	0.510 ± 0.008
24	Brown	Crystalline powder	95.12 ± 1.14	0.10 ± 0.007	0.624 ± 0.007

$: Mean of triplicate analysis with standard deviation.

Table 4.13: Results: Thermal stability of GLZ at 150°C.

Duration (hours)	Appearance		Content (%) $		
	Color	Nature	GLZ	Impurity F	Total impurities
Initial	White	Crystalline powder	99.58 ± 1.08	0.05 ± 0.005	0.12 ± 0.008
6	White	Crystalline powder	99.31 ± 1.24	0.05 ± 0.003	0.14 ± 0.007
12	White	Crystalline powder	99.08 ± 1.08	0.09 ± 0.004	0.21 ± 0.007
24	White	Crystalline powder	97.28 ± 1.37	0.3 ± 0.007	0.43 ± 0.008

$: Mean of triplicate analysis with standard deviation.

Table 4.14: Results: Thermal stability of GPD at 150°C.

Duration (hours)	Appearance		Content (%) $		
	Color	Nature	GPD	Sulfonamide	Total impurities
Initial	White to off white	crystalline powder	99.58 ± 1.20	0.04 ± 0.003	0.10 ± 0.008
6	White to off white	crystalline powder	99.41 ± 1.08	0.05 ± 0.004	0.11 ± 0.007
12	White to off white	crystalline powder	99.38 ± 1.31	0.05 ± 0.006	0.13 ± 0.005
24	White to off white	crystalline powder	99.30 ± 1.40	0.06 ± 0.005	0.16 ± 0.007

$: Mean of triplicate analysis with standard deviation.

Table 4.15: Results: Photo-stability of MFH.

Duration	Content (%)$		
	MFH	Cyanoguanidine	Total impurities
Initial	99.51 ± 1.24	0.10 ± 0.003	0.36 ± 0.005
7th day	99.52 ± 1.09	0.10 ± 0.002	0.35 ± 0.004

$: Mean of triplicate analysis with standard deviation.

Table 4.16: Results: Photo-stability of GLZ.

Duration	Content (%)$		
	GLZ	Impurity F	Total impurities
Initial	99.58 ± 1.18	0.05 ± 0.004	0.31 ± 0.002
7th day	99.56 ± 1.07	0.06 ± 0.005	0.29 ± 0.003

$: Mean of triplicate analysis with standard deviation.

Table 4.17: Results: Photo-stability of GPD.

Duration	Content (%)$		
	GPD	Sulfonamide	Total impurities
Initial	100.21 ± 1.14	0.07 ± 0.008	0.35 ± 0.003
7th day	100.10 ± 1.11	0.07 ± 0.005	0.34 ± 0.004

$: Mean of triplicate analysis with standard deviation.

Table 4.18: Results obtained by study of MFH in strong oxidizing conditions.

Duration (hours)	Content (%)$		
	MFH	Cyanoguanidine	Total impurities
Initial	99.51 ± 1.05	0.10 ± 0.007	0.37 ± 0.005
24	81.83 ± 1.12	3.02 ± 0.06	8.53 ± 0.07
48	67.62 ± 1.09	9.75 ± 0.04	19.05 ± 0.05
72	66.47 ± 1.27	9.46 ± 0.05	20.1 ± 0.94

$: Mean of triplicate analysis with standard deviation.

Table 4.19: Results obtained by study of GLZ in strong oxidizing conditions.

Duration (hours)	Content (%)$		
	GLZ	Impurity F	Total impurities
Initial	99.58 ± 1.27	0.056 ± 0.004	0.16 ± 0.009
24	53.2 ± 1.14	1.0 ± 0.05	36.82 ± 1.20
48	46.50 ± 1.07	1.50 ± 0.07	36.84 ± 1.09
72	30.80 ± 1.10	2.20 ± 0.08	36.98 ± 1.11

$: Mean of triplicate analysis with standard deviation.

Table 4.20: Results obtained by study of GPD in strong oxidizing conditions.

Duration (hours)	Content (%)[$]		
	GPD	Sulfonamide	Total impurities
Initial	99.10 ± 1.30	0.06 ± 0.004	0.12 ± 0.009
24	98.2 ± 1.08	0.27 ± 0.006	0.42 ± 0.007
48	97.8 ± 1.16	0.53 ± 0.008	0.78 ± 0.006
72	39.6 ± 1.01	40.5 ± 0.99	48.51 ± 1.04

$: Mean of triplicate analysis with standard deviation.

Table 4.21: Solubility studies of MFH, GLZ and GPD at different pH values.

pH of the media	Concentration (mg/ml)[$]		
	MFH	GLZ	GPD
1.20	50.71 ± 1.57	68.24 ± 2.37	37.25 ± 3.40
2.0	53.42 ± 0.83	72.95 ± 1.41	41.30 ± 2.70
4.5	67.82 ± 1.37	77.19 ± 0.82	43.81 ± 3.75
6.8	100.65 ±2.21	94.75 ± 0.77	79.88 ± 2.68
7.2	99.53 ±1.31	101.37 ± 1.56	90.86 ± 1.22

$: Mean of triplicate analysis with standard deviation.

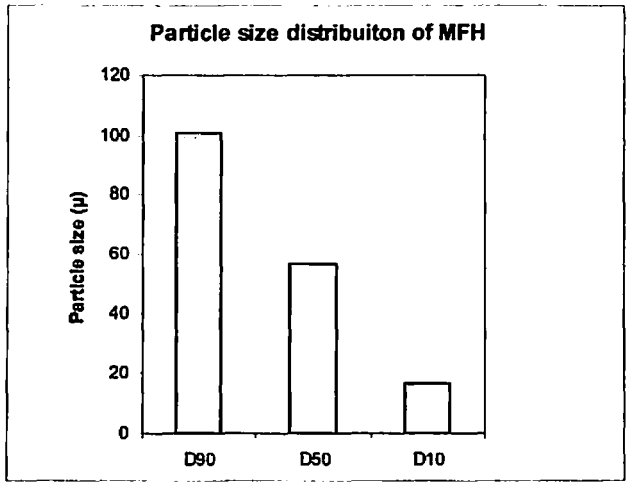


Fig. 4.1: Particle size distribution of MFH.

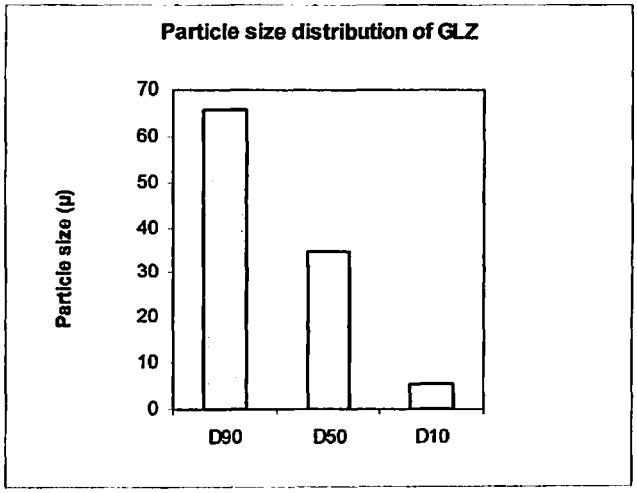


Fig. 4.2: Particle size distribution of GLZ.

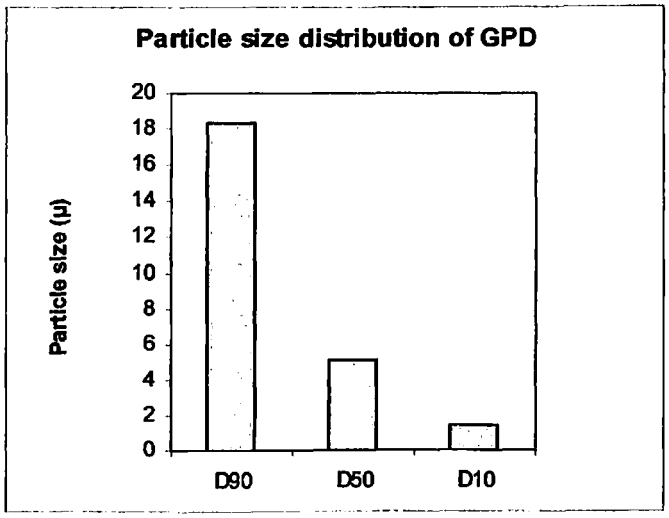


Fig. 4.3: Particle size distribution of GPD.

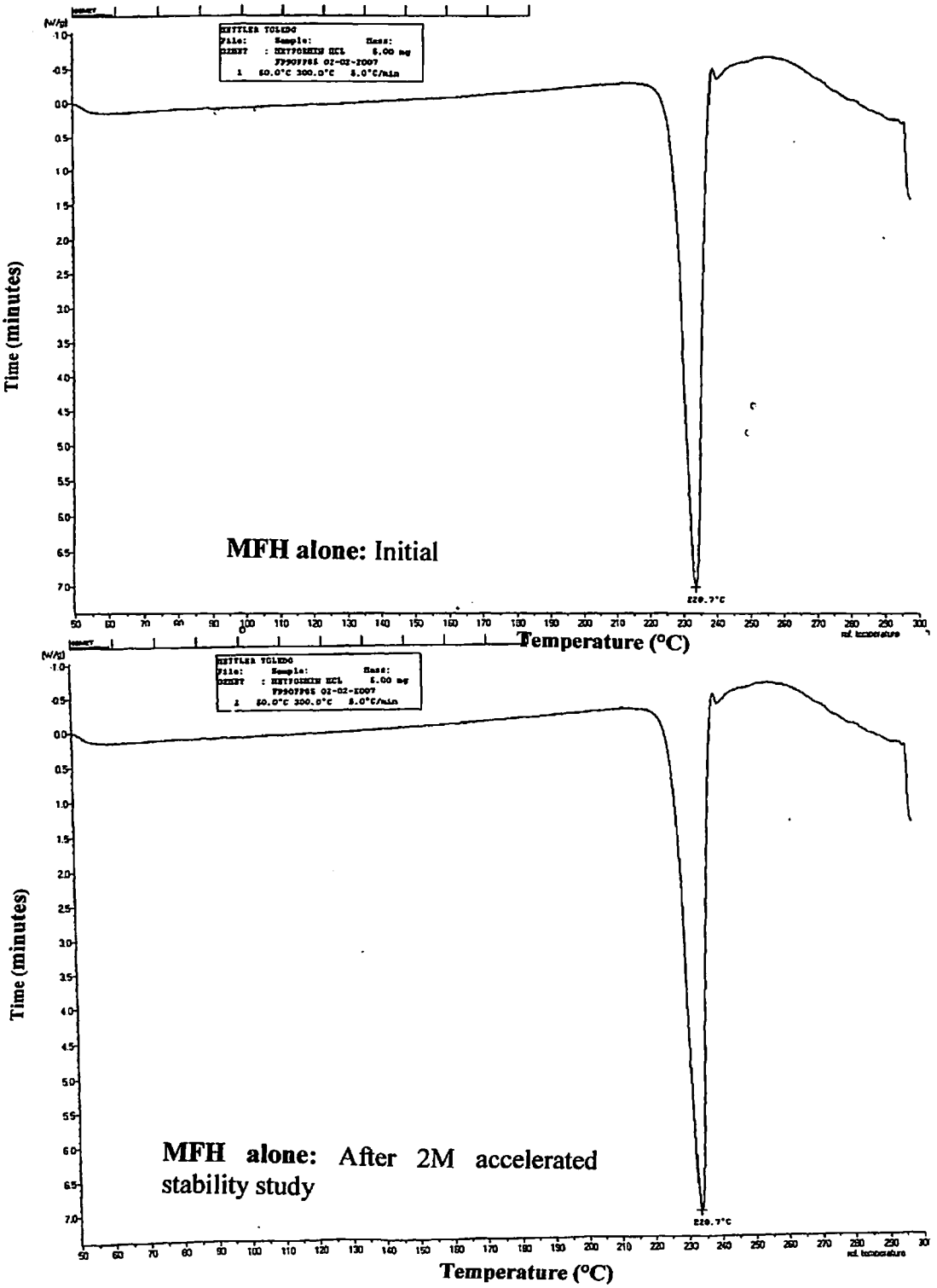


Fig. 4.4: DSC thermograms for MFH alone.

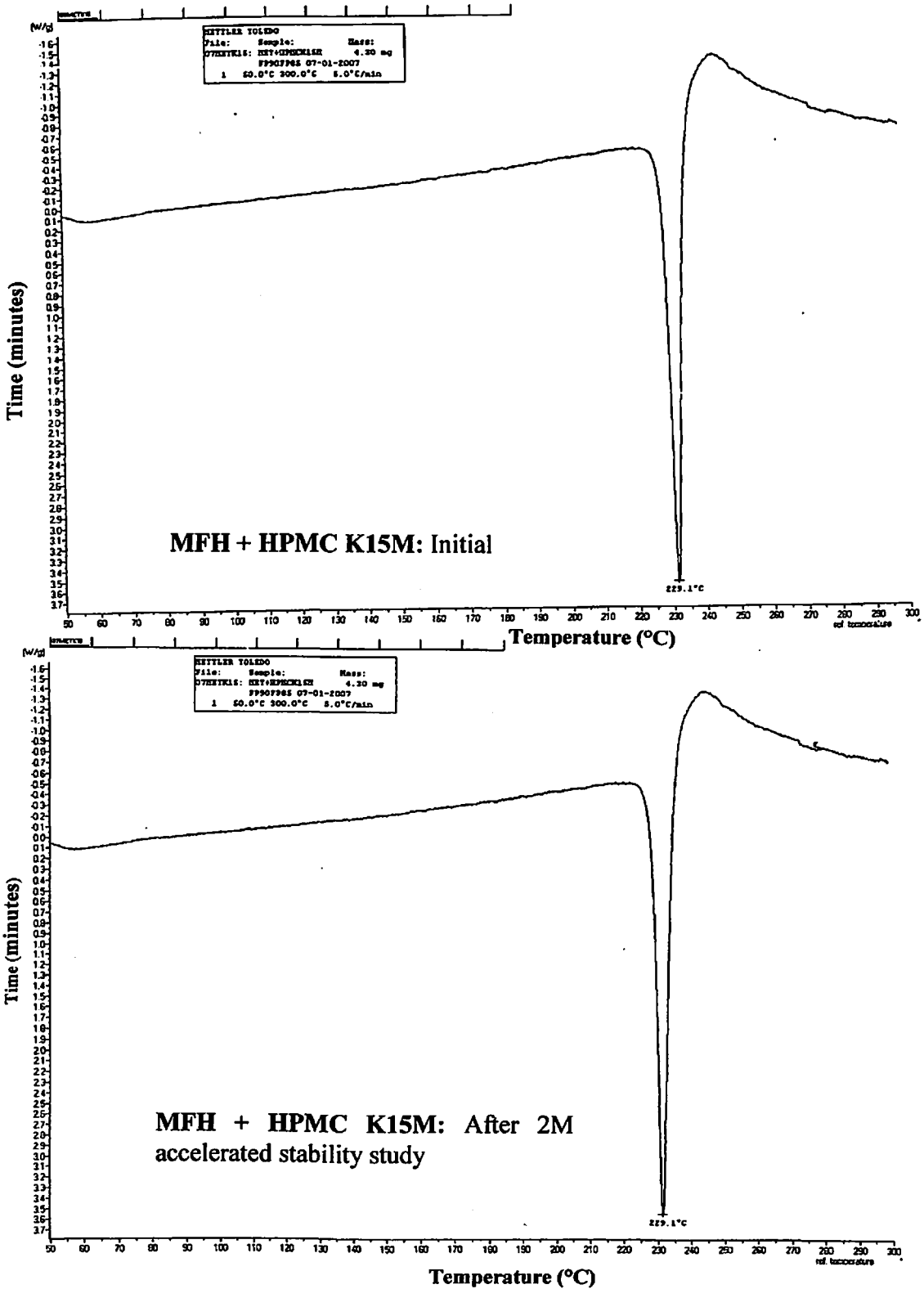


Fig. 4.5: DSC thermograms for MFH + HPMC K15M.

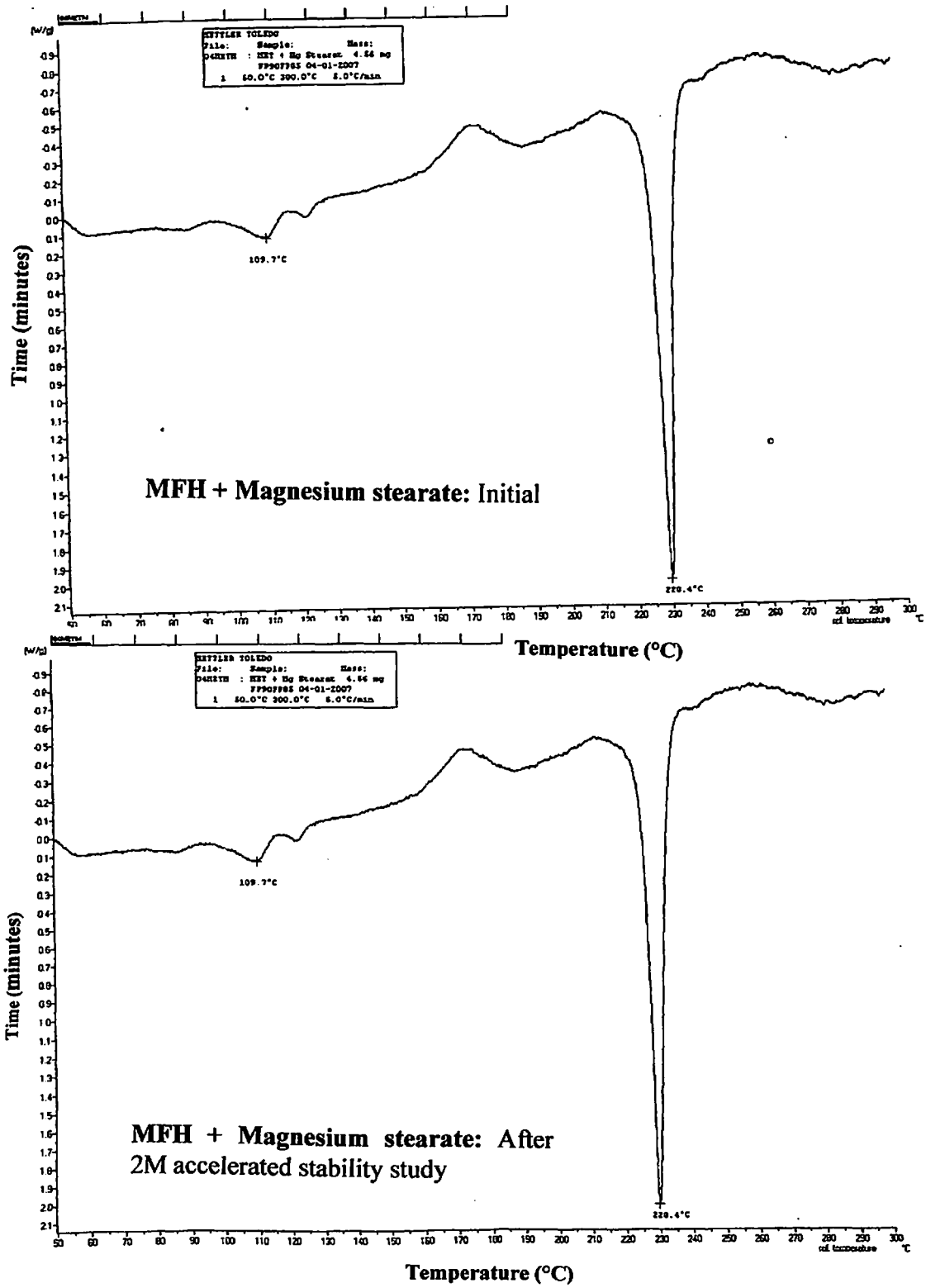


Fig. 4.6: DSC thermograms for MFH + Magnesium stearate.

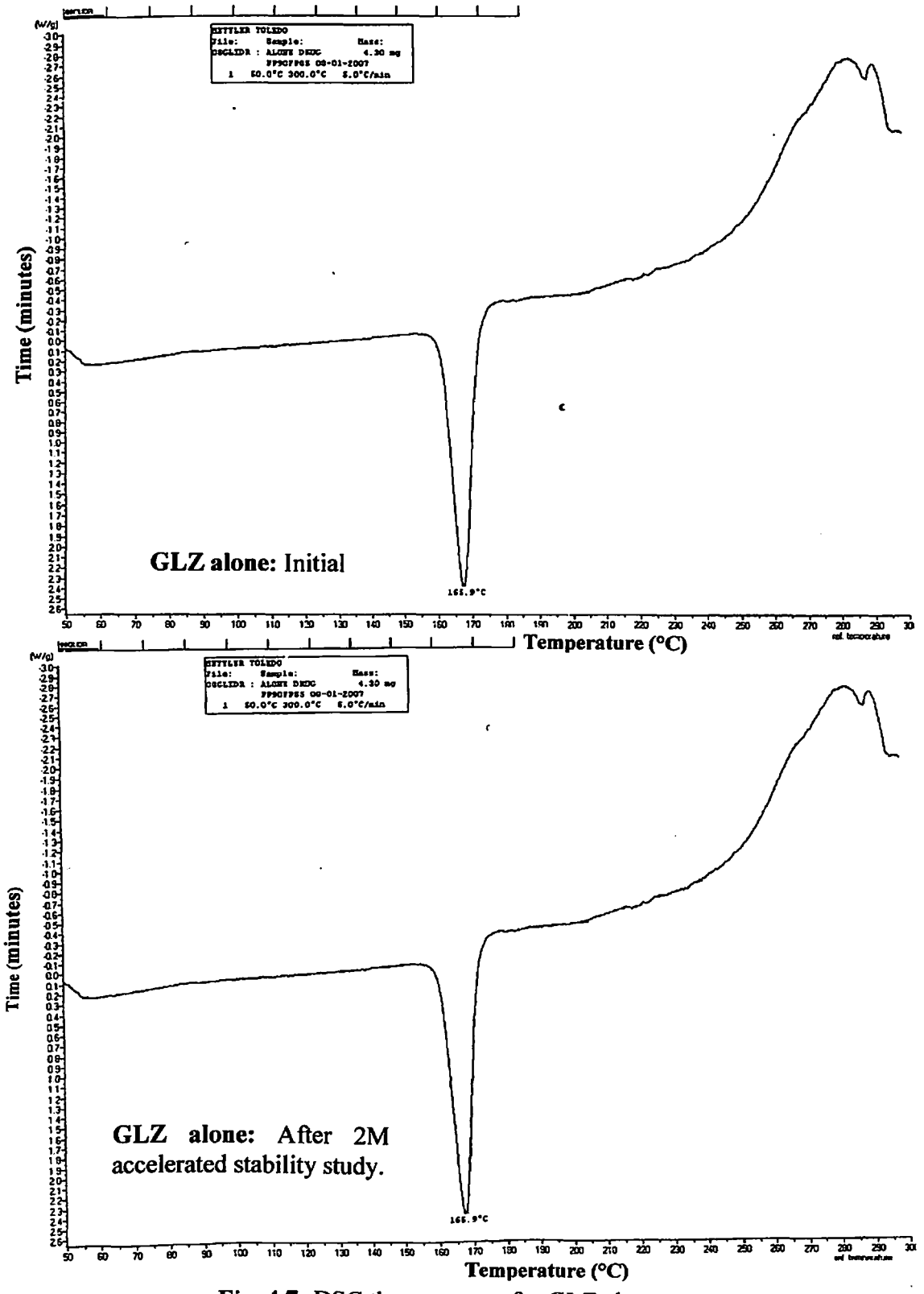


Fig. 4.7: DSC thermograms for GLZ alone.

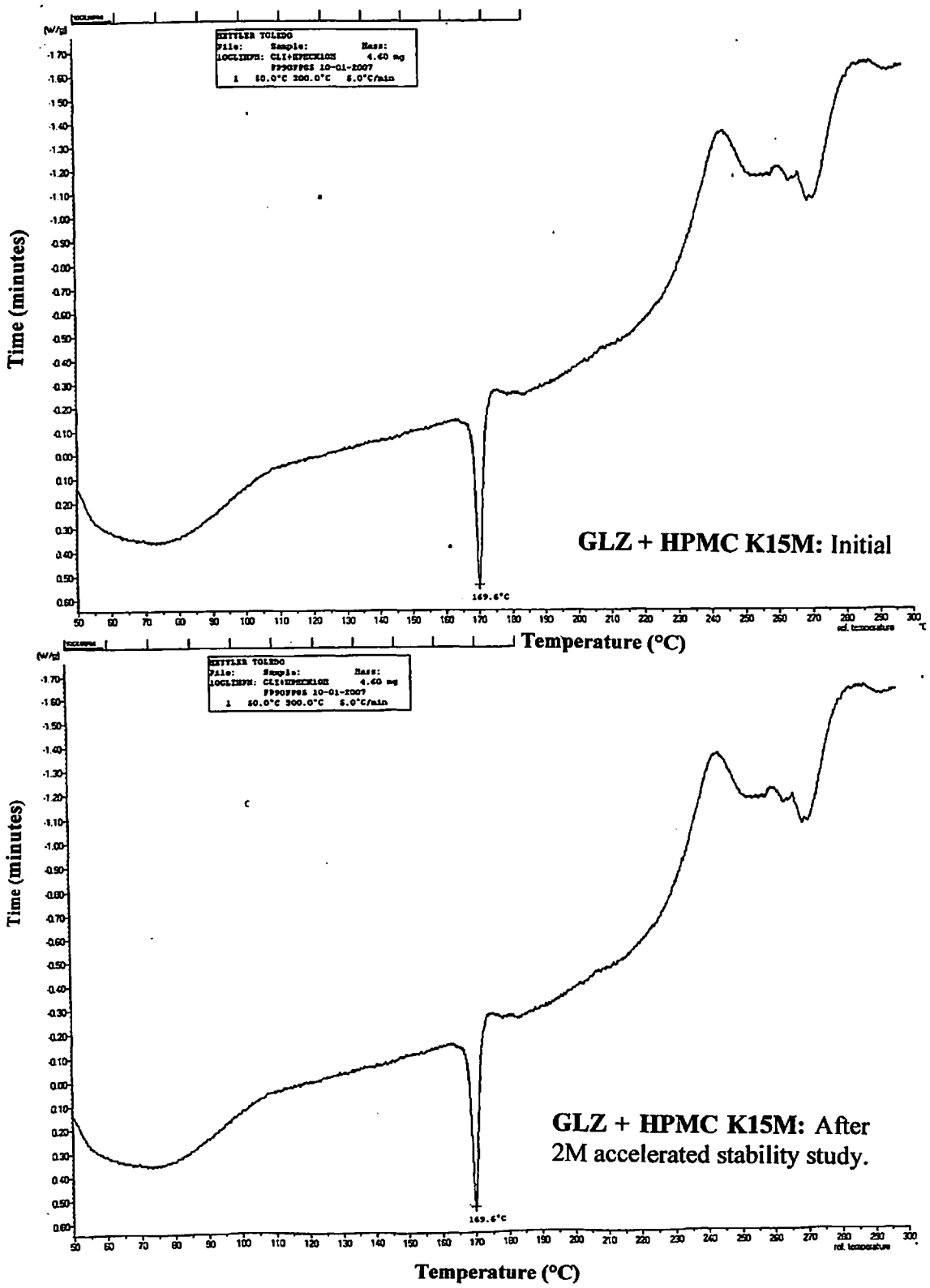


Fig. 4.8: DSC thermograms for GLZ + HPMC K15M.

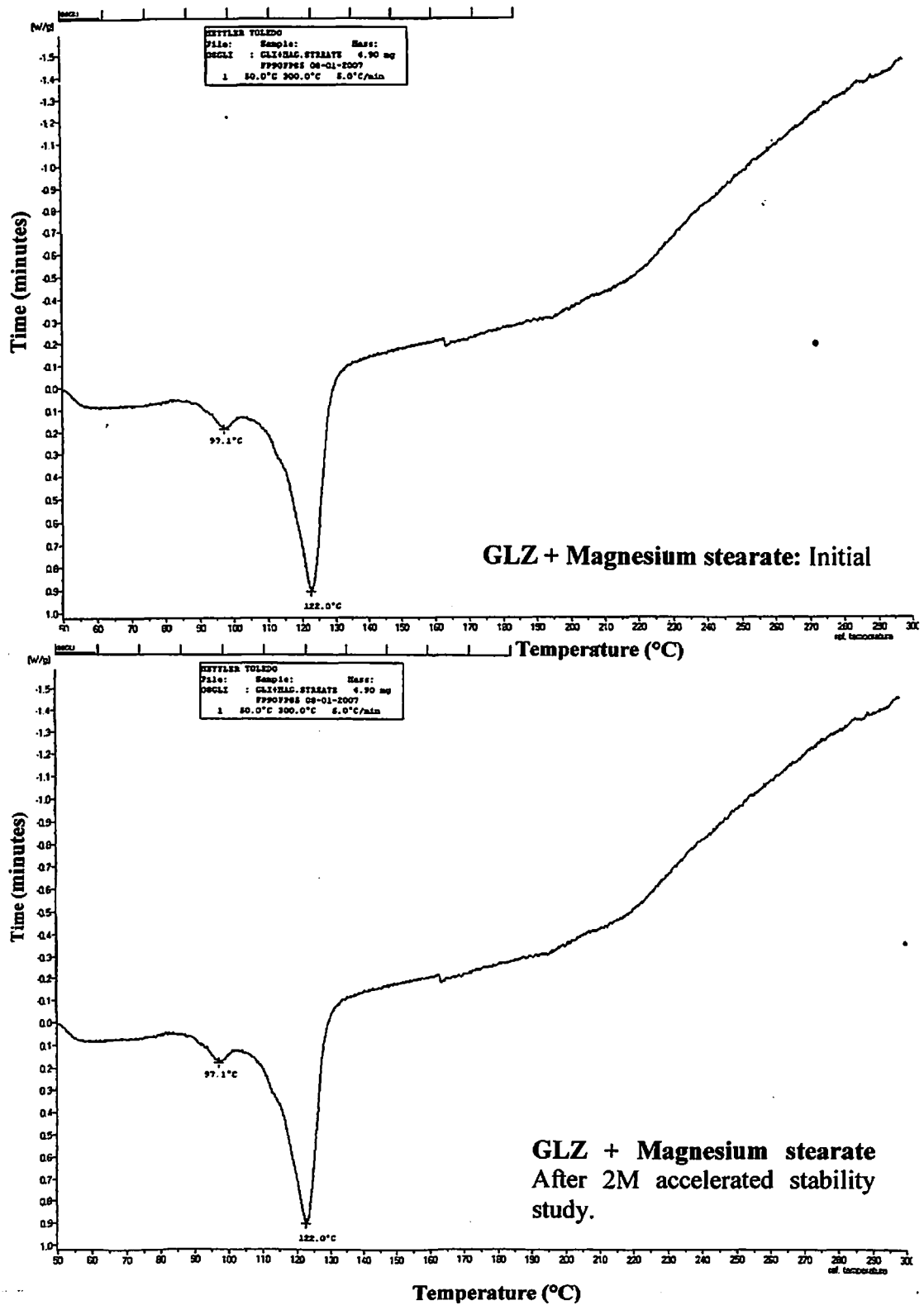


Fig. 4.9: DSC thermograms for GLZ + Magnesium stearate.

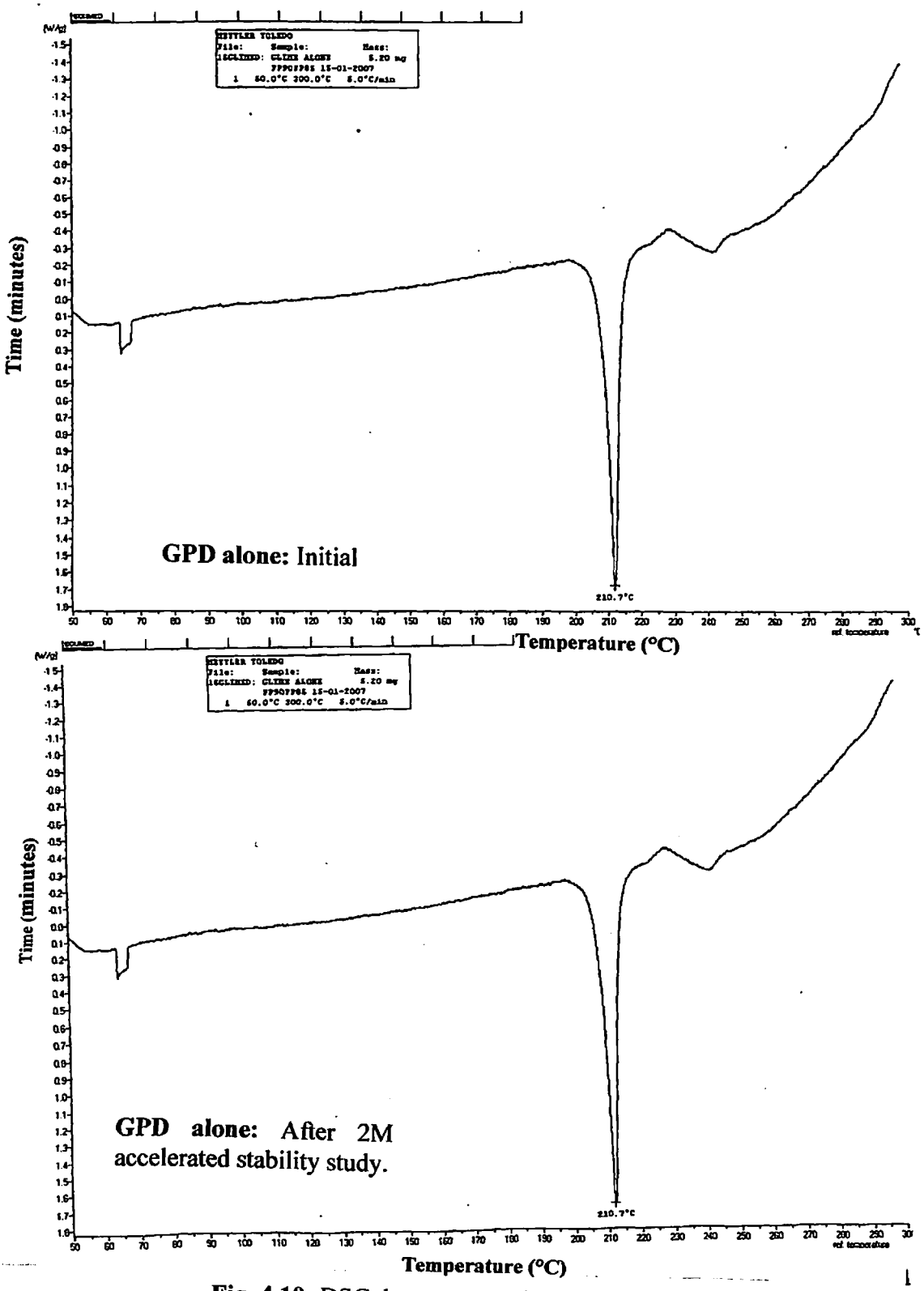


Fig. 4.10: DSC thermograms for GPD alone.

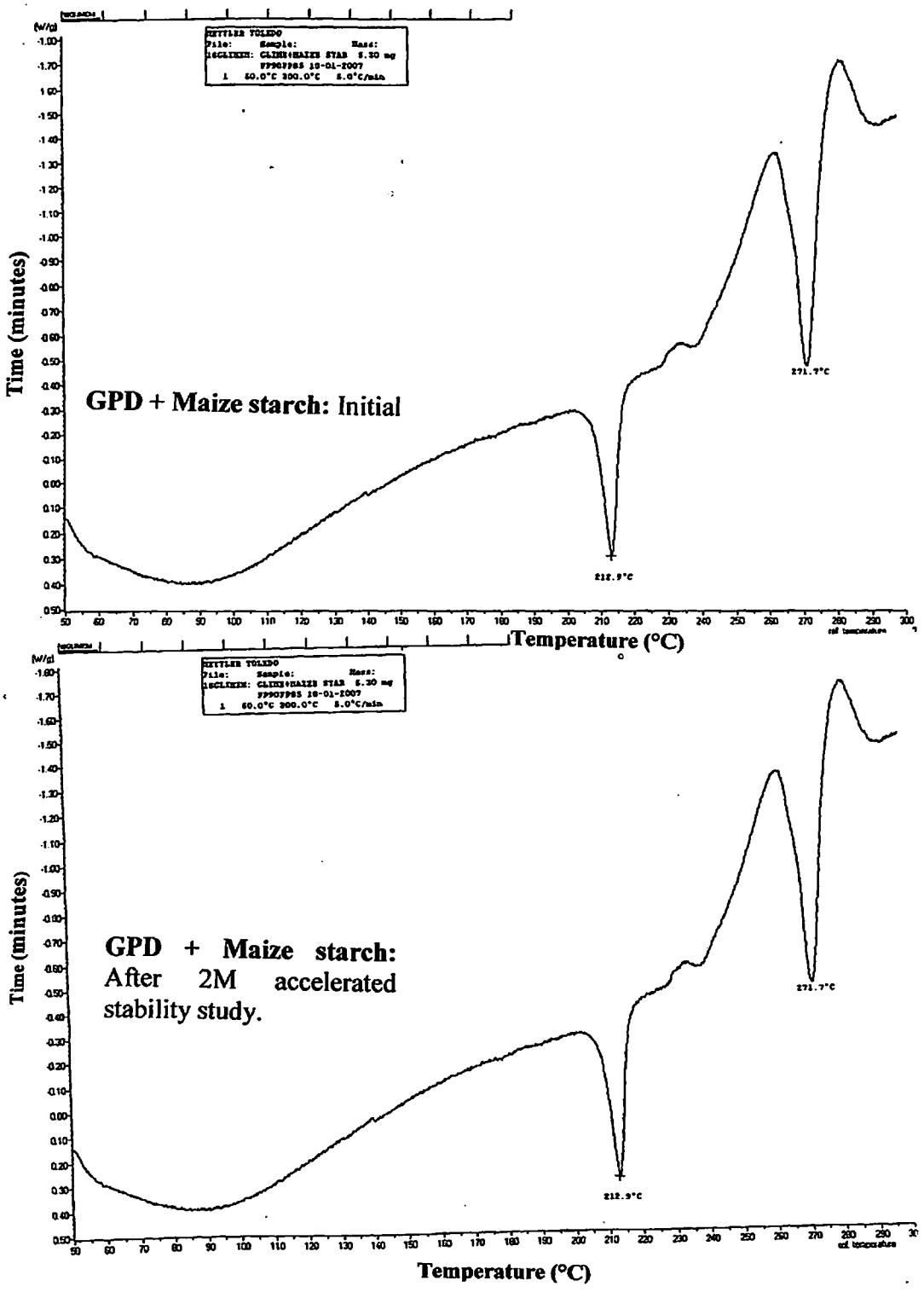


Fig. 4.11: DSC thermograms for GPD + Maize starch.

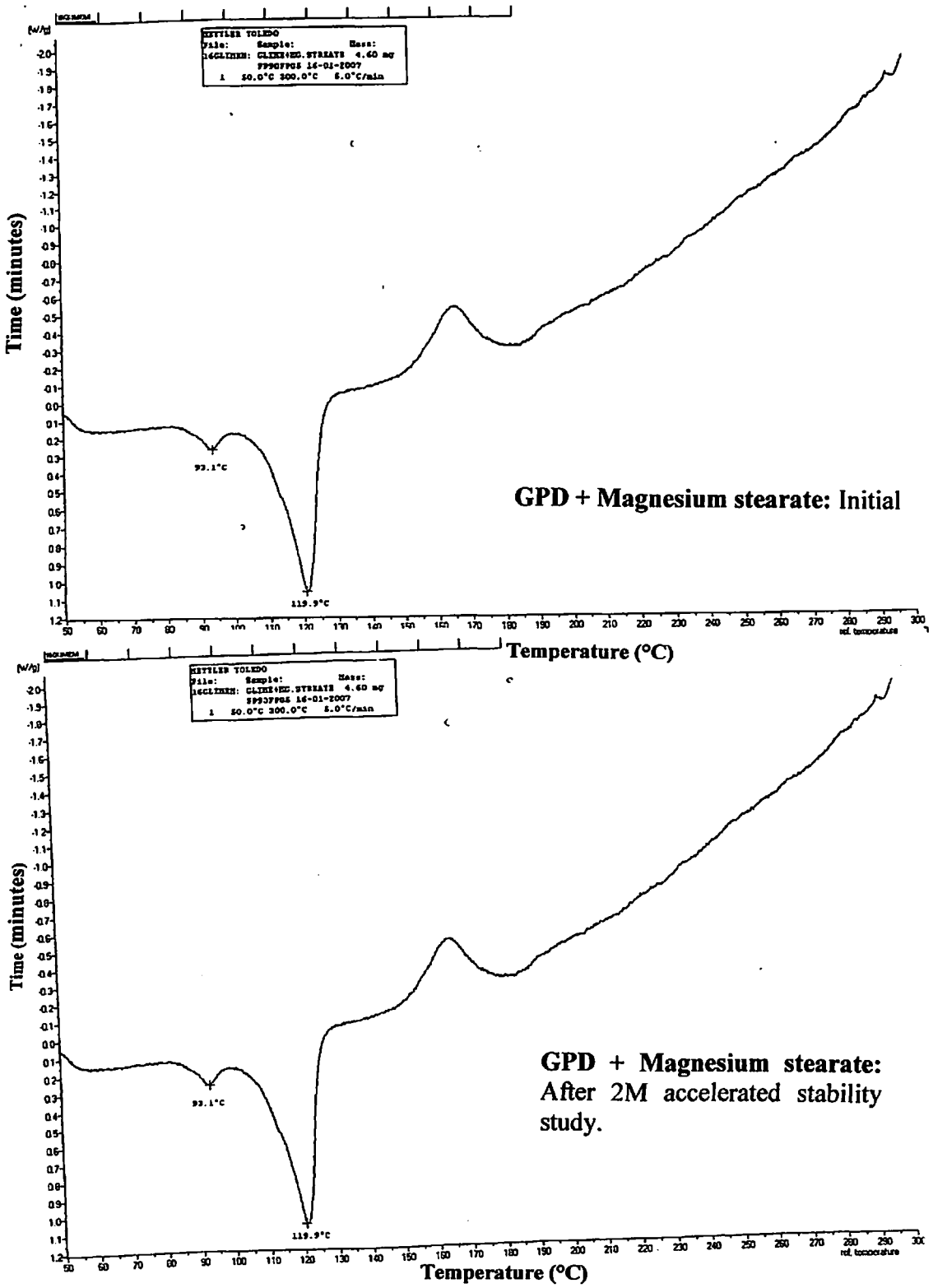


Fig. 4.12: DSC thermograms for GPD + Magnesium stearate.

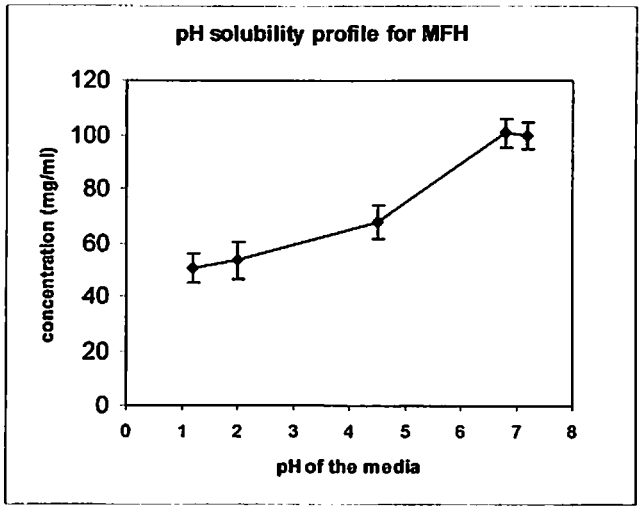


Fig. 4.13: pH solubility profile of MFH.

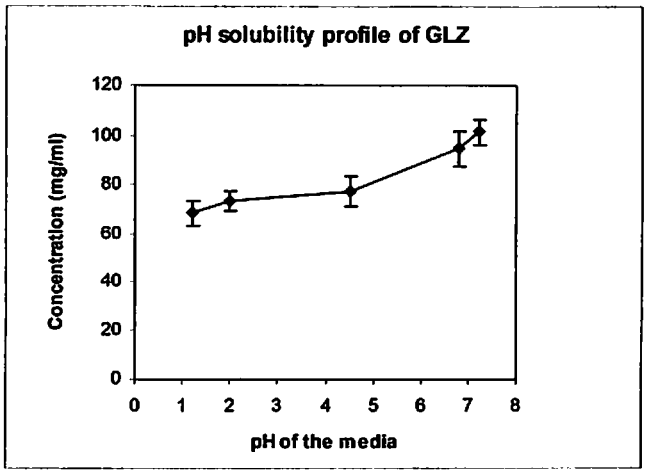


Fig. 4.14: pH solubility profile of GLZ.

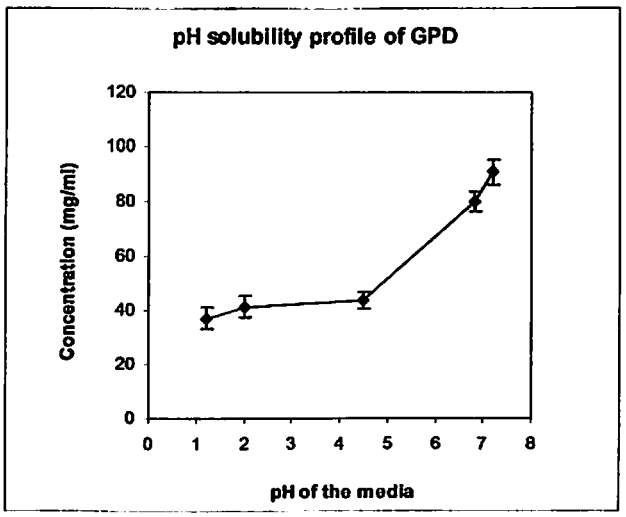


Fig. 4.15: pH solubility profile of GPD.

Chapter 5

Design and Development of Oral Extended Release Formulations

MFH belongs to a class of oral hypoglycemic agents known as biguanides. MFH has a valuable role in treatment of diabetes mellitus because of its hypoglycemic effect even in absence of insulin which makes it a drug of choice in obese diabetics whose hyperglycemia is more due to insulin resistance. The conventional formulation of MFH suffers the disadvantages (discussed in Chapter 1) which forms the rational of development of extended release formulations of MFH. Extended release form of MFH can improve patient compliance and reduce total dose requirement in addition to several other distinct advantages.

At present, the management of diabetes includes "Polychemotherapy" as a pivotal tool. The patient is prescribed with the combinations of hypoglycemic agents having complimentary actions which obviously indicate the need for combination formulations. It becomes a very logical step to design, develop and study a combination product using innovative manufacturing technologies like say multi-layered tablets. The rational, need and advantage of polychemotherapy have been emphasized time and again.

The present study is a stepping stone towards designing a platform technology for extended release MFH tablets, which in turn can be combined with other anti-diabetic drugs i.e. GLZ and GPD to formulate a stable, safe and efficacious unit dosage form.

The attempts have been made to design each component of a unit dosage form in such a way that it can be linearly scaled up and scaled down for multiple strengths required by the clinicians based on the dosage recommendations.

Following is the description of attempts made to design, develop and evaluate extended release formulations of anti-diabetic drugs alone and in combination.

Materials

The active materials MFH, GLZ and GPD used for formulation development studies were obtained from M/s. Ipca Laboratories Ltd, India. Other inactive excipients used in the study were procured from the approved vendors of M/s. Ipca labs Ltd, Mumbai, India, as per the Standard Operating Procedure (SOP). All other chemicals and reagents used were of pharmaceutical or analytical grade.

Instruments / equipments

Tablet compression was carried out on rotary bi-layered compression machine (manufactured by: M/s Cadmach) using suitable capsule shaped punches. The dissolution was carried out by using dissolution test apparatus of M/s Electrolab, India (Model: TDT 06P). Other equipments used are fluidized bed drier (M/s Adams Engineering Works, Mumbai), rapid mixer granulator (M/s Saral Engineering Works, Mumbai) and octagonal blender (M/s Saral Engineering Works, Mumbai).

Analytical method

Analysis of MFH, GLZ and GPD was done by the methods described in Chapter 3, Analytical methodology.

5.1 Formulation of MFH extended release tablets

5.1.1 Experimental

Design inputs

The present study was aimed at development of extended release formulation as a Monolithic matrix formulation, the release of drug through which is governed by drug diffusion technology. The Pharmacokinetic data of MFH obtained through extensive literature search was taken as a guiding scale to decide the target dissolution profile of MFH. The targeted dissolution profile is given in Table 5.1.

Formulation design using single hydrophilic polymer

Extended release matrix tablets with different proportions [5%w/w (M01), 10%w/w (M02), 25%w/w (M03)] of Hydroxypropyl Methyl Cellulose (HPMC K15M) were formulated by wet granulation method (Table 5.2). The drug, MCC and HPMC K15M (passed through 40 #) were mixed uniformly and granulated with isopropyl alcohol (IPA), using Polyvinyl Pyrrolidone (PVP K30) as binder dissolved in solvent. The wet mass was dried at 50-55°C in a Fluidized bed drier to achieve moisture content in the range 1.0 to 3.0% w/w. The final granules were rasped through 16 # mesh and lubricated with Magnesium Stearate (0.6% w/w) and compressed on 16 stations rotary compression machine using suitable capsule shaped punches. The tablets were evaluated for its physical and chemical parameters. The in-vitro release profile is presented in Fig. 5.1.

Formulation design using combination of hydrophilic polymers

Formulation was also prepared with combination of hydrophilic polymers. HPMC K15 M was used in fixed proportion (10% w/w) with varied proportions of HPMC K100M [5% w/w (M06), 10% w/w (M07) and 25% w/w (M08)]. The drug, MCC and HPMC K15M (passed through 40 #) were mixed uniformly and granulated with isopropyl alcohol (IPA), using Polyvinyl Pyrrolidone (PVP K30) as binder dissolved in solvent. The wet blend was dried at 50-55°C in a Fluidized bed drier to achieve moisture content in the range 1.0% to 3.0% w/w. The final granules were rasped through 16 # and blended with different proportions of HPMC K100M and finally lubricated with Magnesium Stearate (0.6% w/w) and compressed on 16 station rotary compression machine using suitable capsule shaped punches. The tablets were evaluated for its physical and chemical parameters (Table 5.3). The in-vitro release profile is presented in Fig. 5.3.

Formulation design using combination of hydrophilic (HPMC K15M and HPMC K100M) and hydrophobic polymer, Hydroxypropyl Methyl Cellulose Phthalate (HPMCP-HP55)

As MFH is freely soluble in water, therefore a hydrophobic polymer, HPMCP HP55, was studied in the formulation to retard the release of the drug in order to achieve target dissolution profile. HPMCP HP55, being a hydrophobic and acid insoluble polymer was used to control release at initial time points. This polymer was used in addition to the combination of hydrophilic HPMC K15M and HPMC K100M (M10). The drug, MCC and HPMC K15M (10% w/w) were passed through 40# and mixed thoroughly. PVP K30 and HPMCP HP55 (5% w/w) were dissolved in IPA and Methylene Chloride and used as a granulating fluid. The wet mass was dried at 50-55°C in a Fluidized bed drier to achieve moisture content in the range of 1.0% to 3.0% w/w. The final granules were rasped through 16 # mesh and blended with HPMC K100M (25% w/w) and finally lubricated with Magnesium Stearate (0.6% w/w) and compressed on 16 stations rotary compression machine using suitable capsule shaped punches. The tablets were evaluated for its physical and chemical parameters (Table 5.4). The in-vitro release profile is presented in Fig.5.4.

Formulation design using combination of hydrophilic (HPMC K15M and HPMC K100M) and hydrophobic polymer, Carbomer USP (Carbopol 971G)

The drug, MCC and HPMC K15M (10% w/w) and Carbopol 971G (10% w/w) were passed through 40# and were mixed together. The dry blend was granulated with PVP K30 dissolved in IPA.

The wet mass was dried at 50-55°C in a Fluidized bed drier to achieve the desired moisture content in the range of 1.0% to 3.0% w/w. The dried granules were rasped through 16 # mesh and further blended with HPMC K100M (25% w/w) and finally lubricated with Magnesium Stearate (0.6% w/w) and compressed on 16 station rotary compression machine using suitable capsule shaped punches. The tablets were evaluated for its physical and chemical parameters. (Table 5.4, M09). The in-vitro release profile is presented in Fig. 5.4.

Physical and chemical evaluation of the tablets

Formulated tablets were studied for its physical parameters and drug content (Table 5.2 to Table 5.4.) The drug content of the product was determined in duplicate as per the Analytical method described in Chapter 3. The weight variation was determined on 20 tablets using electronic balance (Mettler Toledo / Afcoset), Tablet hardness was determined for minimum 5 tablets using Dr. Schleuniger hardness tester (M 8 model), Friability was determined with 20 Tablets using Friability apparatus (M/s Electrolab) for 100 revolutions (25 rpm).

In vitro release studies

Release studies were carried out using pH 6.8 phosphate buffer. The volume of dissolution medium was 900 ml and stirring speed was 100 rpm (USP Type 2: Paddle). At predetermined time period (viz. 2,4,8,12 hours)10 ml samples were withdrawn and replaced with fresh aliquot. After appropriate dilution, the samples were analyzed. Cumulative percent of drug released was calculated, and mean of six tablets were used for data analysis. (Fig. 5.1 to 5.4).

The formulation design was selected based on the above experiments. It contained the combination of hydrophilic polymers [HPMC K15M (10% w/w) and HPMC K100M (25% w/w)] and a hydrophobic polymer [Carbopol 971G (10% w/w) using PVP K30 in IPA as a binding solution. The effect of various parameters was studied on this selected formulation

design in order to optimize the design and to fine tune the formulation. These trials are discussed as follows:

Optimization of the design for extended release formulation

(a) Effect of viscosity of polymer.

The different viscosity grades of the polymer (HPMC K 100 M) were used to study the effect of viscosity on the in-vitro release of MFH (Table 5.5). The different grades used for the study were HPMC K100M (LV) (M04) and HPMC K100M (CR) (M05). The findings obtained from the study are shown in Fig. 5.2.

(b) Effect of granulating solvent

In order to study the effect of granulating fluid, wet granulation was done by using aqueous and non-aqueous solvent (Table 5.6). In case of aqueous solvent study (M12), Polyvinyl Pyrollidone (PVP K30) was dissolved in purified water and was used as the binder for extended release tablet formulation. For the study of non-aqueous solvent (M09), Polyvinyl Pyrollidone (PVP K30) was dissolved in IPA (Isopropyl alcohol) and was used as a binder. The findings obtained from the study are shown in Fig 5.5.

Optimization of the manufacturing process technology

(a) Effect of manufacturing technology

The effect of manufacturing technology was studied by varying granulation techniques (Table 5.7). The proportion of the polymer in both the experiment was maintained constant. In case of direct compression, drug along with the release controlling polymers, and other excipients including the binder PVP K 30 was blended together (M11). In case of wet granulation, drug and release controlling polymer were granulated using PVP K30/ IPA as a binder (M09). In both the cases the tablets were compressed and in-vitro dissolution was studied. The in-vitro release profile is presented in Fig 5.6.

(b) Effect of compression force

In order to study the effect of compression force on the in-vitro release, the tablets were compressed by using common blend at three different hardness levels, 125N to 150N (M13),

175N to 200N (M14) and 225N to 250N (M15) and in vitro dissolution rate was studied. The findings are presented in the Fig 5.7.

(c) Effect of extent of granulation

The extent of granulation was varied by using varying quantity of granulating fluid and kneading time. In order to achieve heavy granulation (M18), the quantity of granulating solvent was increased by approx. 20% and kneading time was also increased by approx.10%. Light granulation (M16) was achieved by adding granulating solvent just enough for getting granules and kneading time was kept minimum to ensure uniform mixing of binder. Wet mass in both the cases was dried at 50-55°C in a Fluidized bed drier. The dried granules were rasped through 16 # mesh and further lubricated with Magnesium Stearate (0.6% w/w) and compressed on 16 station rotary compression machine using suitable capsule shaped punches. The findings are presented in Fig 5.8.

Batch reproducibility

Three batches of the selected and finalized formulation were manufactured (Table 5.8) (M19, M20 and M21) and their quality and respective release profile was evaluated under the same conditions as prescribed in previous sections. In vitro release data pertaining to reproducibility studies are shown in Fig 5.9.

Effect of pH of dissolution media.

In-vitro release of MFH from the extended release tablet formulation (M21) was studied over the range of pH. Different pH media were selected simulating the pH conditions along the GI tract. In vitro dissolution profile was carried out in following dissolution media: SGF buffer of pH 1.2, Acetate buffer of pH 4.5, Phosphate buffer pH 6.8. Method used was USP type II (paddle, 100rpm) using 900 ml of dissolution media. (Fig.5.10).

Effect of hydrodynamic conditions (Stirring speed of paddle)

The study was undertaken on the formulation with target dissolution profile (M21). In-vitro release studies were carried out at two different stirring speeds, 50rpm and 100rpm keeping all other parameters same in pH 6.8 Phosphate buffer. The results are presented in Fig 5.11.

Stability Studies

Three batches of the formulation with target dissolution were kept under accelerated and long term stability study (as per ICH guideline) [M19: MXT (500) 19/05, M20: MXT (500) 20/05, M21: MXT (500) 21/05]. The storage conditions used for the studies were controlled room temperature (CRT : 25 ± 2°C and 60 ± 5 % RH), accelerated condition (40 ± 2°C and 75 ± 5 % RH) and intermediate , fall back condition (30 ± 2 °C and 65 ± 5% RH). The samples were withdrawn from each time point at predetermined time interval (1, 2, 3 months) and analyzed for physical characteristic, in vitro dissolution study and assay. The dissolution profile of stability batches are shown in Table 5.9.

5.1.2 Results and Discussions:

Formulation design for extended release tablet

From preformulation studies it was observed that none of the inactive excipients (including the polymers) studied has got any deleterious effect on the stability of MFH. Therefore a variety of release retarding polymers could be used to achieve target dissolution profile. As expected, the formulation designed with single polymer (HPMC K15M) could not achieve the target release profile in all the tried proportions (Fig. 5.1); since MFH is highly soluble in water and the drug content is also very high (500mg).

The desired dissolution profile could not be achieved even after using the combination of hydrophilic polymers (HPMC K 15M and HPMC K100M).Although the release profile was retarded upto 4 hours (approx. 40% to 45%) (Fig.5.3), the release was about 90% within 8 hours, which is almost an upper limit of targeted release profile (75% to 90% in 8 hours).

The combination of hydrophilic (HPMC K15M and HPMC K100M) and hydrophobic polymer (HPMCP HP55 or Carbopol 971G) produced the dissolution profile very close to the targeted one. Fig 5.4 shows the comparison between the dissolution profiles of the designed formulation with Carbopol 971G (M09) and HPMCP HP 55 (M10). The release of drug from tablets with HPMCP HP55 polymer was observed to be up to 90% in 8 hours and with Carbopol 971G about 75% in 8 hours. The release was found to be better controlled in the formulation with Carbopol 971G.

It is clear from the above discussion, that the combination of hydrophilic polymers [HPMC K15M (10% w/w) and HPMC K100M (25% w/w)] and a hydrophobic polymer [Carbopol 971G

(10% w/w)] using PVP K30 in IPA as a binding solution produced MFH ER tablets giving in-vitro release profile very close to the targeted release profile.

Study of release character

Study of release profile indicated that the release of MFH from the designed tablets followed mainly first order as release rate found to decrease with time. Use of different HPMC and their combination, carbopol and HPMCP, all showed somewhat first order release character.

Physical and chemical evaluation of the tablets

The physical characterization of the tablet forms the preliminary basis for selecting the formulation for further study. Physical appearance, tablet hardness, friability, weight variation and Assay were evaluated for all the trials. It is clear from the data presented in Table 5.2 to 5.4 that the physical characteristics for all the trials were satisfactory. The normal range of hardness obtained for all the trials was between 150N to 200N. The friability in all the cases was less than 0.3% w/w. The flow properties of the granules were excellent that was confirmed by very low weight variation observed in all the trials.

Optimization of the design for extended release formulation

(a) Effect of viscosity of polymer

As shown in Figure 5.2, change in viscosity has very little effect on control of drug release probably due to high water solubility of MFH as both products M04 and M05 produced same release profile.

(b) Effect of granulating solvent

It is clear from Fig. 5.5, that in the formulation designed with aqueous granulating fluid (water), the in vitro release was restricted to less than 80% after 12 hours (M12) whereas, the release was close to 100% after 12 hours when non-aqueous solvent (IPA) was used as the granulating fluid (M09). The lower release in case of aqueous granulation technique could be attributed to the hardening phenomenon due to high solubility of MFH in aqueous granulating fluid (water).

Optimization of the manufacturing process technology

(a) Effect of manufacturing technology

The findings from study of effect of manufacturing technology are presented in Table 5.7. The in-vitro release was found to be faster in dry granulation technology than wet granulation method. The complete release (more than 95%) was observed within 8 hours in case of direct compression method (M11) as compared to wet granulation process (M09) where it is less than 80% in same time. The behavior can be attributed to previous swelling of polymer in presence of solvent in case of wet granulation which might have helped in release due to solvent binding effect. The graphical representation is given in Fig 5.6.

(b) Effect of compression force

The compression force or hardness of the tablet is an important physical parameter which controls drug release. MFH is freely water soluble drug therefore the change in hardness did not change the release profile to a significant extent as observed in profile presented in Fig 5.7.

(c) Effect of extent of granulation

The effect of extent of granulation was studied to determine the ruggedness and robustness of the formulation design. From Fig 5.8, it is clear that, extent of granulation had no significant effect on the physical parameters of the tablet and in-vitro dissolution profile, since the drug is highly water soluble and granulating fluid is non aqueous solvent. This proves that the formulation design is very robust and release profile will not alter even if the extent of granulation is taken to higher or lower side during commercial manufacture. It makes the formulation design ideal for the commercial extrapolation.

Batch reproducibility:

The physical properties of the tablets from all three batches (M19, M20 and M21) showed low standard deviation values for the drug content, friability, weight variation and hardness. These low standard deviation values for all physical properties showed that there was excellent batch-to-batch reproducibility and absence of significant variations among the three batches. In-vitro release profile pertaining to reproducibility studies were compared by f_2 (similarity factor) values (M19: 73.81, M20: 75.13, M21: 81.26). No significant difference was observed in the release

profiles of the formulations among the three batches. The graphical representation of the release profile of the formulations from three batches is given in Fig 5.9. Thus the batch reproducibility indicated that the formulation methodology employed is suitable for manufacturing good quality extended release tablets of MFH.

Effect of pH of dissolution media

The dissolution pattern of MFH extended release tablets in different pH media simulating the pH conditions along the GI tract is presented in Fig.5.10, which shows that there is no effect of pH medium on the release pattern of the formulation (M21) and the drug is getting released independent to the pH of the medium.

Effect of hydrodynamic conditions (Stirring speed of paddle):

The results are shown in Fig 5.11. The difference in the dissolution values at all time points was less than 10% at different stirring paddle speeds (i.e. 50 and 100 rpm). This difference is statistically insignificant (f_2=69.12) probably due to high water solubility of MFH. The observed variation in the release with change in the stirring speed could also be attributed to the difference in hydrodynamic stress around the surface of the tablet undergoing dissolution. At lower stirring rpm, (50), there was a slow fluid (release media) motion and formation of stable stagnant layer surrounding the tablet. This prevented the easy entry of release medium into the tablet and also the release of the drug outside the tablet. But as the stirring rpm was increased to 100 rpm there was a greater fluid flow that resulted in the increased attrition of the tablet matrix at the swelling/dissolution interface. This phenomenon caused increased erosion of the matrix and decrease in the stagnant diffusion layer thickness that ultimately resulted in the increased drug release.

Stability studies:

Three months accelerated stability data showed no degradation of MFH and no significant effect on dissolution profile of the product in comparison to the initial values in the studied primary pack (Aluminum blister packing). The product was found to be stable under the studied conditions (Table 5.9).

5.2 Formulation of GLZ extended release tablets

5.2.1 Experimental

Design inputs

The aim of the present study was to design and develop a stable and effective ER formulation for GLZ independently and then combine the same with previously developed MFH ER part developed as platform technology for antidiabetic combination formulation. Thus the theoretical in-vitro dissolution profile of MFH was kept constant as discussed earlier and dissolution profile of GLZ was decided based upon the pharmacokinetic data of GLZ (Table 5.10). The method for this combined preparation provides a dual release pharmaceutical formulation. The product was developed as bi-layered tablet.

Formulation design using various grades of HPMC (single polymer study)

GLZ is practically insoluble in water therefore hydrophilic HPMC polymer was obvious choice for preparing extended release matrix tablets. Different trials were taken with HPMC K4M and HPMC K15M separately as release controlling polymers. The proportion of HPMC K4M and HPMC K15M was varied in the range, 10%w/w, 20%w/w and 25% w/w (Table 5.11 and 5.12 respectively). The granulation for both the trials was done separately by wet granulation method using PVPK 30 in water as binding solution. The drug, excipients e.g. Lactose (q.s.), starch (q.s.) and polymer were passed through 40 #, mixed uniformly and granulated using suitable granulator. The wet blend was dried at 50-55°C to achieve moisture content in the range of 1.50% to 3.50% w/w. The final granules were rasped through 16 # and further blended with colloidal silicon dioxide (0.7 % w/w) and finally lubricated with Magnesium Stearate (0.75%). The lubricated blend was compressed on 16 station rotary compression machine, using suitable capsule shaped punches.

Formulation design using combination of (HPMC) polymers

Formulation was studied using combination of two hydrophilic polymers. HPMC K4M was used in fixed proportion (10% w/w) and proportion of HPMC K15M was varied in the range of 5%w/w (MGL 08), 10% w/w (MGL 09) and 25% w/w (MGL 10). The drug, excipients (in same proportion as used in the previous experiments) and polymers were passed through 40 #, mixed uniformly and granulated with purified water, using polyvinyl pyrrolidone (PVP K30) as binder.

The wet blend was dried at 50-55°C to achieve moisture content in the desired range. The final granules were rasped through 16 # and further blended with colloidal silicon dioxide (0.7 % w/w) and finally lubricated with Magnesium Stearate (about 0.75%). The lubricated blend was compressed on 16 station rotary compression machine, using suitable capsule shaped punches. The physical characteristics and dissolution profile of Glyclazide ER tablet was studied. The experimental trial results are presented in Table 5.13 and dissolution profile is presented in Fig.5.13.

Study of different viscosity grades of HPMC

Three separate experiments were taken using different grades of HPMC in each trial to study the effect of viscosity of the polymer on the release pattern. The polymers used were HPMC K4M (MGL 03), HPMC K15M (MGL 06) and HPMC K100M (MGL 07). The concentration of each polymer in the trial was kept constant as 25% w/w. The physical characteristics of the formulation are presented in Table 5.14 and in-vitro dissolution is presented in Fig 5.14.

Formulation design using different binders

Formulation was studied with different binders like PVP K30 (MGL 11) and Polyethylene glycol 6000 (PEG 6000) (MGL 12) separately. The trial was also taken without the use of binder using only Purified water as the granulating fluid (MGL 13). The drug, excipients (in same proportion like previous experiments) and polymers, HPMC K4M and HPMC K15M were passed through 40 #, mixed uniformly and granulated separately using different binders. The wet blend of each trial was dried at 50-55°C to achieve required moisture content. The final granules were rasped through 16 # and further blended with Colloidal silicon dioxide (0.7 % w/w) and finally lubricated with Magnesium Stearate. (about 0.75%). The lubricated blend of each trial was compressed on 16 station rotary compression machine, using suitable capsule shaped Punches. The physical characteristics of each experiment was studied and presented in Table 5.15.

Formulation using different concentrations of PVP K30.

Study using different concentrations of PVP K30 was carried out to study its effect on physical characteristics of tablets. The proportions of PVP K30 were varied between 2 % w/w to 6 % w/w. The data is presented in Table 5.16.

Physical characterization and evaluation of the tablets

Formulated tablets were subjected to different physical characterization studies (Table 5.11 to Table 5.16) The drug content of the product was determined in duplicate as per stated analytical method. The weight variation was determined on 20 tablets using electronic balance (Mettler Toledo / Afcoset). Tablet hardness was determined for minimum 5 tablets using Dr. Schleuniger hardness tester (M 8 model) Friability was determined with 10 Tablets using Friability apparatus (M/s Electrolab) at 100 revolutions (25 rpm).

In vitro release studies

Release studies were carried out in pH 7.5 buffer at 37.5 ± 0.5°C. The volume of dissolution medium was 900 ml and stirring speed was 75 rpm (USP Type 2: Paddle) At predetermined time period (viz. 2,4,8,12 hours)10 ml samples were withdrawn and replaced with fresh dissolution media. After appropriate dilution, the samples were analyzed. Cumulative percent of drug released were calculated, and mean of six tablets were used for data analysis.

The formulation design was selected based on the above experiments. It contained the combination of hydrophilic polymers [HPMC K4M (10% w/w) and HPMC K15M (10% w/w)] using PVP K30 in water as a binding solution. The effect of various parameters was studied on this selected formulation design in order to optimize the design and to fine tune the formulation. These trials are discussed as follows:

Optimization of the manufacturing process technology

(a) Effect of compression force

In order to study the effect of compression force on the in-vitro release, the tablets were compressed by using common blend prepared for the selected formulation at three different

hardness levels viz. 60N-80 N (MGL 18), 80N-100N (MGL 19), 100N to 130N (MGL 20) and in vitro dissolution rate was studied. The findings are presented in the Fig 5.15.

(b) Effect of extent of granulation

The effect of extent of granulation was studied to fine tune the process parameters. The extent of granulation was varied by using varying quantity of granulating fluid and kneading time. In order to achieve heavy granulation (MGL 23), the quantity of granulating solvent was increased by approx.20% and kneading time was also increased by approx.10%. Light granulation (MGL 21) was achieved by adding granulating solvent just enough for getting granules and kneading time was kept minimum to ensure uniform mixing of binder. Wet mass in both the cases was dried at 50-55°C in a Fluidized bed drier (M/s Adams engineering works) to achieve the desired moisture content. The dried granules were rasped through 16 # mesh and further lubricated with Magnesium Stearate (0.75% w/w) and compressed on 16 station rotary compression machine using suitable capsule shaped punches. The findings are presented in Fig 5.16.

Batch reproducibility

Three batches (MGL 24, MGL 25 and MGL 26) of the selected formulation (Table 5.19) with target dissolution profile were manufactured and their quality and respective release profile was evaluated under the same conditions as prescribed in previous sections. In vitro release data pertaining to reproducibility studies is shown in Fig 5.17.

Effect of pH of dissolution media.

The effect of pH of dissolution media was studied by generating a multipoint multimedia dissolution profile of MGL 26 in three different media: pH 1.2 buffer, pH 4.5 phosphate buffer and pH 7.5 buffer. The comparative profile is presented in Fig.5.18.

Effect of hydrodynamic conditions (Stirring speed of paddle)

The study was undertaken on the selected formulation (MGL 26). In-vitro release studies were carried out at two different stirring speeds, 50rpm and 100rpm keeping all other dissolution parameters the same. The results are presented in Fig 5.19.

Stability Studies

Accelerated stability studies were performed as per ICH guideline of Stability Testing of New Drug Substances and products [Q 1A (R2)]. Three batches of the finalized formulation were kept on accelerated and long term stability study (as per ICH guidelines). The stability protocol mentioned in Table 5.18 was followed for carrying out the studies. The results of stability studies are summarized in Table 5.20.

5.2.2 Results and Discussions:

Formulation design with different viscosity grades of HPMC polymers, alone and in combination

It is clear from Fig. 5.12 that HPMC K4M and HPMC K15M alone were not sufficient to retard the release of the drug when used in proportions upto 25%w/w. The effect was better with HPMC K15M when compared with lower viscosity grade HPMC K4M. Increased concentration of the individual polymer in both the cases had "slow down" affect on the dissolution profile but could not retard the drug release upto 12 hours. The release was 85% in 8 hours in MGL 06, (25% HPMC K15M) and 88% in MGL 03, (25% HPMC K4M) in the same time, which is close to the upper limit of targeted release profile in 8 hours.

The combination of HPMC K4M (10%w/w) and HPMC K15M (10%w/w) retarded the release upto 12 hours in line with the design inputs. Keeping the HPMC K4M constant and varying the proportions of HPMC K15M (5% and 25%w/w) did not give the satisfactory results. With 5% w/w HPMC K15M the dissolution was faster (above 90% in 8 hours) wherein with 25% HPMC K15M, the profile was on the lower side (less than 80% in 12 hours). (Fig.5.13)

Fig. 5.14 explains the behavior of different viscosity grades of HPMC polymers. The viscosity of the polymer had direct effect on the dissolution profile of the product. Higher the viscosity grade used in the product, slower was the dissolution. The tablet prepared with HPMC K100M (25% w/w, MGL 07) gave about 70% release in 12 hours, which is very low when compared with targeted release profile.

Study of release character

Release character, when studied, of the extended release GLZ tablets, using different grade of HPMC, produced first order profile. Combination of fixed proportion of HPMC K4M and

varying proportion of HPMC K15M also showed first order release character except in 25% HPMC K15M where the release was found to be nearly zero order

Formulation design with different binders with varying proportions

It was observed that PVP K30 (4%w/w) produced the tablets with desired physical parameters (MGL 11). PVP K30 gave suitable binding properties resulting in optimum granulation. Optimum granulation of the dry mix helped in getting good flow properties of the granules, desired hardness of the tablet (80 to 100N), low friability (0.20% w/w) and minimum weight variation (±2.1% w/w). (Table 5.15)

The granulation was incomplete with Polyethylene glycol-6000 (PEG 6000), which resulted in excessive fines, poor flow of granules, low hardness (40 to 50N), high friability (0.68% w/w) and weight variation on the higher side (± 4.2% w/w). (Table 5.15)

Similar unsatisfactory observations were made with the trial batch manufactured using only purified water without any binder.

The trials were not satisfactory with respect to the above parameters when PVP K30 was used in lower concentration (2% w/w) as a binder (MGL 14). The tablet properties were practically similar in a trial batch manufactured using 4% w/w (MGL 15) and 6% w/w PVP K30 (MGL 16). Therefore, PVP K30 (4% w/w) was selected as a binder based on the optimum physical characters obtained with the same (Table 5.16).

Optimization of the manufacturing process technology of GLZ ER tablet

(a) Effect of compression force

It was observed that there was no effect of hardness on in-vitro release profile because MGL 18, MGL 19 and MGL 20 gave similar release profile. (Fig. 5.15). The release profile did not show any significant changes based upon the hardness of the tablets.

(b) Effect of extent of granulation

It was observed that even by increasing water quantity (granulating fluid) by 20% and kneading time by 10% over the standard decided process parameters, there was no effect observed on the release profile of the extended release GLZ tablet. Light granulation also did not affect the release profile (Fig. 5.16). The reason for this phenomenon can be attributed to the insolubility of

GLZ, in the granulating fluid (water), therefore, by increasing granulating solvent there was no change in dissolution profile.

Batch reproducibility

The tablets showed low standard deviation values for the drug content, friability, weight variation and hardness from three different batches (MGL 24, MGL 25 and MGL 26) prepared separately. These low standard deviation values for all physical properties showed that there was excellent batch-to-batch reproducibility and absence of any significant variation among the three batches (Table 5.19). Also, no significant difference was observed in the in-vitro release profile of the three reproducible batches, calculated from the f_2 (similarity factor) (MGL 24: 70.14, MGL 25: 71.49, MGL 26: 69.84) (Fig.5.17). Thus the batch reproducibility indicated that the formulation methodology employed (aqueous granulation of GLZ by using combination of two hydrophilic polymers, HPMC K4M and HPMC K15M) is suitable for manufacturing good quality extended release matrix tablets of GLZ.

Effect of pH of dissolution media

As discussed in Drug substance, GLZ has maximum solubility in alkaline pH. The fact was confirmed when GLZ extended release tablet (MGL 26) showed complete dissolution (more than 90%) at the end of 12 hours in alkaline pH 7.5 whereas the release was found to be incomplete (around 60%) even at the end of 12 hours in acidic pH 1.2 and pH 4.5 (Fig 5.18).

Effect of hydrodynamic conditions (Stirring speed of paddle):

As is clear from the Fig.5.19, change in rpm, had affected the in vitro profile of the product (MGL 26). At 50 rpm, approx.75% of the drug had released at the end of 12 hours whereas at 100 rpm, practically, the entire drug had come out in 12 hours (approx.98%).

The observed variation in the release with change in the stirring speed can be attributed to the difference in hydrodynamic stress around the surface of the tablet undergoing dissolution and solubility characteristic of GLZ. At lower stirring rpm, (50 rpm), there was a slow fluid (release media) motion and formation of stable stagnant layer surrounding the tablet. This prevented the easy entry of release medium into the tablet and also the release of the drug outside the tablet. In addition to this, GLZ is not soluble in water, which decreased further dissolution in the medium.

But as the stirring rpm was increased to 100 rpm there was a greater fluid flow that resulted in the increased attrition of the tablet matrix at the swelling/dissolution interface. This phenomenon caused increased erosion of the matrix and decrease in the stagnant diffusion layer thickness that ultimately resulted in the increased drug release.

Stability Studies

Three months accelerated stability data of MGL 24, MGL 25 and MGL 26 showed no degradation of GLZ and no significant effect on dissolution profile of the product in comparison to the initial values in the studied primary pack (Aluminum blister packing). The product was found to be stable under the studied conditions (Table 5.20).

5.3 Formulation of MFH ER and GLZ ER bi-layer tablet

5.3.1 Experimental

Design inputs

The separately developed platform technologies for MFH ER (formulation similar to M 21) and GLZ ER (formulation similar to MGL 26) were combined together in the form of a single multi-layered tablet using the sophisticated and innovative tablet pressing machine. The bi-layered tablets were studied for its physical and chemical properties (Table 5.21). As the technology was innovative, therefore a number of experimental trials were taken to fine tune the process parameters. The various factors were studied keeping in mind the technological aspects of manufacturing on a large scale production. As the platform technologies were individually developed and studied, therefore, emphasis was given on the machinability part for producing the bi-layered tablet.

Effect of compression and pre-compression force

In order to study the effect of pre-compression and compression force during the compression of a bi-layered tablet; the compression was carried out at different pre-compression and compression force levels (Table 5.22). The pre-compression force was measured in terms of hardness of single layer and compression force was measured in terms of hardness of both the layers together, as a tablet. The pre-compression force on the MFH component was varied at three different levels: 20N to 30N, 30N to 40N and 40N to 50N. The final compression force

after addition of GLZ component was varied in the range of 200N to 250N, 250N to 350N and 350N to 400N. The results are presented in Table 5.22.

Effect of speed of machine

The speed of the machine was identified as critical factor affecting the physical parameters of the bi-layered tablet. Therefore the effect of speed of machine was studied at three different levels: 5 to 8rpm, 8 to 12rpm and 12 to 15rpm keeping the pre-compression and compression force constant (30 to 40N and 250 to 350N respectively) derived from the results of the above experiment (Table 5.23).

Physical characterization of the bi-layered tablets

The bi-layered tablets were analyzed for drug content by using method described in Chapter 3. Hardness, friability and weight variation of each layer and bi-layered tablet were measured and studied. The weight variation for individual layer and for bi-layered tablet was determined on 20 tablets. Tablet hardness was determined on 5 tablets using Dr. Schleuniger hardness tester (M 8 model). Friability was determined by using M/s Electrolab friability apparatus. Separation of two layers and breaking of tablets was the main criteria for physical evaluation. The results are presented in Table 5.21 to 5.23.

Batch reproducibility of the bi-layered tablets

Three batches of bi-layered tablets were manufactured by keeping the same composition, the manufacturing process, process and machine parameters. The batches were evaluated for the drug content, in-vitro release profile and physical parameters. The results are presented in Table 5.24.

Stability studies of bi-layered tablets

The above three batches produced were packed in Aluminum blisters and subjected to accelerated stability conditions (as per ICH guidelines). The storage conditions were kept as per Table 5.18. The samples were withdrawn at each predetermined time interval (Table 5.18) and analyzed for physical characteristics, in-vitro dissolution test and drug content. The stability study results are presented in Table 5.25.

5.3.2 Results and Discussion:

Effect of manufacturing technology and machine parameters

The in vitro release was faster with the product manufactured by direct compression method than the wet granulated product (Fig.5.20). The faster release profile in case of direct compression could be attributed to the absence of effect of solvent binding and fragile tablets obtained because of that. The physical characteristics of the tablet with direct compression were not satisfactory. Poor flow of the granules, led to high weight variation (Table 5.17). Even the hardness of the tablets was too low causing lamination (separation of the two functional layers of the bi-layered tablet). High proportion of fines in case of direct compression design, led to mixing of two layers that subsequently caused interference with the release pattern of individual layer (Fig.5.20). It is clear from Fig.5.20, that the in-vitro release was found faster (more than 90% in 8 hours) in dry granulation technology than wet granulation method (about 70% in 8 hours). The behavior can be attributed to previous swelling of polymer in presence of solvent in case of wet granulation which might have helped due to effect of solvent binding.

The hardness of the tablet is the important parameter for a bi-layered product. At lower compression force of 200N to 250N, the bi-layered tablet suffered from friability. The physical parameters obtained at hardness range 250N to 350N were satisfactory with friability less than 0.2% w/w. But at the hardness more than 350N, separation of two functional layers of tablet was observed therefore operating range of hardness was typically restricted to maximum hardness of 350N (Table 5.22).

The ideal recommended operating speed of the machine is 8 to 12 rpm. At high speed of 15 rpm, mixing of two layers was observed, which is not desirable. Therefore, the speed of the machine was optimized in the range of 8 to 12rpm. In this range, there was no mixing of layers and the tablets also had good physical characteristics (Table 5.23).

Batch reproducibility of the bi-layered tablets

The physical evaluation of tablets from all the three batches showed low standard deviation values for the drug content, friability, weight variation and hardness. These low standard deviation values for all physical and chemical parameters showed that there was excellent batch-to-batch reproducibility and absence of any significant variation (Table 5.24). The process followed was user-friendly. The f_2 values of in-vitro release profile were observed to be

sufficiently high (more than 50%) to prove the similarity among the three reproducible batches (Table 5.24) [MGR (500+60)04/02: 69.52, MGR (500+60) 05/02: 70.21, MGR (500+60) 06/02: 70.54].

Stability studies of bi-layered tablets

Three months accelerated stability data showed no degradation of MFH and GLZ and no significant effect on dissolution profile of the combination bi-layered tablet in comparison to the initial values in the studied primary pack (Aluminum blister packing). The product was found to be stable under the studied conditions (Table 5.25).

5.4 Formulation of GPD immediate release (IR) tablets

5.4.1 Experimental

Design inputs:

In-line with the previously discussed strategy for development of a combination bi-layered tablet, this study was also aimed at development of a GPD IR tablet independently and then combining it with previously developed platform technology of MFH ER tablet. The final aim of the study was to obtain safe, stable and effective combination bi-layered tablet of MFH ER and GPD IR. The dissolution profile and dissolution medium of MFH part was targeted as discussed previously. Based upon the pharmacokinetic parameters, dissolution of GPD IR part was targeted as NLT 75% in 45 minutes.

Study of different binders and granulating solvents

In an attempt to develop GPD IR tablet, various experiments were carried out using different binders. Two commonly used binders were tried separately in different trials.

First set of experiments was taken using different concentrations of PVP K30 [2% w/w (MG 03) and 4% w/w (MG 04)] in Isopropyl alcohol (IPA) as the granulating fluid. Lactose monohydrate and Microcrystalline cellulose (MCC) were used as the diluents. Lubricants included, Colloidal silicon dioxide (Aerosil-200) (1.5% w/w) and magnesium stearate (0.5 % w/w). The wet granulated manufacturing process was followed for making the granules. The granules were dried in Fluid bed drier to achieve LOD between 1.0 to 3.0% w/w. Sodium starch glycollate

(SSG) and Cross-carmellose sodium (Ac-di-sol) (3% w/w each) were selected as superdisintegrants.

In the second trial everything was kept the same, except PVP K30 which was replaced with starch paste (as binder) in different concentrations [1% w/w (MG 01A), 3% w/w (MG 01B) and 6% w/w (MG 02)]. The average weight of the tablet was also kept the same. The process and process parameters were in-line with first set of experiment where PVP K30 was used as binder. The summary of different trials taken and their observations are presented in Tables 5.26, 5.27 and 5.28. The dissolution profile in both the trials is given in Fig.5.21 and 5.22.

Study of surfactant:

GPD has limited water solubility; therefore, there was a strong need to incorporate suitable surfactant into the formulation to achieve the required dissolution for the IR formulation. Commonly used surfactant in the pharmaceutical industry, Sodium lauryl sulfate (SLS) was tried in different concentrations [0.5% w/w (MG 05A), 1.0%w/w (MG 05) and 1.5%w/w (MG 05B)], added intra-granularly. The results are presented in Table 5.29 and Fig. 5.23.

Effect of sequence of addition of SLS

The selected proportion of SLS (1%w/w) was added in different sequences in three different experiments (Table 5.30). In the first experiment, 1% w/w SLS was added intra-granularly (MG 05). In the second experiment, 1% w/w SLS was added extra-granularly (MG 06). In the third experiment (MG 07), the quantity of SLS was divided in two equal parts (0.5% w/w each) and each part was added intra (0.5% w/w) and extra granularly (0.5 w/w). Other formulation components and manufacturing process and process parameters were kept constant. Fig.5.23 graphically represents the results of the experiment.

Effect of particle size of GPD

The effect of particle size was studied by using two different lots of GPD with two different particle sizes range for manufacturing GPD tablets. One lot of GPD was used having particle size: D_{90} = 18.29μm, D_{50} = 5.08μm and D_{10} = 1.40μm (MG 07) and second lot used was having particle size: D_{90} = 98.09μm, D_{50} = 45.87μm and D_{10} = 28.51μm (MG 19). Other components of the formulation and manufacturing process and process parameters were kept constant and the

dissolution profile of both the trials were studied. The results are graphically presented in Fig. 5.24.

The formulation design was selected based on the above experiments. In this design, PVP K30 (2% w/w) in IPA was used as a binding solution, SLS (1% w/w) was used intra and extra-granularly (0.5% w/w each) and GPD was micronised (D_{90} less than 20μm). The effect of various parameters was studied on this selected formulation design in order to optimize the design and to fine tune the formulation. These trials are discussed as follows:

Study of process parameters affecting in-vitro release of GPD IR tablets

(a) Extent of granulation

In order to achieve heavy granulation (MG 15), the quantity of granulating solvent was increased by approx.20% and kneading time was also increased by approx.10%. Light granulation (MG 13) was achieved by adding granulating solvent just enough for getting granules and kneading time was kept minimum to ensure uniform mixing of binder. Wet mass in both the cases was dried at 50-55°C in a Fluidized bed drier till the LOD was achieved within 1.0% to 3.0% w/w. The dried granules were rasped through 30# and further blended with super-disintegrants, SSG and Cross-carmellose sodium (3.0% w/w each). The blend was further blended with glidant Colloidal silicon dioxide (1.5% w/w) and a lubricant magnesium stearate (0.5 % w/w). The results are presented in Fig.5.25.

(b) Effect of hardness

In order to study the effect of compression force on the in-vitro release, the tablets were compressed at three different hardness levels, 40N to 60N (MG 10), 60N to 90N (MG 11) and 90N to 110N (MG 12) and in vitro dissolution rate was studied. The results are presented in Fig.5.26.

(c) Effect of mixing time of Magnesium stearate

To study the effect of mixing time of magnesium stearate on dissolution profile of the product, blend of GPD was prepared without magnesium stearate by keeping other formulation components and process parameters constant. This blend was subdivided into two lots. Magnesium stearate was mixed for 2 minutes with first lot (MG 20A) and for 5 minutes with

second lot (MG 20B). The compression parameters were kept constant for compressing both the lots. The effect was studied on the release profile of GPD tablets of both the lots. The results are presented in Fig 5.27.

Effect of pH of dissolution media

The effect of pH of dissolution media was studied by generating a dissolution profile of MG 18 in three different media: pH 1.2 buffer, pH 4.5 phosphate buffer and pH 7.2 buffer. The result is presented in Fig 5.28.

Physical characterization of GPD IR tablets

Formulated tablets were evaluated for different physical and chemical parameters. (Table 5.27 to 5.30). The drug content of the product was determined in duplicate as per the Analytical method described in Chapter 3. The weight variation was determined on 20 tablets. Tablet hardness was determined for 5 tablets using Dr. Schleuniger hardness tester (M 8 model). Friability was determined with 20 tablets using Friability apparatus (M/s Electrolab).

Batch reproducibility for GPD tablets:

Three batches of the selected formulation (MG 16, MG 17 and MG 18) with targeted dissolution profile were manufactured and their quality and respective release profile were evaluated under the similar conditions described in previous sections. The physical and chemical parameters and in vitro release data pertaining to reproducibility study is shown in Table 5.31 and Fig 5.29 respectively.

Stability studies for GPD tablets

The above three batches produced (MG 16, MG 17 and MG 18) were packed in Clear PVC blisters and subjected to accelerated stability conditions. The storage conditions were kept as per Table 5.18 in line with ICH guidelines. The samples were withdrawn at each predetermined time interval (Table 5.18) and analyzed for physical characteristics, in-vitro dissolution test and drug content. The stability study results are presented in Table 5.32.

5.4.2 Results and Discussion:

Study of different binders and granulating solvents

The results were not satisfactory in the trial where Maize starch was used as the binder. The tablets had good physical characteristics (Table 5.28) when produced with 3% w/w (MG 01B) and 6% w/w (MG 02) Maize starch (paste), as a binder. However, the dissolution was on the lower side, less than 70% released in 45 minutes (Fig. 5.21). The probable reason could be hard granules retarding the release of GPD, in addition to the limited water solubility of GPD. Reducing the concentration of Maize starch (to 1% w/w) improved the dissolution profile (Fig.5.21) of the product (MG 01A) but it had poor compressibility, high friability (0.7%) and low hardness (20N to 30N) as shown in Table 5.28. Based on the above facts, it was concluded that starch paste was not the right choice as a binder for this formulation.

Study with PVP K30 in different proportions gave better results in terms of dissolution profile (Fig 5.22) and good physical parameters (Table 5.27). Tablets with higher concentration of PVP K30 (4% w/w) (MG 04) showed lower dissolution profile (less than 70%) as compared with tablets produced with low concentration of PVP K30 (2% w/w) (MG 03).

Study of surfactant and sequence of addition:

SLS being a surfactant improved the dissolution profile of GPD IR tablets produced with PVP K30 (2%w/w) as binder. But study at different proportions of SLS showed that the in vitro release did not improve beyond 80% even with higher concentration of SLS (upto 1.50% w/w) (MG 05B), there was practically no difference in the dissolution profile (Fig.5.23).

The sequence of addition of SLS had a significant impact on in vitro release profile. 1%w/w SLS when used by equally dividing intra-granularly and extra-granularly (as 0.5% w/w each) produced tablets releasing almost 100% of the drug in 45 minutes (Fig.5.23, MG 07).

Effect of particle size

It is evident from Fig. 5.24 that the product (MG 07) manufactured with micronised grade of material (D90 less than 20μ) had better dissolution (about 100%) in comparison with the trial (MG 19) taken with coarser grade of material. This phenomenon could be explained because of increased surface area of micronised GPD.

Study of process parameters affecting in-vitro release profile of GPD tablet

(a) Extent of granulation

From Fig 5.25, it is clear that; extent of granulation had no adverse effect on in-vitro dissolution of the product as the release profile of MG 13, MG 14 and MG 15 was almost similar. This proves that the formulation design is very robust and release profile will not be altered during scaling up. It makes the formulation design ideal for exploring the commercial viability.

(b) Effect of hardness

The study of hardness at different levels showed that there was no significant effect of hardness on the in-vitro release of GPD. The dissolution profile was not significantly changed (Fig 5.26) even at higher hardness of 90N to 110N (MG 12). But beyond the hardness value of 100N; capping was observed in selected tablets. Therefore the functional hardness range for the GPD tablet was selected as 40N to 100N.

(c) Effect of mixing time of Magnesium stearate

It was observed that when Magnesium stearate was mixed for longer time (5minutes) (MG 20B); there was a drop in the dissolution results at initial time point (till 20 minutes) but at later time points the dissolution was well within the desired specifications. The behavior can be explained as Magnesium stearate tends to form a hydrophobic layer on the granules, retarding the dissolution of the product (Fig.5.27).

Effect of pH of dissolution media

Results of multipoint-multimedia dissolution profile of MG 18 explained that the dissolution is poor in acidic media (pH 1.2 buffer and pH 4.5 phosphate buffer). There was almost100% drug release in pH 7.2 buffer. In acidic media, probably the drug is precipitating out due to solubility.

Batch reproducibility for GPD tablets:

The tablets showed low standard deviation values for the drug content, friability, weight variation and hardness from three different batches (MG 16, MG 17 and MG 18) prepared separately. These low standard deviation values for all physical properties showed that there was excellent batch-to-batch reproducibility and absence of any significant variation among the three

batches (Table 5.31). Also, no significant difference was observed in the in-vitro release profile (Fig. 5.29) of the three reproducible batches, calculated from the f_2 (similarity factor) (MG 16: 71.86, MG 17: 68.33, MG: 68.88).

Stability studies for GPD tablets

Three months accelerated stability data showed no degradation of GPD and no significant effect on dissolution profile of IR tablet in comparison to the initial values in the studied primary pack (Clear PVC blister). The product was found to be stable under the studied conditions (Table 5.32).

5.5 Formulation design for combination of MFH ER and GPD IR tablets

5.5.1 Experimental

Design inputs

The separately developed platform technologies for MFH ER (similar to M 21) and GPD IR (similar to MG 18) were combined together in the form of a single bi-layered tablet using the sophisticated and innovative tablet pressing machine. The bi-layered tablets were studied for its physical properties; drug content (Table 5.33) and dissolution profile. Based upon the experience with the bi-layered tablet of MFH ER and GLZ ER, the machine parameters were identified as critical for formulating a good quality bi-layered tablet at large scale. Therefore emphasis was given on fine tuning of the machine parameters. The experiments carried out are as follows.

Effect of compression and pre-compression force

In order to study the effect of pre-compression and compression force during the compression of a bi-layered tablet; the compression was carried out at different pre-compression and compression force levels. The pre-compression force was measured in terms of hardness of single layer and compression force was measured in terms of hardness of both the layers together, as a final bi-layered tablet. The pre-compression force on the MFH component was varied at three different levels (20N to 30N, 30N to 40N and 40N to 50N). The final compression force of the bi-layered tablet was varied in the range of 200N to 275N, 275N to 350N and 350N to 400N (Table 5.34).

Effect of speed of machine

The effect of speed of machine was studied on the physical parameters of the bi-layered tablet mainly hardness and friability and also possibility of mixing of two layers during the manufacturing operation. The effect was studied at three different levels of machine speed (5 to 8rpm, 8 to 12rpm and 12 to 15rpm) keeping the pre-compression and compression force constant (30 to 40N and 275 to 350N respectively) derived from the results of the above experiment (Table 5.35).

Effect of granulation technology for GDP part of the bi-layered tablet

The effect of granulation technology for GPD part of the bi-layered tablet was studied by taking trials with dry granulated (MG 08) blend and wet granulated blend (MG 09). The composition and average weight of GPD tablets in both the trials was kept constant. The physical parameters of these trials are presented in Table 5.36.

Physical characterization of the bi-layered tablets.

The bi-layered tablets were analyzed for drug content by using analytical method described in Chapter 3. Hardness, friability and weight variation of each layer and bi-layered tablet were also studied. The weight variation for individual layer and for bi-layered tablet was determined by selecting 20 tablets. Tablet hardness was determined for 5 tablets using Dr. Schleuniger hardness tester (M 8 model). Friability was determined by using M/s. Electrolab friability apparatus. The physical parameters of bi-layered tablets are presented in Table 5.33 to Table 5.36.

Batch reproducibility of the bi-layered tablets

Three batches of bi-layered tablets were manufactured by keeping the same composition, the manufacturing process and machine parameters. The batches were evaluated for the drug content, in-vitro release profile and physical parameters. The results are presented in Table 5.37.

Stability studies of bi-layered tablets

The above three batches produced were packed in Aluminum blisters and subjected to accelerated stability conditions (as per ICH guidelines). The storage conditions were kept as per Table 5.18. The samples were withdrawn at each predetermined time interval (Table 5.18) and

analyzed for physical characteristics, in-vitro dissolution test and drug content. The stability study results are presented in Table 5.38.

5.5.2 Results and Discussions:

Effect of manufacturing technology and machine parameters

It is clear from the physical parameters presented in Table 5.36 that the tablets produced by using dry granulation method (MG 08) showed high friability (0.8% w/w) and low hardness (35N to 45N). Also, higher proportion of fines in direct compression technology resulted in mixing of GPD IR and MFH ER layer during compression that subsequently caused interference with the release pattern of ER layer, especially at 2 hours sampling point (Fig. 5.30). The release of MFH was about 60% after 2 hours which is not desirable.

At lower pre-compression force (20 to 30N), the granules of MFH were compacted into a loose packing structure, which could not form a definite separation structure in the form of two distinct layers. Therefore at lower pre-compression force, appearance was not good and friability of the MFH layer was observed to be high. When pre-compression force was increased to 40N to 50N, the packing of the MFH granules was more compact which lost its adhesiveness with the GPD layer resulting in the separation of the two functional layers. The optimized range of pre-compression force was determined to be between 30N to 40N (Table 5.34). Within this range, the MFH granules showed optimum packing with better adhesive properties for GPD layer.

At lower compression force of 200N to 275N, the bi-layered tablet showed high friability (0.83%). At hardness more than 350N, separation of two functional layers of tablet was observed therefore functional range of hardness was typically restricted to maximum hardness of 350N (Table 5.34).

At 12-15 rpm, mixing of granules of immediate release GPD took place with the layer of MFH ER causing interference with the individual release profile of the respective layer. Therefore machine speed was optimized at 8 rpm to 12 rpm, at which there was no mixing (Table 5.35).

Batch reproducibility of the bi-layered tablets

The bi-layered tablets from the three batches showed low standard deviation values for the drug content, friability, weight variation, hardness and in vitro release. These low standard deviation values for all physical properties showed that there was excellent batch-to-batch reproducibility

and absence of significant variations among the three batches (Table 5.37). In-vitro release data pertaining to reproducibility studies were compared by f_2 (similarity factor) values. The f_2 values of in-vitro release profile were observed to be sufficiently high (more than 50%) to prove the similarity among the three reproducible batches (Table 5.37) [MGL (500+2)05/02: 73.40, MGL (500+2) 06/02: 68.11, MGL (500+2) 07/02: 71.59].

Stability studies of bi-layered tablets

It was observed during stability, rise in temperature has very little effect on dissolution profile or assay of the product (Table 5.38). It was also observed that in the studied primary packing (Aluminum blister packing) there is no significant effect of humidity and temperature on the drug content and release profile of the tablet.

5.6 Conclusion:

The designed tablet formulations of MFH ER, GLZ ER, GPD IR, MFH ER + GLZ ER and MFH ER + GPD IR showed excellent compliance to predetermined specifications with respect to physical parameters, drug content with variable release profile. The composition, manufacturing process and process parameters for all the tablet products were finalized based on number of experimental trials taken and after critical review of each experimental findings the products were found to have required release profile. Commonly used excipients in tablet manufacturing technology were selected for designing the formulations. These excipients were easily available at affordable price to ensure cost effective formulations. The excipients were used in recommended proportions in various standard text books. Three reproducible batches of each tablet dosage form showed low standard deviation values for all the testing parameters. It was concluded from the experimental trials that manufacturing process followed is user-friendly and can be explored for large scale commercial manufacturing. The process optimization of critical parameters in each formulation proved that the formulation design is very robust and reproducible.

The combination of polymers provided better control on the dissolution profile of the drugs instead of single polymer. Designed formulations of both MFH ER tablets and GLZ ER tablets showed first order release profile except, GLZ ER tablets with 25% HPMC K15M showed

nearly zero order release profile. Also the results were much better in a wet granulated process when compared with the dry granulation method.

The effect of particle size of the drug was significant in GLZ and GPD based formulations because of limited solubility of these drugs in water. However, such effect was negligible in case of MFH due to its high water solubility. Multipoint-multimedia dissolution profile was established for each drug product (alone and in combination) simulating the physiological conditions to assess the solubility pattern. The hydrodynamic condition in the dissolution media was found to have significant effect on the dissolution release profile. Increase in the stirring rpm increased rate of dissolution confirming the erosion mechanism of matrix.

The bi-layer technology was preferred to combine two drugs in a single unit dosage form. It had distinct advantages over the other tried approaches (tab-in-tab technology and drug loading). This technique also can prevent interaction between drugs particularly for incompatible drugs. Release of the drugs also will be independent. The aesthetic appearance of the tablet was very good, simple process, easy to reproduce and simultaneous release of both the drugs from two distinct layers of the bi-layer product. As the bi-layer technology is innovative, great emphasis was given to standardize the machine parameters (like, pre-compression force, compression force and machine speed) and physical parameters (hardness, friability and layer separation) to achieve tablets of required specifications.

All the designed tablet formulations of MFH ER, GLZ ER, GPD IR, MFH ER + GLZ ER and MFH ER + GPD IR were stable with respect to physical parameters, drug content and dissolution testing at accelerated and controlled temperature and humidity conditions, as per ICH guidelines in the recommended packs for the studied period. Variation in the drug content at different time points is statistically insignificant. Also there was no significant change in the release profiles at different intervals studied. It was concluded that the designed formulations are stable with insignificant variation in the testing values of various parameters. The stability data indicated that there is no drug incompatibility in the combination products in single unit dosage form.

Use of different colors provided better product identification and improved aesthetic look. All the colors used were food grade approved by the Foods and Drug Administration (FDA).

Table 5.1: Target dissolution profile of MFH.

Sr.No.	Time (hours)	Target dissolution rate profile range (%)
1	2	35-50
2	4	55-75
3	8	75-90
4	12	NLT 90

Table 5.2: Formula and physical properties of MFH ER tablets prepared with single polymer, (HPMC K 15M).

Formulations	M01	M02	M03
Components[@]			
Drug (mg)	500	500	500
HPMC K15M[#] (%)	5	10	25
Physical properties.			
Average tablet weight (mg)	750.00mg	750.00mg	750.00mg
Weight variation (%)[*]	± 2.1	± 1.8	± 1.7
Hardness (N)[a]	150 N –200 N	150 N –200 N	150 N –200 N
Friability (%)	0.1	0.2	0.1
Assay (%) [$]	100.78 ± 0.9	101.53 ±0.74	99.97 ± 0.99

@: Also contains 0.6%w/w of Magnesium stearate as lubricant 3.0% w/w PVP K30 as binder and MCC (q.s.) as diluent.
#: % w/w of drug content. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.

Table 5.3: Formula and physical properties of MFH ER tablets designed with combination of polymers HPMC K 100 M and HPMC K15 M.

Formulations	M06	M07	M08
Components[@]			
Drug (mg)	500	500	500
HPMC K100M[#] (%)	5	10	25
HPMC K15M[#] (%)	10	10	10
Physical properties.			
Average tablet weight (mg)	750.00mg	750.00mg	750.00mg
Weight variation (%)[*]	± 2.3	± 2.4	± 1.27
Hardness (N)[a]	150 N –200 N	150 N –200 N	150 N –200 N
Friability (%)	0.15	0.21	0.1
Assay (%) [$]	101.68± 1.32	99.53 ± 1.04	100.97 ± 1.25

@: Also contains 0.6%w/w of Magnesium stearate as lubricant 3.0% w/w PVP K30 as binder and MCC (q.s.) as diluent.
#: % w/w of drug content. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.

Table 5.4: Formula and physical properties of MFH ER tablets prepared for selecting a hydrophobic polymer (HPMCP or Carbopol 971G).

Formulation	M09	M10
Components[@]		
Drug (mg)	500	500
HPMC K15M [#] (%)	10	10
HPMC K100M [#] (%)	25	25
Carbopol 971G [#] (%)	10	-
HPMCP (HP55) [#] (%)	-	5
Physical properties		
Average tablet weight (mg)	750.00	750.00
Wt. Variation (%)[*]	± 2.1	± 2.5
Hardness (N) [a]	150 N –200 N	150 N –200 N
Friability (%)	0.18 %	0.2%
Assay (%) [$]	101.68 ± 1.07	99.53 ± 1.24

@: Also contains 0.6%w/w of Magnesium stearate as lubricant and 3.0% w/w, PVP K30 as binder and MCC (q.s.) as diluent.
#: % w/w of drug content. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.

Table 5.5: Formula and physical properties of MFH ER tablets prepared with different viscosity grades polymer, HPMC K 100 M (LV) and HPMC K100M (CR) grade.

Formulation	M04	M05
Components[@]		
Drug (mg)	500	500
HPMC K100M[#] LV (%)	25	-
HPMC K100M CR[#] (%)	-	25
Physical properties		
Average tablet weight (mg)	750.00	750.00
Wt. Variation (%)[*]	±1.9	±2.3
Hardness (N) [a]	150 N –200 N	150 N –200 N
Friability (%)	0.1 %	0.15%
Assay (%) [$]	101.23 ± 1.04	100.65 ± 0.91

@: Also contains 0.6% w/w of Magnesium stearate as lubricant, 3.0% w/w PVP K30 as binder and MCC (q.s.) as dilent.
#: % w/w of drug content. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.

Table 5.6: Formula and physical properties of MFH ER tablets prepared with different granulating fluids [aqueous solvent (water) and non aqueous solvent (IPA)].

Formulation	M09	M12
Components[@]		
Drug (mg)	500	500
HPMC K15 M [#] (%)	10	10
HPMC K100M[#] (%)	25	25
Carbopol 971 [#] (%)	10	10
Physical properties		
Average tablet weight (mg)	750.00	750.00
Wt. Variation (%)[*]	± 2.1	± 3.5
Hardness (N) [a]	150 N –200 N	200 N –225 N
Friability (%)	0.21 %	0.05 %
Solvent for granulation [b]	Non aqueous	Aqueous
Assay (%) [$]	100.98 ± 0.82	101.53 ± 1.33

@: Also contains 0.6% w/w of Magnesium stearate as lubricant and 3.0% w/w, PVP K30 as binder and MCC (q.s.) as diluent
#: % w/w of the drug content. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.
b: In both of the experiments polymer quantities were same, only differed in method of granulation.

Table 5.7: Formula and physical properties of MFH ER tablets prepared with different methods of granulation (direct compression and wet granulation).

Formulation	M09	M11
Components[@]		
Drug (mg)	500	500
HPMC K15M [#] (%)	10	10
HPMC K100M[#] (%)	25	25
Carbopol 971G [#] (%)	10	10
Physical properties		
Average tablet weight (mg)	750.00	750.00
Wt. Variation (%)[*]	±2.1	±3.5
Hardness (N) [a]	150 N –200 N	100 N –125 N
Friability (%)	0.21 %	0.32 %
Method of granulation [b]	Wet granulation	Direct compression
Assay (%) [$]	101.68 ± 1.10	102.53 ± 1.18

@: Also contains 0.6% w/w of Magnesium stearate as lubricant 3.0% w/w PVP K30 as binder and MCC (q.s.) as diluent.
#: % w/w of the drug content. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.
b: In both of the experiments polymer quantities were same, only differed in method of granulation.

Table 5.8: Batch reproducibility of three batches of MFH ER tablet.

Formulation	MXT (500) 19/05 (M19)	MXT (500) 20/05 (M20)	MXT (500) 21/05 (M21)	Standard deviation (%)
Components[@]				
Drug (mg)	500	500	500	Not applicable
HPMC K15M [#] (%)	10	10	10	
HPMC K100M[#] (%)	25	25	25	
Carbopol 971G [#] (%)	10	10	10	
Physical properties				
Average tablet weight (mg)	750.00	750.00	750.00	Not applicable
Wt. Variation (%)[*]	± 2.1	±1.9	± 1.53	Not applicable
Hardness (N) [a]	145 N – 184 N (164.5N)[b]	151-187 (169N)[b]	149-174 (161.5N)[b]	± 3.08[c]
Friability (%)	0.21	0.18	0.24	± 0.024
Assay (%) [$]	98.04 ± 1.05	99.94 ± 1.64	100.04 ± 0.9	± 0.84

@: Also contains 0.6%w/w of Magnesium stearate, 3.0% w/w PVP K30 as binder and MCC (q.s.) as diluent.
#: % w/w of drug content. $: Mean of triplicate with standard deviation. *: % variation from the mean.
a: Range of 10 tablets. b Average taken from hardness values of 5 tablets c Standard deviation value calculated considering average hardness values.

Table 5.9: Stability Summary report of MFH ER tablet (500 mg) in accelerated condition 40°C / 75% RH.

Test	Specification	Batch No.	Initial	1 month	2 months	3 months
Appearance	White colored, capsule shaped tablets with both sides plain	MXT (500) 19/05	Complies	Complies	Complies	Complies
		MXT (500) 20/05	Complies	Complies	Complies	Complies
		MXT (500) 21/05	Complies	Complies	Complies	Complies
Cumulative release (%)	2 hours 35% to 50% 4 hours 55% to 75% 8 hours 75% to 90% 12 hours NLT 90%	MXT (500) 19/05	44 68 83 99	42 65 80 95	45 66 83 98	44 63 81 97
		MXT (500) 20/05	41 66 84 96	43 67 82 99	44 65 84 97	42 63 81 98
		MXT (500) 21/05	42 64 83 97	41 65 81 95	42 64 81 98	43 61 82 98
Related compounds (%)	Cyanoguanidine: NMT 0.1% Unknown impurity: NMT 0.1% Total impurities: NMT 0.6%	MXT (500) 19/05	0.002 0.001 0.013	0.001 0.003 0.011	0.001 0.002 0.013	0.002 0.002 0.012
		MXT (500) 20/05	0.002 0.002 0.011	0.002 0.002 0.015	0.001 0.003 0.011	0.002 0.001 0.012
		MXT (500) 21/05	0.004 0.001 0.014	0.002 0.003 0.015	0.001 0.002 0.013	0.002 0.001 0.011
Assay (%)	95.0% to 105.0%	MXT (500) 19/05	98.04	97.09	99.16	98.88
		MXT (500) 20/05	99.94	100.08	99.98	100.10
		MXT (500) 21/05	100.04	99.87	100.80	101.31

Conclusion: The product was found to be stable for the studied 3 months period.

Table 5.10: Target dissolution profile of GLZ.

Sr.No.	Time (hours)	Target dissolution rate profile range (%)
1	2	10-40
2	4	30-70
3	8	60-90
4	12	NLT 75

Table 5.11: Formula and physical properties of GLZ ER tablets prepared with single polymer, HPMC K 4 M.

Formulation	MGL 01	MGL 02	MGL 03
Components[@]			
Drug (mg)	60	60	60
HPMC K4M[#] (%)	10	20	25
Physical properties			
Average tablet weight (mg)	300.00	300.00	300.00
Wt. Variation (%)[*]	± 2.1	± 2.3	± 1.9
Hardness (N) [a]	80 -100	80 -100	80 -100
Friability (%)	0.2	0.2	0.1
Assay (%) [$]	99.98 ± 1.02	100.53 ± 1.1	99.1 ± 1.1

@: Also contains 0.75% w/w of Magnesium stearate, Lactose monohydrate and Maize starch (q.s.), 4% w/w PVP K30 and 0.7% w/w of Colloidal silicon dioxide #: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.

Table 5.12: Formula and physical properties of GLZ ER tablets prepared with single polymer, HPMC K 15 M.

Formulation	MGL 04	MGL 05	MGL 06
Components[@]			
Drug (mg)	60	60	60
HPMC K15M [#] (%)	10	20	25
Physical properties			
Average tablet weight (mg)	300.00	300.00	300.00
Wt. Variation (%)[*]	± 1.9	± 2.2	± 2.1
Hardness (N) [a]	80 -100	80 -100	80 -100
Friability (%)	0.18	0.2	0.21
Assay (%) [$]	101.7 ± 1.08	99.53 ± 0.9	100.5 ± 1.2

@: Also contains 0.75% w/w of Magnesium stearate, Lactose monohydrate and Maize starch (q.s.), 4% w/w PVP K30 and 0.7% w/w of Colloidal silicon dioxide #: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.

Table 5.13: Formula and physical properties of GLZ ER tablets prepared with combination polymer HPMC K4M and HPMC K15 M.

Formulation	MGL 08	MGL 09	MGL 10
Components[@]			
Drug (mg)	60	60	60
HPMC K4M [#] (%)	10	10	10
HPMC K15M [#] (%)	5	10	25
Physical properties			
Average tablet weight (mg)	300.00	300.00	300.00
Wt. Variation (%)[*]	± 2.0	± 2.1	± 2.1
Hardness (N) [a]	80 -100	80 -100	80 -100
Friability (%)	0.2	0.19	0.15
Assay (%) [$]	100.2	99.53	101.5

@: Also contains 0.75% w/w of Magnesium stearate, Lactose monohydrate and Maize starch (q.s.), 4% w/w PVPV K30 and 0.7% w/w of Colloidal silicon dioxide #: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.

Table 5.14: Formula and physical properties of GLZ ER tablets prepared with polymer of different viscosity grades (HPMC K4 M, HPMC K 15 M, HPMC K100 M).

Formulation	MGL 03	MGL 06	MGL 07
Components[@]			
Drug (mg)	60	60	60
HPMC K4M [#] (%)	25	-	-
HPMC K15M [#] (%)	-	25	-
HPMC K100M [#] (%)	-	-	25
Physical properties			
Average tablet weight (mg)	300.00	300.00	300.00
Wt. Variation (%)[*]	± 2.1	± 2.3	± 1.9
Hardness (N) [a]	80 -100	80 -100	80 -100
Friability (%)	0.18	0.2	0.21
Assay (%) [$]	99.7 ± 1.05	100.53 ± 1.20	99.5 ± 0.9

@: Also contains 0.75%w/w of Magnesium stearate, Lactose monohydrate and Maize starch (q.s.), 4% w/w PVP K30 and 0.7% of Colloidal silicon dioxide. #: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets

Table 5.15: Formula and physical properties of GLZ ER tablets prepared with different binders.

Formulation	MGL 11	MGL 12	MGL 13
Components[@]			
Drug (mg)	60	60	60
HPMC K4M [#] (%)	10	10	10
HPMC K15M [#] (%)	10	10	10
PVP K 30 # (%)	4	-	-
PEG 6000 [#] (%)	-	8	-
Physical properties			
Average tablet weight (mg)	300.00	300.00	300.00
Wt. Variation (%)[*]	± 2.1	± 4.2	± 3.1
Hardness (N) [a]	80 -100	40 -50	45 -60
Friability (%)	0.2	0.68	0.59
Assay (%) [$]	101.2 ± 1.8	102.7 ± 1.2	99.5 ± 1.3

@: Also contains 0.75% w/w of Magnesium stearate, Lactose monohydrate and Maize starch (q.s.) and 0.7% w/w of Colloidal silicon dioxide. #: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets

Table 5.16: Formula and physical properties of GLZ ER tablets prepared with PVP K 30 of varied quantity.

Formulation	MGL 14	MGL 15	MGL 16
Components[@]			
Drug (mg)	60	60	60
HPMC K4M [#] (%)	10	10	10
HPMC K15M [#] (%)	10	10	10
PVP K 30 [#] (%)	2	4	6
Physical properties			
Average tablet weight (mg)	300.00	300.00	300.00
Wt. Variation (%)[*]	± 3.9 %	± 1.7 %	± 1.8 %
Hardness (N) [a]	40 -50	80 -100	80 -100
Friability (%)	0.58 %	0.21%	0.20%
Assay (%) [$]	99.7	100.53	99.5

@: Also contains 0.75% w/w of Magnesium stearate, Lactose monohydrate and Maize starch (q.s.) and 0.7% w/w of Colloidal silicon dioxide. #: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.

Table 5.17: Formula and physical properties of MFH ER + GLZ ER bi-layered tablets with varied method of granulation for GLZ ER part.

Formulation	MGL 15	MGL 17
Components[@]		
Drug (mg)	60	60
HPMC K4M [#] (%)	10	10
HPMC K15M [#] (%)	10	10
PVP K 30 # (%)	4	4
Physical properties		
Average tablet weight (mg)	300.00	300.00
Wt. Variation (%)[*]	± 2.1	± 2.9
Hardness (N) [a]	260-280	150 -180
Friability (%)	0.2	1.3
Assay (%) [$]	100.4 ±1.3	101.7 ±1.05

@: Also contains 0.75% w/w of Magnesium stearate, Lactose monohydrate and Maize starch (q.s.) and 0.7% Colloidal silicon dioxide. #: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.

Table 5.18: Stability protocol as per ICH guidelines.

Sr.No.	Study	Storage Condition	Frequency of testing
1.	Long Term	25°C ± 2°C / 60 ± 5% RH	3M, 6M, 12M, 18M, 24M, 36M
2.	Intermediate	30°C ± 2°C / 65 ± 5% RH	3M, 6M ,9M, 12M
3.	Accelerated	40°C ± 2°C / 75 ± 5% RH	1M, 2M, 3M, 6M

Table 5.19: Batch reproducibility of three batches of GLZ ER tablets.

Formulation	MGL 24	MGL 25	MGL 26	Standard deviation (%)
Components[@]				
Drug (mg)	60	60	60	Not applicable
HPMC K4M [#] (%)	10	10	10	
HPMC K15M [#] (%)	10	10	10	
PVP K 30 [#] (%)	4	4	4	
Physical properties				
Wt. Variation (%)[*]	± 2.1	± 2.4	± 2.9	Not applicable
Average tablet weight (mg)	300.00	300.00	300.00	Not applicable
Hardness (N) [a]	82-94 (88)[b]	88-97 (93)[b]	87-99 (93)[b]	± 4.16[c]
Friability (%)	0.20	0.27	0.22	± 0.04
Assay (%) [$]	99.29 ±0.9	101.25 ± 1.7	99.25 ± 1.1	± 1.06

@: Also contains 0.75% w/w of Magnesium stearate, Lactose monohydrate and Maize starch (q.s.) and 0.7% w/w of Colloidal silicon dioxide. #: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.

b Average taken from hardness values of 5 tablets

c Standard deviation value calculated considering average hardness values.

Table 5.20: Stability Summary report of GLZ tablet ER in accelerated condition 40°C / 75% RH.

Test	Specification	Batch No.	Initial	1 month	3 months	6 months
Appearance	White colored, capsule shaped tablets with both sides plain.	**MGL 24**	Complies	Complies	Complies	Complies
		MGL 25	Complies	Complies	Complies	Complies
		MGL 26	Complies	Complies	Complies	Complies
Cumulative release (%)	2 hours 10% to 40% 4 hours 30% to 70% 8 hours 60% to 90% 12 hours NLT 75%	**MGL 24**	22.3 43.5 75.0 92.0	21.5 45.0 77.4 95.5	21.0 46.5 78.6 94.7	20.5 49.5 73.0 95.0
		MGL 25	24.0 39.5 79.5 101.0	24.5 42.9 78.0 96.6	23.9 41.5 77.9 95.0	23.2 42.3 77.4 97.0
		MGL 26	22.5 47.0 75.2 91.5	21.8 47.9 76.9 94.0	22.4 46.8 78.0 93.4	21.0 48.5 76.5 94.5
Related compounds (%)	Impurity F: NMT 0.2% Unknown impurity: NMT 0.1% Total impurities: NMT 0.4%	**MGL 24**	0.07 0.07 0.225	0.08 0.08 0.252	0.09 0.09 0.274	0.115 0.095 0.289
		MGL 25	0.06 0.088 0.135	0.07 0.095 0.173	0.09 0.1 0.210	0.125 0.114 0.254
		MGL 26	0.078 0.108 0.204	0.08 0.11 0.224	0.10 0.115 0.249	0.128 0.117 0.265
Assay (%)	95.0% to 105.0%	**MGL 24**	99.29	98.15	98.65	99.35
		MGL 25	101.25	99.25	98.97	98.96
		MGL 26	99.25	99.97	98.67	98.53

Conclusion: GLZ extended release tablet was found to be stable for the studied period of time.

Table 5.21: Physical and chemical parameters of a bi-layered, MFH ER and GLZ ER bi-layered tablet.

Tests	Observed values for bi-layered tablets
Assay (%) [$]	99.7 ±0.8
Average tablet weight (mg)	1050.00
Wt. Variation (%)*	± 2.1
Hardness (N) [a]	270-284 (277)[b]
Friability (%)	0.15

$: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.
b Average taken from hardness values of 5 tablets

Table 5.22: Observations of Effect of compression and pre-compression force on MFH ER + GLZ ER bi-layered tablet.

Machine parameter	Tablet parameter	
Pre-compression force (N)	**Friability (%)**	**Weight variation***
20-30	1.5	± 1.8
30-40	0.8	± 1.2
40-50	0.5	± 1.4
Compression force (N)		
200-250	0.7	± 2.2
250-350	0.18	± 1.1
350-400	Separation of two functional layers.	Not done

*: % variation from the mean.

Table 5.23: Observations of Effect of speed of machine on MFH ER + GLZ ER bi-layered tablet.

Machine parameter	Tablet parameter	
Speed of the machine (rpm)	**Friability (%)**	**Weight variation***
5-8	0.10	± 0.8
8-12	0.12	± 1.1
12-15$	0.11	± 4.4

*: % variation from the mean. $: At 12-15 rpm, mixing of two functional layers was also observed in addition to high weight variation.

Table 5.24: Batch reproducibility of three batches of MFH ER + GLZ ER bi-layered tablet.

Physical properties		**MGR (500+60) 04/02**	**MGR (500+60) 05/02**	**MGR (500+60) 06/02**	**Standard deviation (%)**
Average tablet weight (mg)		1050.00	1050.00	1050.00	Not applicable
Wt. Variation (%)*		± 2.1	± 2.4	± 2.9	Not applicable
Hardness (N) [a]		260-300 (285)[b]	265-281 (273)[b]	277-285 (281)[b]	± 2.16[c]
Friability (%)		0.2	0.27	0.22%	± 0.04
f_2 values (Similarity factor)		69.52	70.21	70.54	Not applicable
Assay (%) [$]	MFH	100.27 ±0.90	99.85 ± 1.70	100.44 ± 1.10	± 1.06
	GLZ	99.68 ±1.40	99.25 ± 0.8	100.85 ± 1.30	± 1.11

$: Mean of triplicate with standard deviation *: % variation from the mean.
a: Range of 10 tablets. b Average taken from hardness values of 5 tablets
c: Standard deviation value calculated considering average hardness values.

Table 5.25: Stability Summary report of MFH ER and GLZ ER bi-layered tablet in accelerated condition 40°C / 75% RH.

Test	Specification			Batch No.	Initial		1 month		3 months		6 months	
Appearance	White - orange colored, capsule shaped tablet with both sides plain.			**MGR (500+60) 04/02**	Complies		Complies		Complies		Complies	
				MGR (500 +60) 05/02	Complies		Complies		Complies		Complies	
				MGR(500 +60) 06/02	Complies		Complies		Complies		Complies	
Cumulative release (%)	Time	MFH	GLZ		MFH	GLZ	MFH	GLZ	MFH	GLZ	MFH	GLZ
	2 hours	35 to 50	10 to 40	**MGR (500+60) 04/02**	44	21.68	43	22.41	43	20.85	42	22.27
	4 hours	55 to 75	30 to 70		68	45.69	63	44.85	65	45.18	66	44.24
	8 hours	75 to 90	60 to 90		83	71.39	82	72.64	84	70.99	81	72.54
	12 hours	NLT 90	NLT 75		99	98.10	98	99.12	99	98.37	98	97.91
				MGR (500 +60) 05/02	41	24.10	39	25.11	42	24.87	40	24.61
					65	43.24	61	40.85	63	42.67	62	41.27
					85	74.29	80	75.28	82	76.14	82	74.29
					99	101.01	95	100.87	98	100.94	98	100.08
				MGR(500 +60) 06/02	43	23.74	42	24.30	40	25.10	44	24.80
					69	42.60	65	43.57	63	44.20	60	43.60
					88	73.90	86	74.30	87	74.10	84	71.90
					97	98.27	98	98.30	99	99.20	95	98.70
Assay (%)	MFH		GLZ	**MGR (500+60) 04/02**	100.2	99.68	99.87	100.1	100.8	99.90	100	100.4
	95.0% to 105.0%			**MGR (500 +60) 05/02**	99.85	99.25	99.28	99.40	99.37	99.82	99.80	97.13
				MGR(500 +60) 06/02	100.4	100.9	99.91	99.71	100.5	98.65	99.27	98.31

Conclusion: Combination of MFH ER and GLZ ER tablet was found to be stable for the studied period of time.

Table 5.26: Summary of trials taken to decide the formulation design for GPD tablet.

Experiment	Observation	Conclusion
1. Trials to decide binder and granulation solvent		
1.1. Trial with Maize starch paste as a binder.	• Poor dissolution profile, which improved by decreasing the binder percentage. • Lowered binder proportion caused poor physical properties in terms of low hardness and high friability.	Maize starch paste could not be used as a binder. Aqueous granulation decreased the release profile.
1.2. Trial with PVP K30 as a binder and Isopropyl alcohol as a granulating solvent.	• Dissolution profile improved with decreased binder (2%w/w) proportion.	Lower proportion of binder is required for good physical properties and improved dissolution profile. But the profile was still incomplete indicating the need to use a surfactant.
2. Trial with Sodium Lauryl Sulphate		
2.1. Proportion of SLS	• Dissolution profile was improved with increase in SLS proportion till 1.0%w/w.	Plateau phase is formed in Dissolution release profile beyond 1.0%w/w SLS quantity.
2.2. Sequence of addition of SLS	• Dissolution profile was better with SLS equally divided intra- and extra-granularly.	Sequence of addition of SLS played a significant role in improving dissolution profile.
3. Particle size of GPD		
Particle size of GPD was Varied.	• Best dissolution profile was obtained with micronised drug with D_{90} value of 18.29μ.	Particle size reduction of GPD is required to improve the dissolution profile.

Table 5.27: Formula and physical properties of GPD tablets prepared with PVP K30 as a binder.

Formulation	MG03	MG04
Components[@]		
Drug (mg)	2	2
PVP K30[#] (%)	2	4
Physical properties.		
Average tablet weight (mg)	120.00	120.00
Wt. Variation (%)[*]	±2.1	±1.9
Hardness (N)[a]	65-75	65-75
Friability (%)	0.21	0.17
Assay (%)[$]	99.75±0.8	100.98±1.21

@: Also contains 0.5% w/w of Magnesium stearate, Lactose monohydrate and MCC (q.s.), 1.5% w/w of Colloidal silicon dioxide, SSG and Ac-di-sol (3% w/w of each) as other additives.
#: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean.
a: Range of 10 tablets.

Table 5.28: Formula and physical properties of GPD tablets prepared with starch Paste as a binder.

Formulation	MG01A	MG01B	MG02
Components[@]			
Drug (mg)	2	2	2
Maize Starch for paste[#] (%)	1	3	6
Physical properties.			
Average tablet weight (mg)	120.00	120.00	120.00
Weight variation (%)[*]	± 5.8	± 2.4	± 1.9
Hardness (N)[a]	20-30	50-80	50 –80
Friability (%)	0.7	0.1	0.2
Assay (%)[$]	99.57 ± 2.9	100.1± 1.02	99.8 ±1.14

@: Also contains 0.5% w/w of Magnesium stearate, Lactose monohydrate and MCC (q.s.), 1.5% w/w of Colloidal silicon dioxide, SSG and Ac-di-sol (3% w/w of each) as other additives.
#: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean
a: Range of 10 tablets.

Table 5.29: Formula for the trials taken to study the effect of surfactant proportion on GPD tablets.

Formulation	MG05A	MG05	MG05B
Components[@]			
Drug (mg)	2	2	2
SLS[#] (%)	0.5	1.0	1.5
PVP K30[#] (%)	2	2	2
Physical properties.			
Average tablet weight (mg)	120.00	120.00	120.00
Wt. Variation (%)[*]	±1.7	±1.5	±1.5
Hardness (N)[a]	65-75	65-75	65-75
Friability (%)	0.15	0.18	0.14
Assay (%)[$]	99.11 ± 1.09	102.21 ±1.33	100.63 ±1.21

@: Also contains 0.5% w/w of Magnesium stearate, Lactose monohydrate and MCC (q.s.), 1.5% w/w of Colloidal silicon dioxide, SSG and Ac-di-sol (3% w/w of each) as other additives.
#: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean
a: Range of 10 tablets.

Table 5.30: Formula and physical properties of GPD tablets prepared by varying sequence of addition of Sodium Lauryl Sulphate (SLS).

Formulation	MG05	MG06	MG07
Components[@]			
Drug (mg)	2	2	2
PVP K30[#] (%)	2	2	2
SLS[#] (%)	Intra : 1	Intra : NIL	Intra :0.5
	Extra : NIL	Extra : 1	Extra : 0.5
Physical properties.			
Average tablet weight (mg)	120.00	120.00	120.00
Wt. Variation (%)[*]	±1.7	±1.5	±1.5
Hardness (N)[a]	65-75	65-75	65-75
Friability (%)	0.15	0.18	0.14
Assay (%)[$]	99.89 ±1.17	100.21 ±1.07	99.43±0.99

@: Also contains 0.5% w/w of Magnesium stearate, Lactose monohydrate and MCC (q.s.), 1.5% w/w of Colloidal silicon di oxide and SSG and Ac-di-sol (3% w/w of each) as other additives.
#: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean.
a: Range of 10 tablets.

Table 5.31: Batch reproducibility of three batches of GPD tablet.

Formulation	**AYC4001F (MG 16)**	**AYC4002F (MG 17)**	**AYC4003F (MG 18)**	**Standard deviation (%)**
Components[@]				
Drug (mg)	2	2	2	Not applicable
PVP K30[#] (%)	2	2	2	
SLS [#]%	Intra :0.5	Intra :0.5	Intra :0.5	
	Extra : 0.5	Extra : 0.5	Extra : 0.5	
Physical properties.				
Average tablet weight (mg)	120.00	120.00	120.00	Not applicable
Wt. Variation (%)[*]	±1.5	± 1.64	± 0.9	Not applicable
Hardness (N) [a]	60-75	59-70	62-74	± 2.12
Friability (%)	0.14	0.18	0.20	± 0.77
Assay (%) [$]	100.24 ±0.99	99.87 ± 1.05	101.24 ± 1.11	± 1.60

@: Also contains 0.5% w/w of Magnesium stearate, Lactose monohydrate and MCC (q.s.), 1.5% w/w of Colloidal silicon di oxide and SSG and Ac-di-sol (3% w/w of each) as other additives.
#: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean.
a: Range of 10 tablets.

Table 5.32: Stability Summary report of GPD tablet 2 mg in accelerated condition 40°C / 75% RH.

Test	Specification	Batch No.	Initial	1 month	3 months	6 months
Appearance	White colored, capsule shaped tablets with both sides plain.	**AYC 4001F**	Complies	Complies	Complies	Complies
		AYC 4002F	Complies	Complies	Complies	Complies
		AYC 4003F	Complies	Complies	Complies	Complies
Cumulative release (%)	NLT 75% in 45 minutes	**AYC 4001F**	102.5	106.5	99.80	101.2
		AYC 4002F	100.1	103.6	103.8	105.7
		AYC 4003F	101	98.50	101.2	99.80
Related compounds (%)	Sulphonamide: NMT 0.1% Unknown impurity: NMT 0.1% Total impurities: NMT 0.4%	**AYC 4001F**	0.053 0.045 0.282	0.051 0.045 0.281	0.055 0.046 0.275	0.050 0.047 0.288
		AYC 4002F	0.041 0.044 0.251	0.047 0.041 0.264	0.048 0.031 0.257	0.045 0.042 0.268
		AYC 4003F	0.040 0.031 0.291	0.047 0.037 0.285	0.049 0.031 0.286	0.046 0.035 0.290
Assay (%)	95.0% to 105.0%	**AYC 4001F**	100.24	100.02	100.12	00.47
		AYC 4002F	99.87	100.57	99.27	101.21
		AYC 4003F	101.24	100.31	99.87	100.57

Conclusion: GPD immediate release tablet was found to be stable for the studied period of time.

Table 5.33: Physical and chemical parameters of a combination bi-layered, MFH ER and GPD tablet.

Tests	Observed values for bi-layered tablets
Assay (%) [$]	98.94 ±0.9
Average tablet weight (mg)	870.00
Wt. Variation (%)*	± 1.9
Hardness (N) [a]	280-290 (285)[b]
Friability (%)	0.22

$: Mean of triplicate with standard deviation. *: % variation from the mean. a: Range of 10 tablets.
b Average taken from hardness values of 5 tablets

Table 5.34: Observations of Effect of compression and pre-compression force on MFH ER and GPD bi-layered tablet.

Machine parameter	Tablet parameter	
Pre-compression force (N)	**Friability (%)**	**Weight variation***
20-30	1.6	± 1.74
30-40	0.75	± 1.11
40-50	0.58	± 1.36
Compression force		
200-275	0.83	± 1.97
275-350	0.15	± 1.23
350-400	Separation of two functional layers.	Not done

*: % variation from the mean.

Table 5.35: Observations of Effect of speed of machine on MFH ER and GPD bi-layered tablet.

Machine parameter	Tablet parameter	
Speed of the machine (rpm)	Friability (%)	Weight variation*
5-8	0.11	± 0.77
8-12	0.14	± 1.21
12-15$	0.18	± 4.9

*: % variation from the mean.
$: At 12-15 rpm, mixing of two functional layers was also observed in addition to high weight variation.

Table 5.36: Formula and physical properties of MFH ER + GPD bi-layered tablets with varied method of granulation for GPD IR part.

Formulation	MG 08	MG 09
Components@		
Drug (mg)	2	2
PVP K30# (%)	2	2
SLS # (%)	Intra : 0.5	Intra : 0.5
	Extra : 0.5	Extra : 0.5
Physical properties.		
Average tablet weight (mg)	120.00	120.00
Wt. Variation (%)*	±4.5	±2.2
Hardness (N) a	35-45	65-75
Friability (%)	0.80	0.10
Assay (%) $	100.89 ±1.22	98.21 ± 3.89

@: Also contains 0.5% w/w of Magnesium stearate, Lactose monohydrate and MCC (q.s.), 1.5% w/w of Colloidal silicon di oxide and SSG and Ac-di-sol (3% w/w of each) as other additives.
#: % w/w of the average tablet weight. $: Mean of triplicate with standard deviation. *: % variation from the mean.
a: Reange of 10 tablets.

Table 5.37: Batch reproducibility of three batches of MFH ER and GPD bi-layered tablet.

Physical properties		**MGL(500 +2) 05/02**	**MGL(500 +2) 06/02**	**MGL(500 +2) 07/02**	**Standard deviation (%)**
Average tablet weight (mg)		120.00	120.00	120.00	Not applicable
Wt. Variation (%)*		± 1.47	± 1.10	± 1.33	Not applicable
Hardness (N) [a]		280-300 (290)[b]	285-308 (293)[b]	287-315 (301)[b]	± 4.16[c]
Friability (%)		0.19	0.17	0.23%	± 0.04
f_2 values (Similarity factor)		73.40	68.11	71.59	Not applicable
Assay (%) [$]	MFH	97.03 ± 0.9	98.20 ± 1.7	99.30 ± 1.1	± 1.06
	GPD	102.0 ± 1.2	100.13 ± 1.19	101.32 ± 1.33	± 1.24

$: Mean of triplicate with standard deviation *: % variation from the mean.
a: Reange of 10 tablets. b Average taken from hardness values of 5 tablets
c Standard deviation value calculated considering average hardness values.

Table 5.38: Stability Summary report of MFH ER and GPD bi-layered tablet (500 mg +2 mg) in accelerated condition 40°C / 75% RH.

Test	Specification			Batch No.	Initial		1 month		3 months		6 months	
Appearance	Orange-white, capsule shaped tablet with both sides plain.			**MGL (500 +2) 05/02**	Complies		Complies		Complies		Complies	
				MGL (500 +2) 06/02	Complies		Complies		Complies		Complies	
				MGL (500 +2) 0702	Complies		Complies		Complies		Complies	
Cumulative release (%)	Time	MFH	GPD		MFH	GPD	MFH	GPD	MFH	GPD	MFH	GPD
	2 hours 4 hours 8 hours 12 hours	35 to 50 55 to 75 75 to 90 NLT 90	NLT 75% in 45 minutes	**MGL (500 +2) 05/02**	49 69 85 99	102.5	47 67 86 99.5	106.5	46 64 84 99	99.8	47 67 85 100	101.2
				MGL (500 +2) 06/02	47 64 87 94	100.1	46.2 70.5 84.3 104.7	103.6	50 68 86 106	103.8	49 73 84 101	105.7
				MGL (500 +2) 0702	47.9 63 86 99	101	49 68 87 99	98.5	47 67 82 100	101.2	46 64 83 99	99.8
Assay (%)	MFH	GPD		**MGL (500 +2) 05/02**	MFH	GPD	MFH	GPD	MFH	GPD	MFH	GPD
	95.0% to 105.0%				97.03	102	101.6	104.4	99.7	100.9	99.45	101.6
				MGL (500 +2) 06/02	98.2	102.2	103.1	103.6	99.6	101.9	101.6	102.9
				MGL (500 +2) 0702	99.3	103.1	97.2	102.3	99.45	101.6	99.7	100.9

Conclusion: Combination of MFH ER and GPD immediate release tablet was found to be stable for the studied period of time.

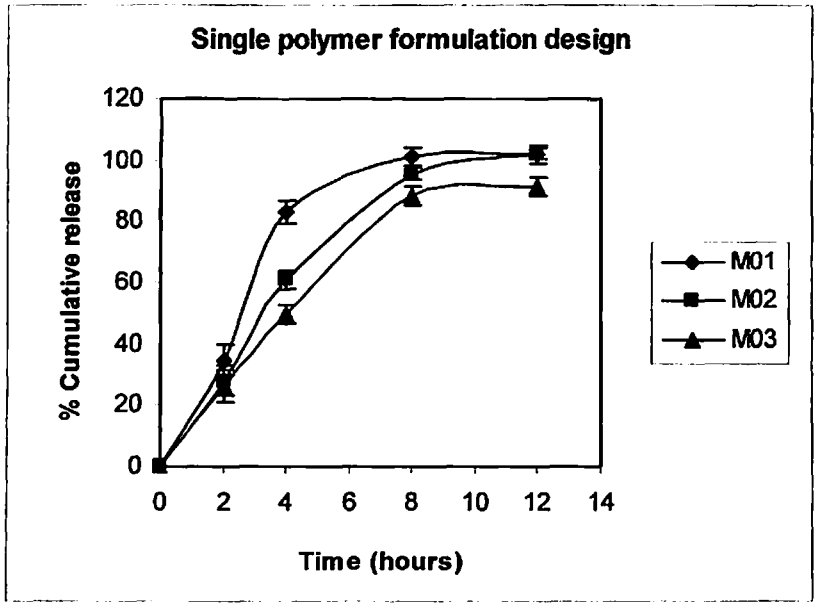


Fig 5.1: Comparative release profile of MFH ER tablets with varying quantity of HPMC K15 M.
M01: 5 % **M02:** 10 % **M03:** 25 %

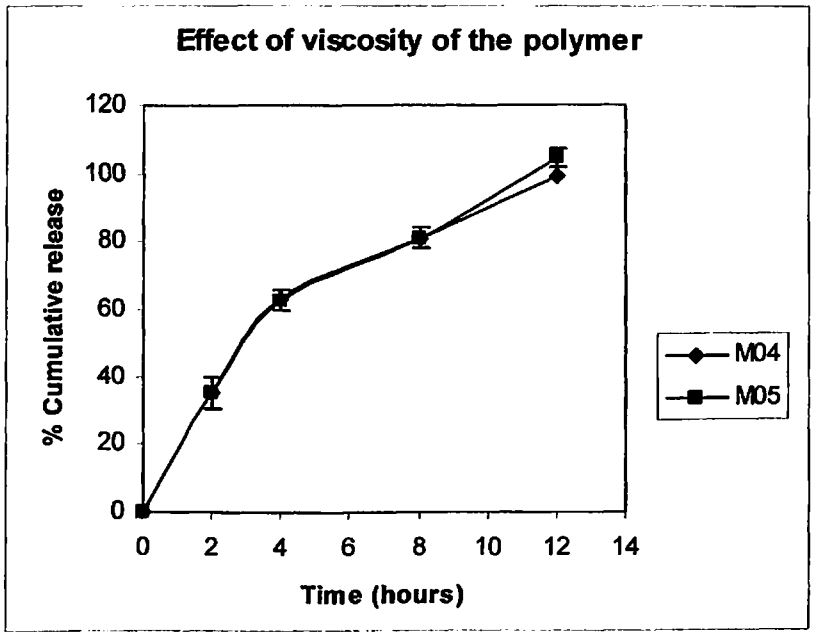


Fig 5.2: Comparative release profile of MFH ER tablets with two viscosity grades of HPMC K100M (25% w/w of average tablet weight).
M04: LV **M05:** CR

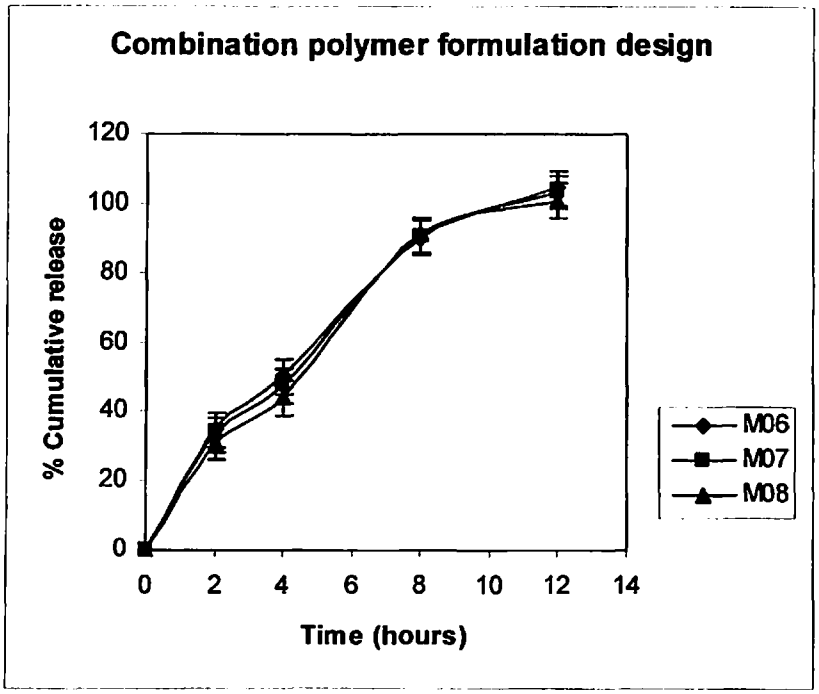


Fig 5.3: Comparison of in vitro release profile of MFH ER tablet with combination of polymers, HPMC K 15M (fixed proportion) and HPMC K 100M (varying proportion).
M06: 5 % **M07:** 10 % **M08:** 25 %

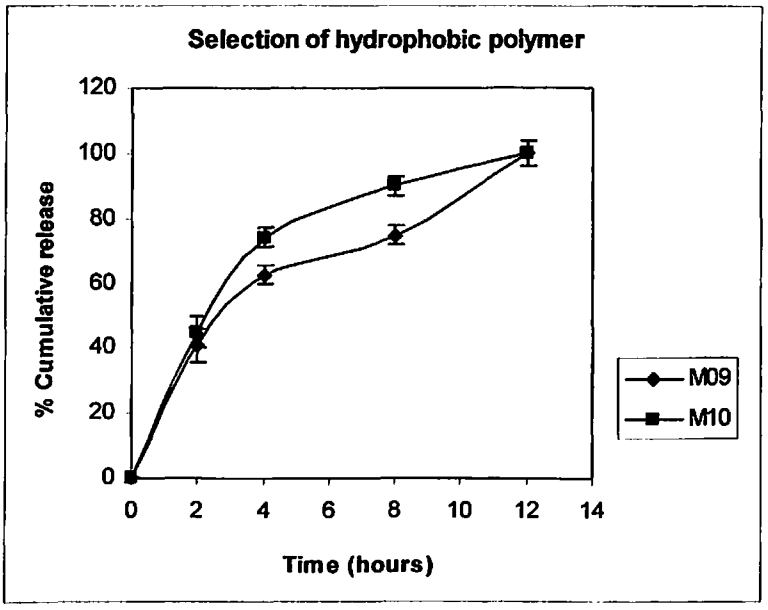


Fig 5.4: Comparison of in vitro release profile of MFH ER tablet in combination with hydrophobic polymer.
M09: 10 % w/w Carbopol 971G **M10:** 5 % HPMCP (HP 55)

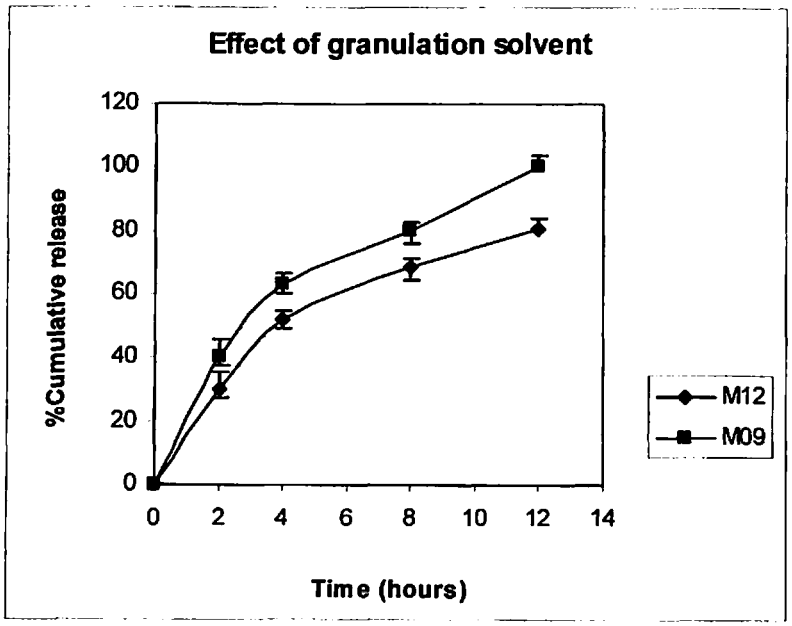


Fig 5.5: In-vitro release profile of MFH ER tablet with different granulating solvents.
M09: Non aqueous solvent (IPA) **M12:** Aqueous solvent (water)

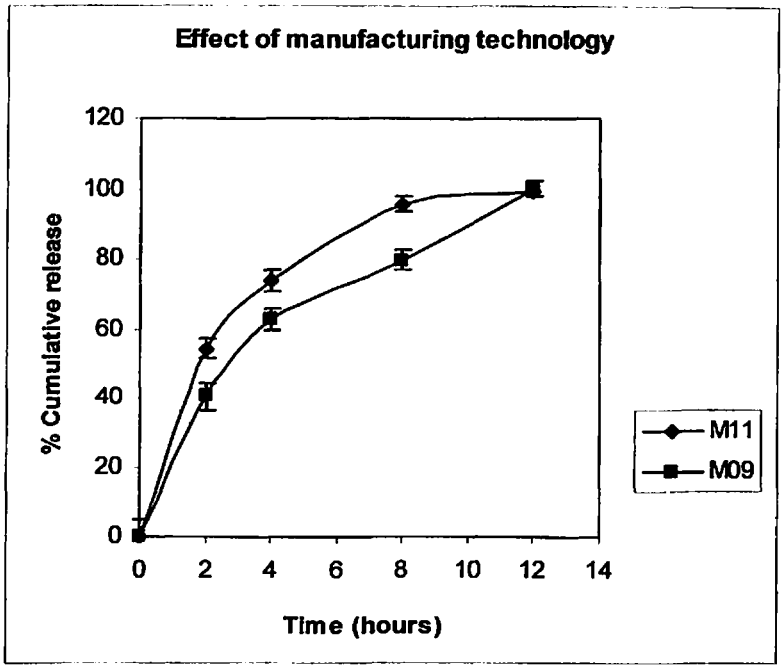


Fig 5.6: In-vitro release profile of MFH ER tablet with different granulation technologies.
M09: Wet granulation **M11:** Direct compression

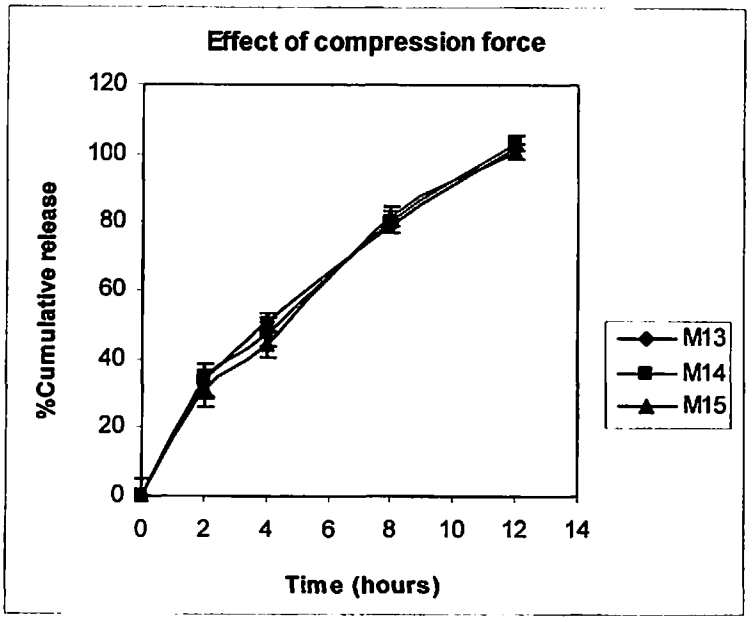


Fig 5.7: Effect of compression force on dissolution profile of MFH ER tablet.
M13: 125-150 N **M 14:** 175 –200 N **M 15:** 225 –250 N

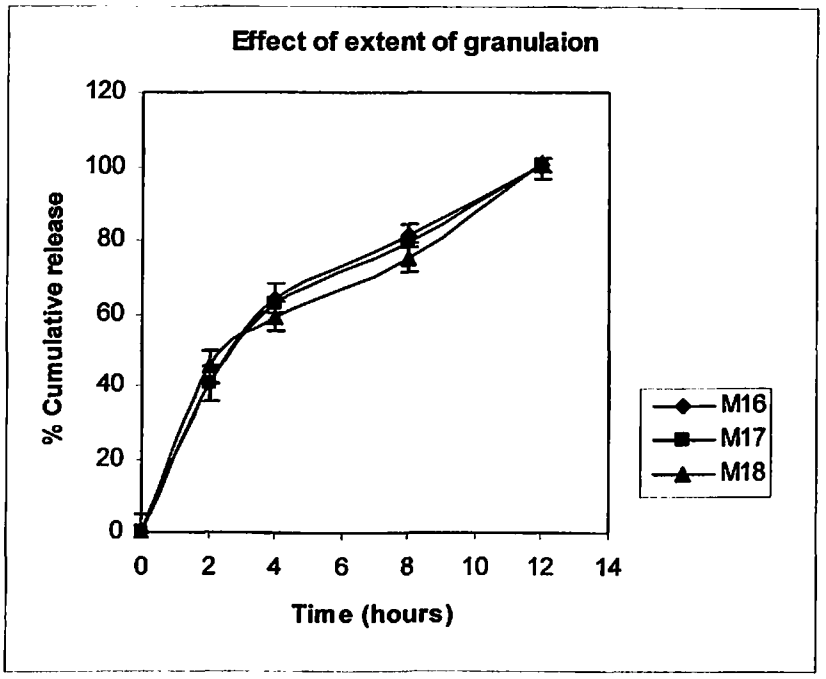


Fig 5.8: Effect of extent of granulation on dissolution profile of MFH ER tablet.
M16: light granulation **M17:** Ideal granulation **M18:** Heavy granulation

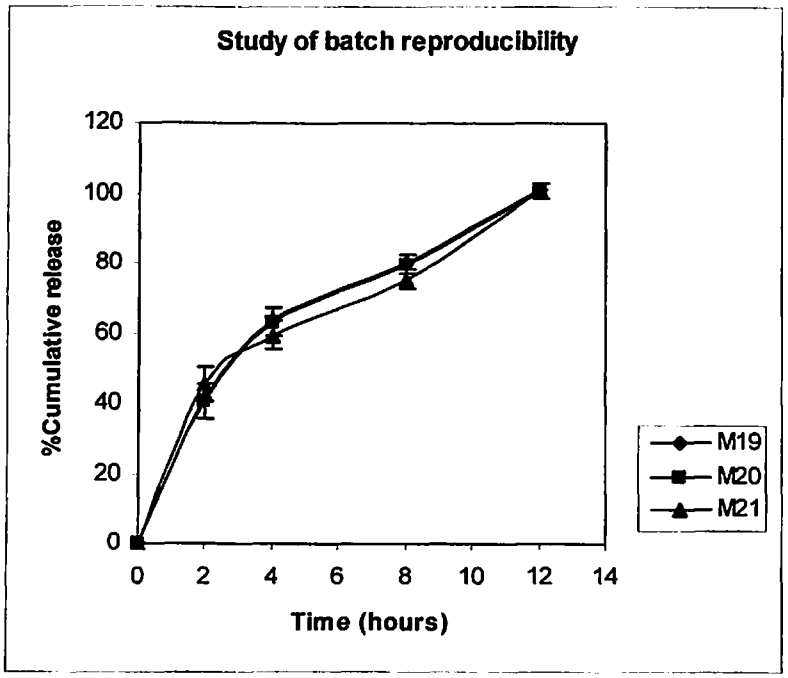


Fig 5.9: Comparative in vitro release profile of reproducible batches of MFH ER tablet. f_2 values in comparison with dissolution profile of prototype design are as follows: **M19:** 73.81 **M20:** 75.13 **M21:** 81.26

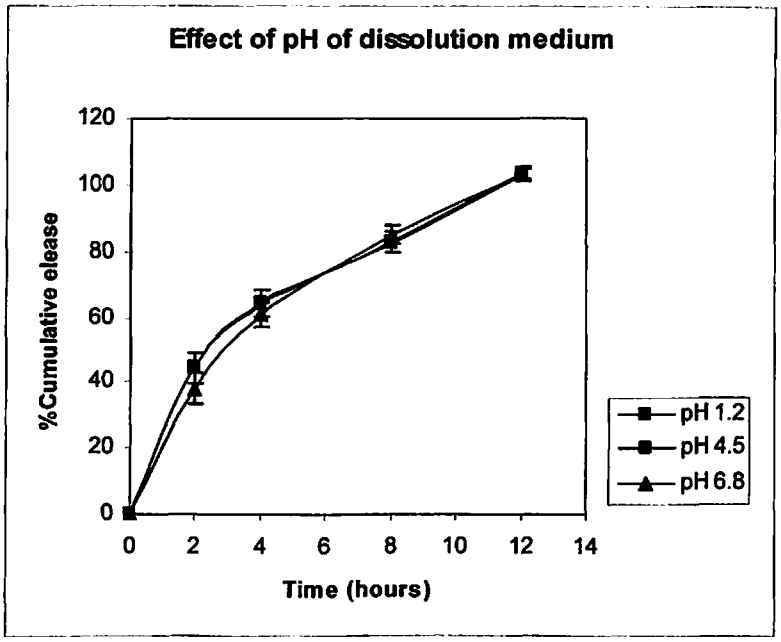


Fig: 5.10: Comparative in vitro release profile of MFH ER tablet in different pH media.

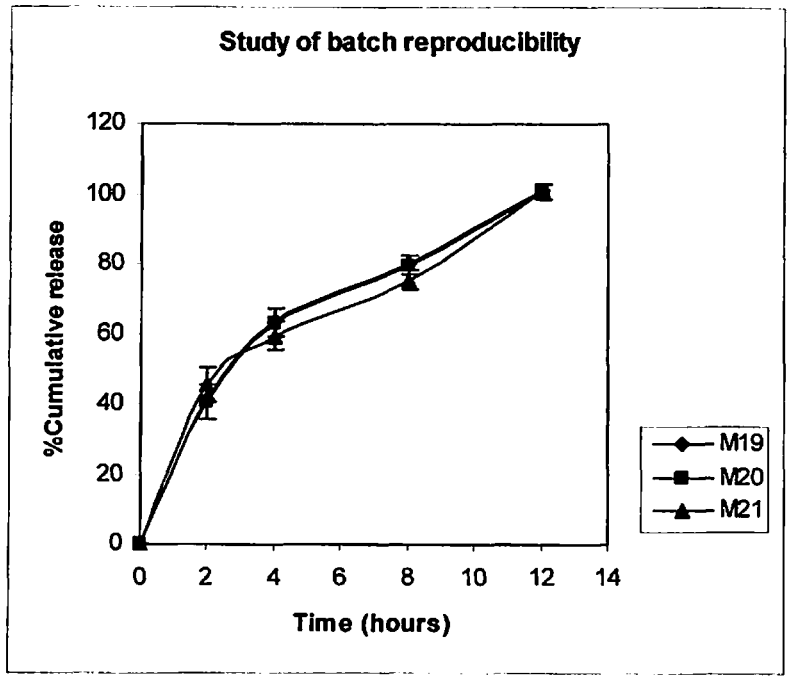


Fig 5.9: Comparative in vitro release profile of reproducible batches of MFH ER tablet. f_2 values in comparison with dissolution profile of prototype design are as follows: **M19:** 73.81 **M20:** 75.13 **M21:** 81.26

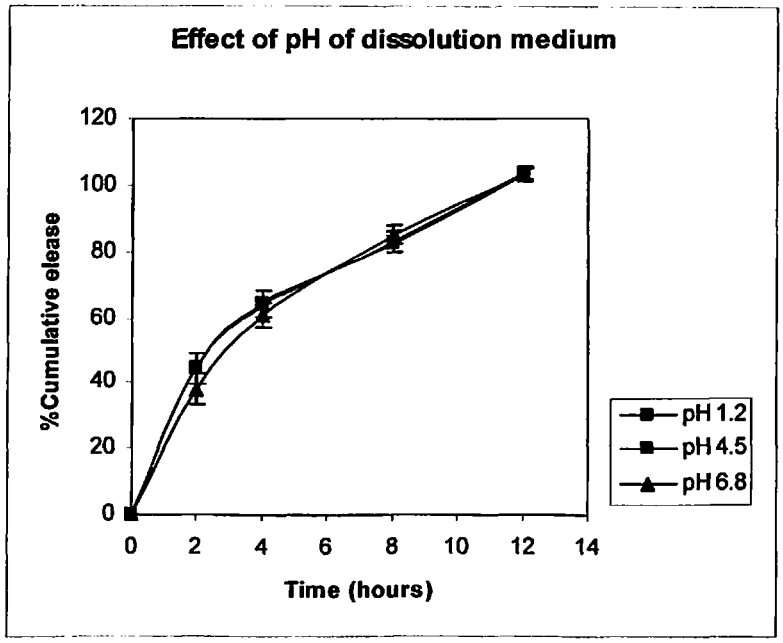


Fig: 5.10: Comparative in vitro release profile of MFH ER tablet in different pH media.

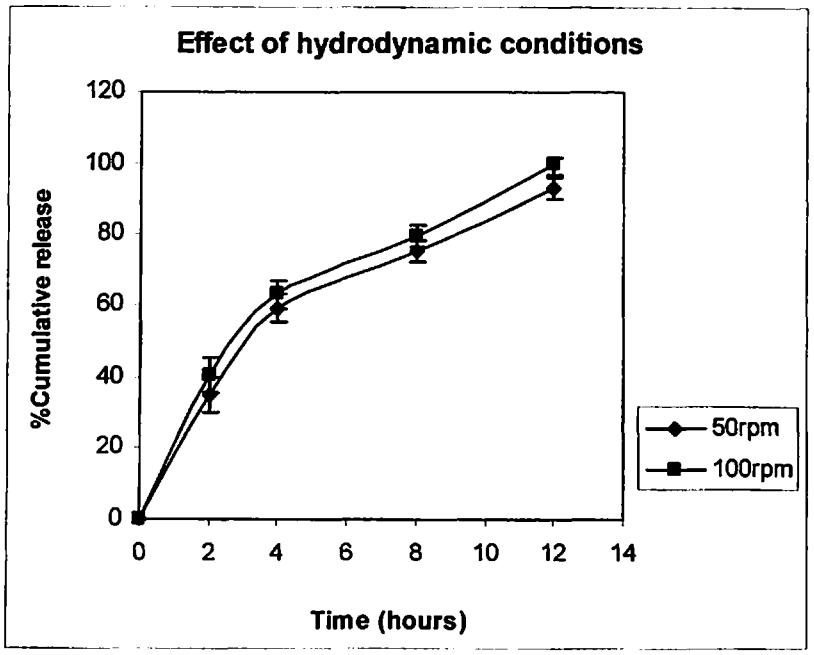


Fig 5.11: Comparative dissolution profile of MFH ER tablet with different stirring speeds.

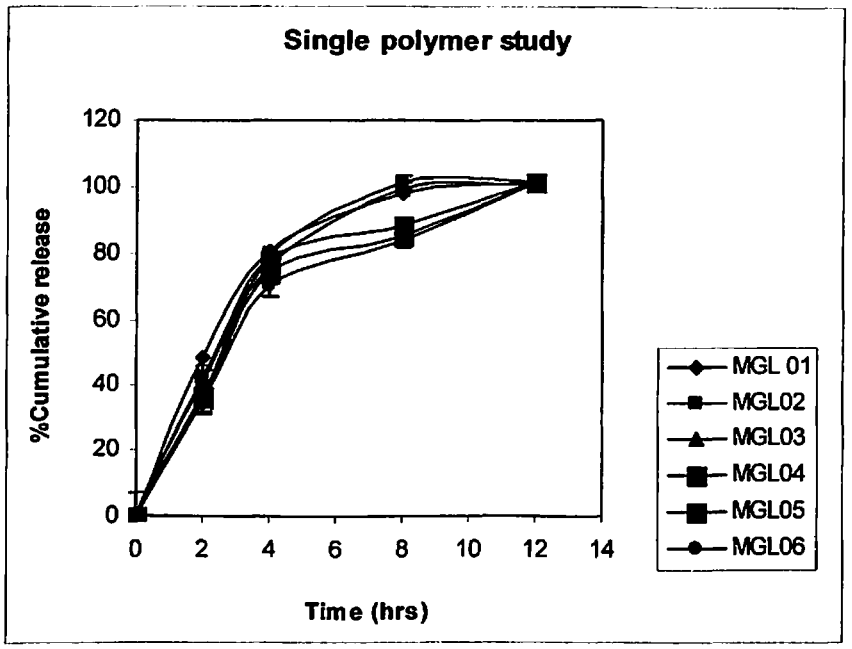


Fig 5.12: In-vitro release profile of GLZ ER tablet during single polymer study.
MGL 01:10 % w/w **MGL 02:** 20 % w/w **MGL 03:** 25 % w/w **(HPMC K4M)**
MGL 04: 10 % w/w **MGL 05:** 20% w/w **MGL 06:** 25% w/w **(HPMC K15M)**

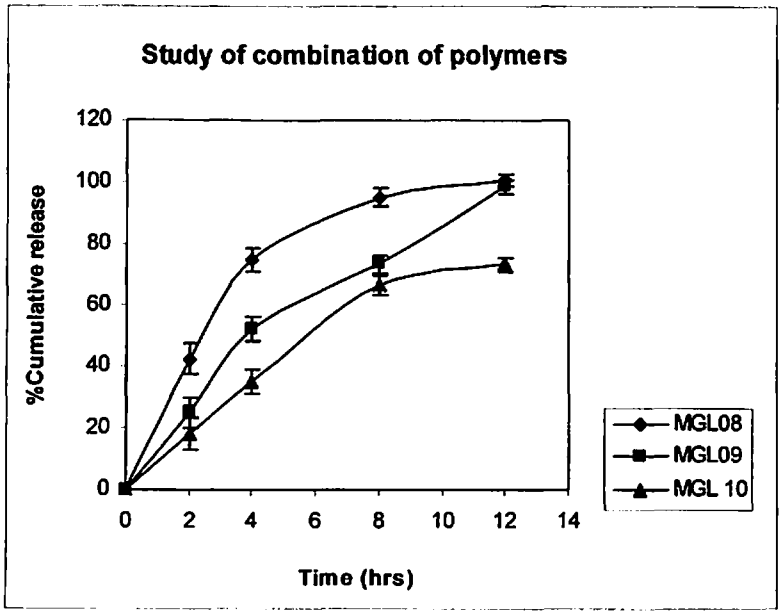


Fig 5.13: In vitro dissolution profile of GLZ ER tablet with combination of polymers, HPMC K4 M (fixed proportion) and HPMC K15M (varying proportion).
MGL 08: 5 % w/w **MGL 09:** 10 % w/w **MGL 10:** 25 % w/w

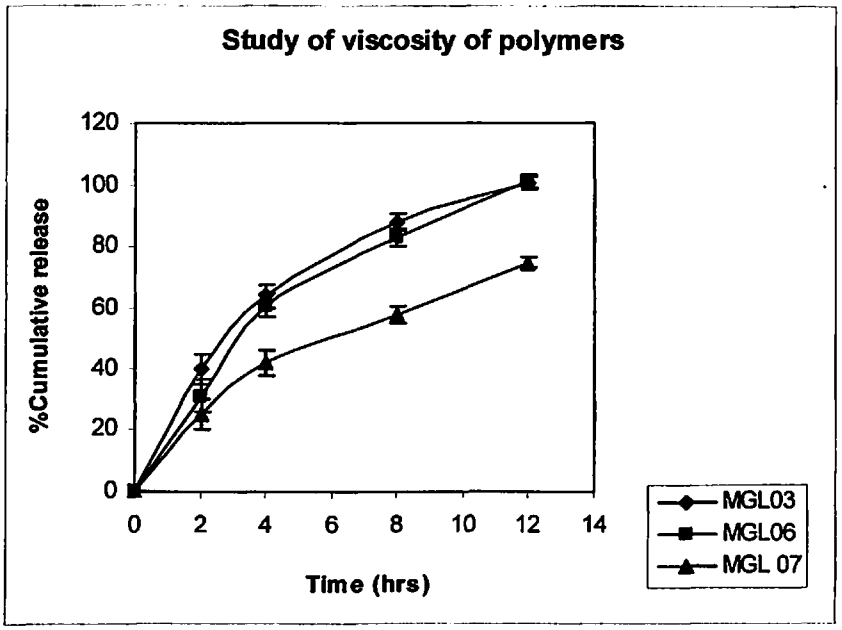


Fig: 5.14: In-vitro release of GLZ ER tablet with different viscosity grades of polymer.
MGL 03: HPMC K4 M **MGL 06:** HPMC K 15 M **MGL 07:** HPMC K 100 M

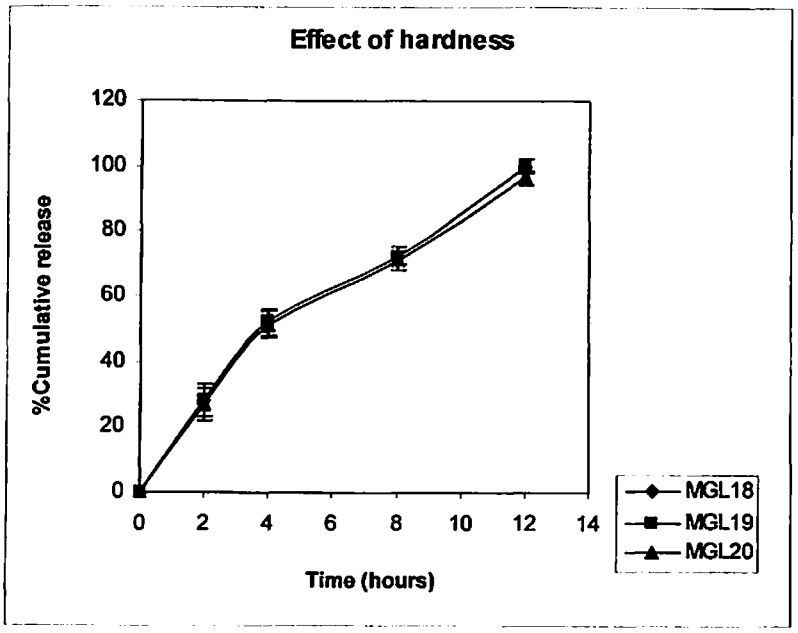


Fig 5.15: In-vitro dissolution profile of GLZ ER tablet at different hardness ranges.
MGL 18: 60N to 80 N **MGL 19:** 80N to 100 N **MGL 20:**100N to 130 N

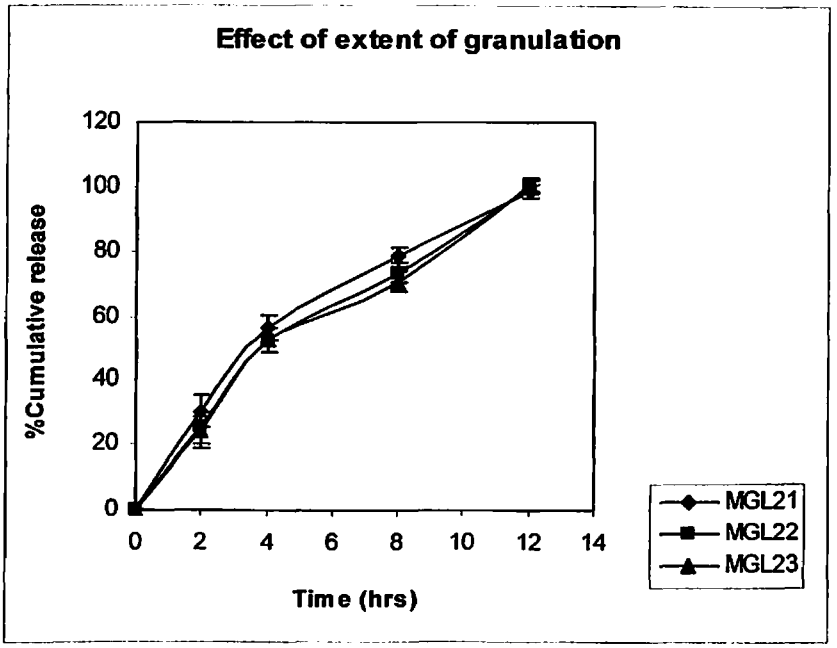


Fig 5.16: In vitro dissolution profile of GLZ extended release tablet with different granulation levels.
MGL 21: Under granulation **MGL22:** Optimum granulation **MGL 23:** Heavy granulation.

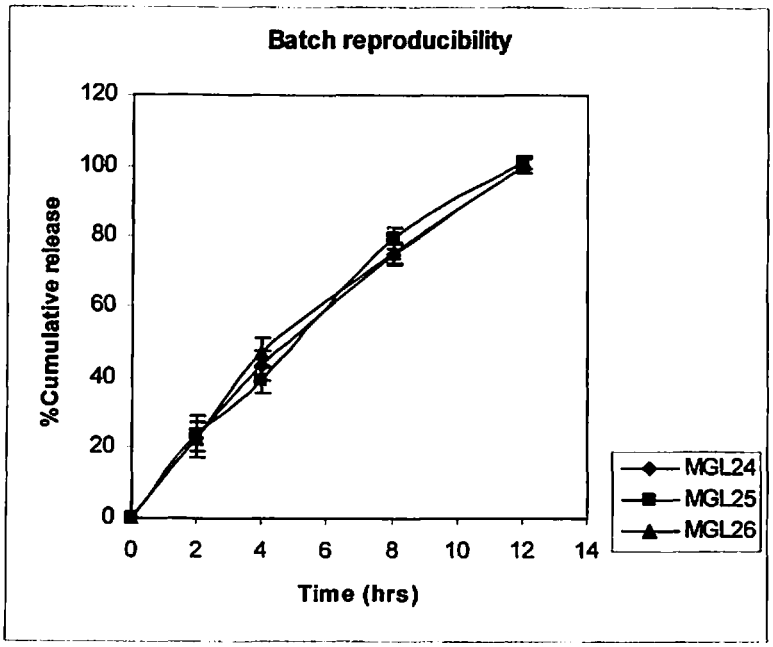


Fig 5.17: Comparative in vitro dissolution study of GLZ ER tablet of reproducible batches. f_2 values in comparison with dissolution profile of prototype design are as follows: **MGL 24:** 70.14 **MGL 25:** 71.49 **MGL 26:** 69.84

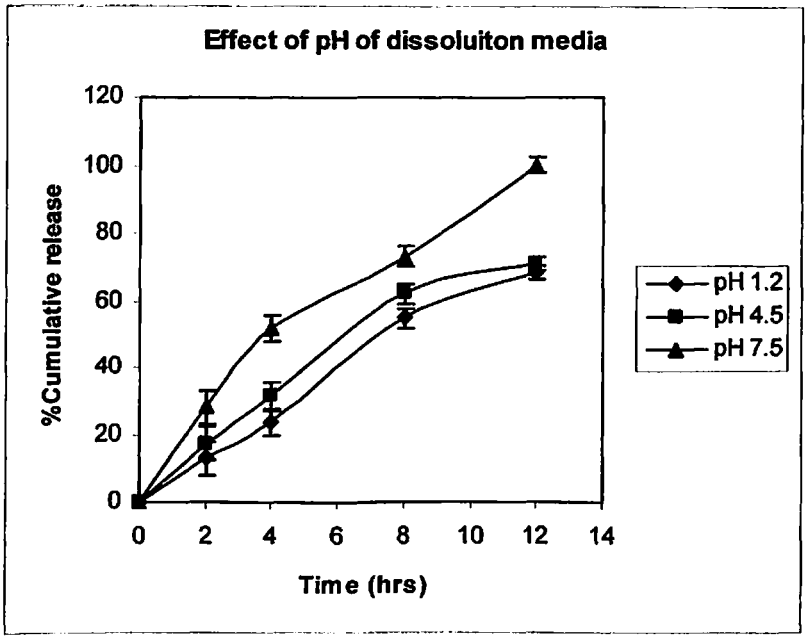


Fig 5.18: Comparison of in-vitro dissolution profile of GLZ ER tablet in media of different pH.

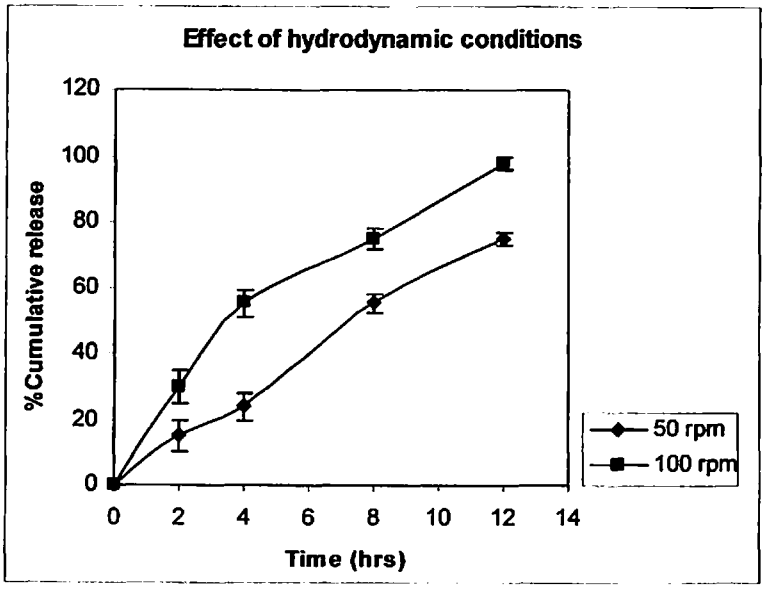


Fig 5.19: Dissolution profile of GLZ ER tablet at different stirring speeds of paddle.

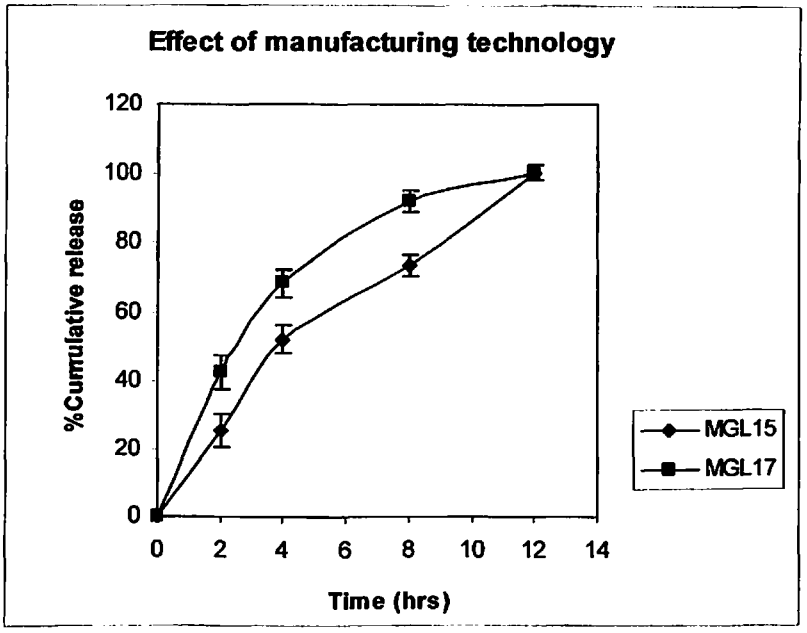


Fig 5.20: In-vitro dissolution profile of GLZ ER part with different manufacturing technologies.
MGL 15: Wet granulation **MGL 17:** Direct compression

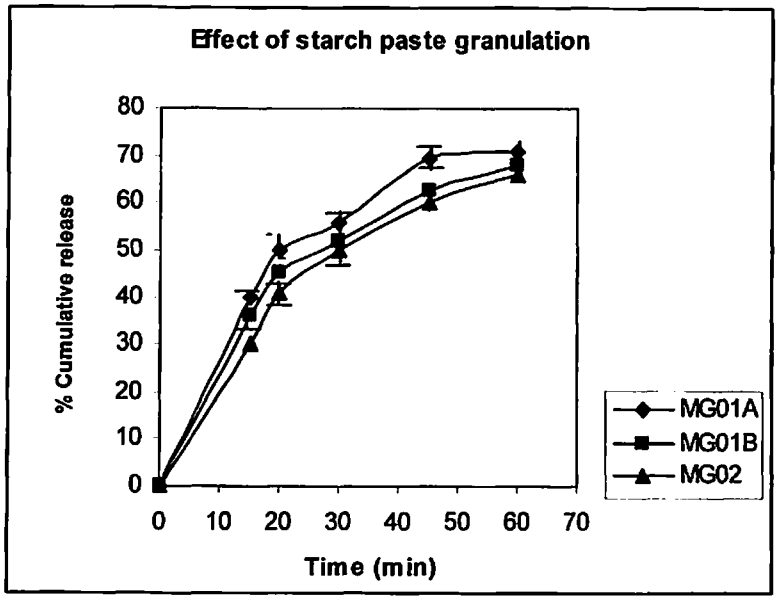


Fig 5.21: In vitro dissolution profile of GPD part with starch paste granulation with different proportion.
MG01A: 1 % starch **MG01B:** 3 % starch **MG 02:** 6% starch

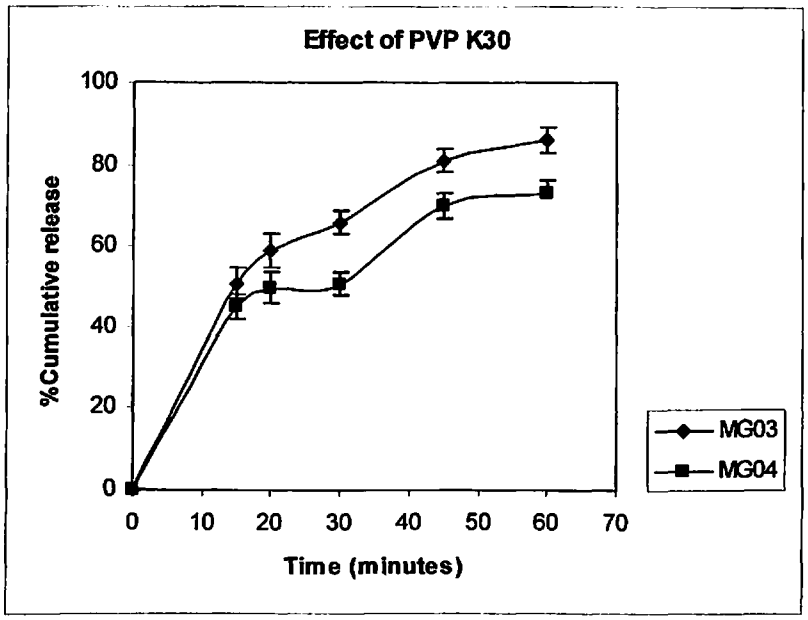


Fig 5.22: In vitro dissolution profile of GPD tablet with different proportions of PVP K30. **MG03:** 2 % PVP K30 **MG04:** 4 % PVP K30

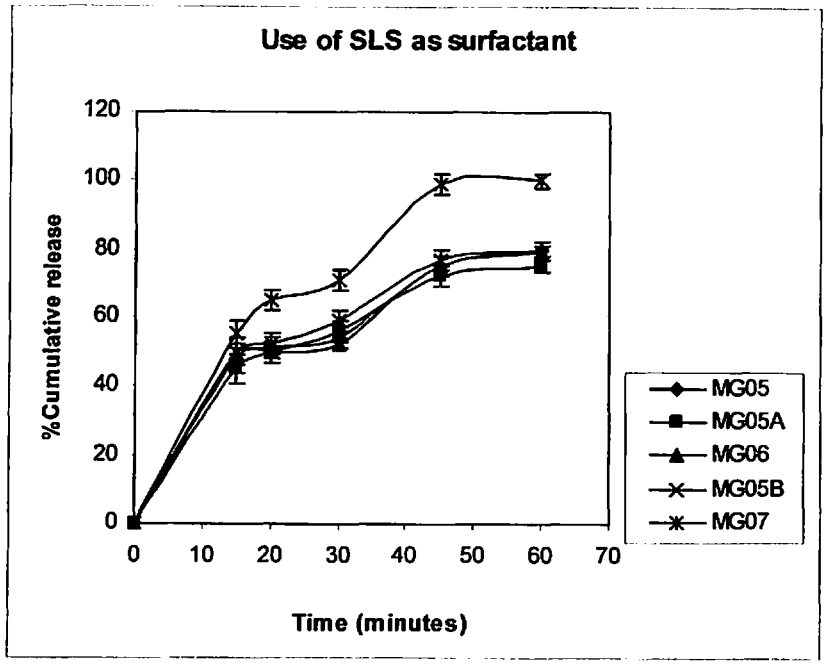


Fig 5.23: In-vitro dissolution profile of GPD tablet with different proportion of (SLS). **MG05:** Intra-granular 1% w/w **MG06:** Extra granular 1% w/w **MG07:** Intra-granular 0.5% w/w and extra granular 0.5% w/w **MG05A:** 0.5% SLS **MG05B:** 1.5% SLS

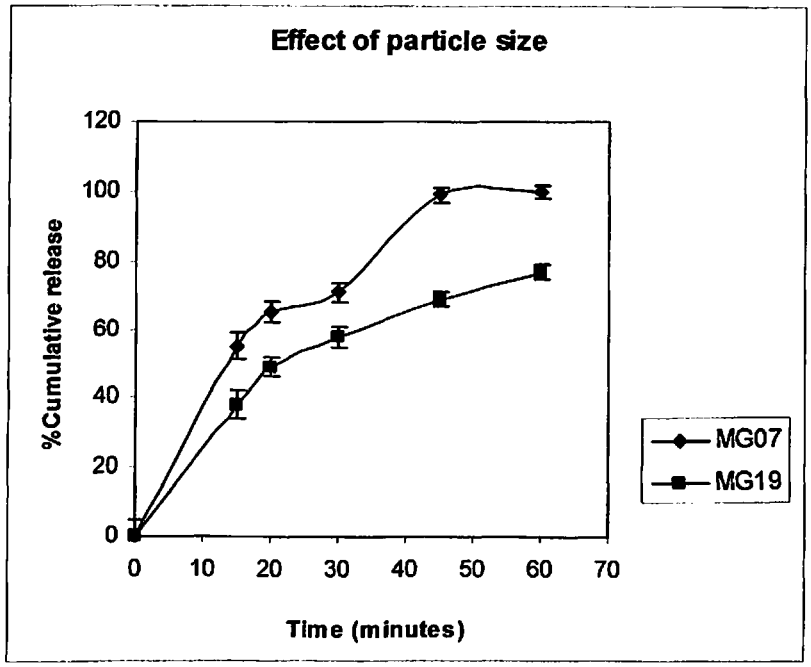


Fig 5.24: Comparative dissolution profile of GPD tablet with GPD raw material of two different particle size distributions. **MG 07:** D_{90}=18.29μ **MG19:** D_{90}= 98.09μ

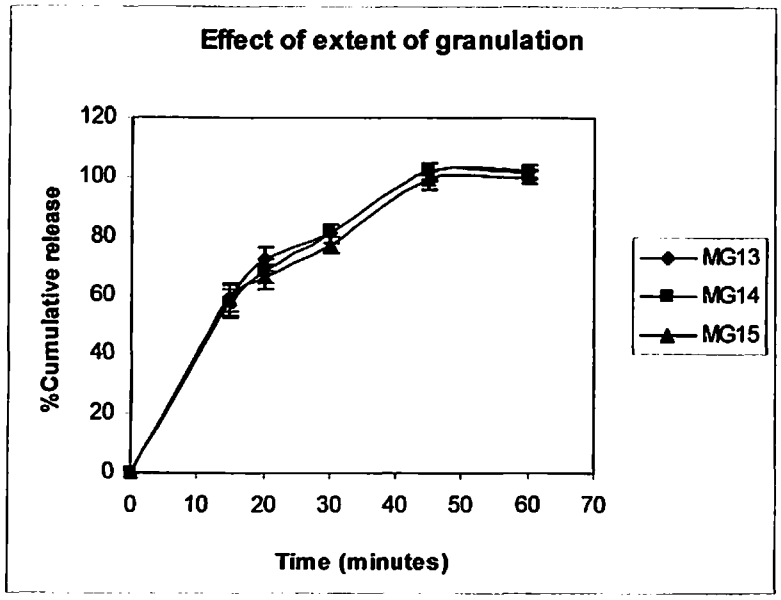


Fig 5.25: Effect of extent of granulation on dissolution profile of GPD tablet.
MG 13: Light granulation **MG14:** Optimum granulation **MG 15:** Over granulation

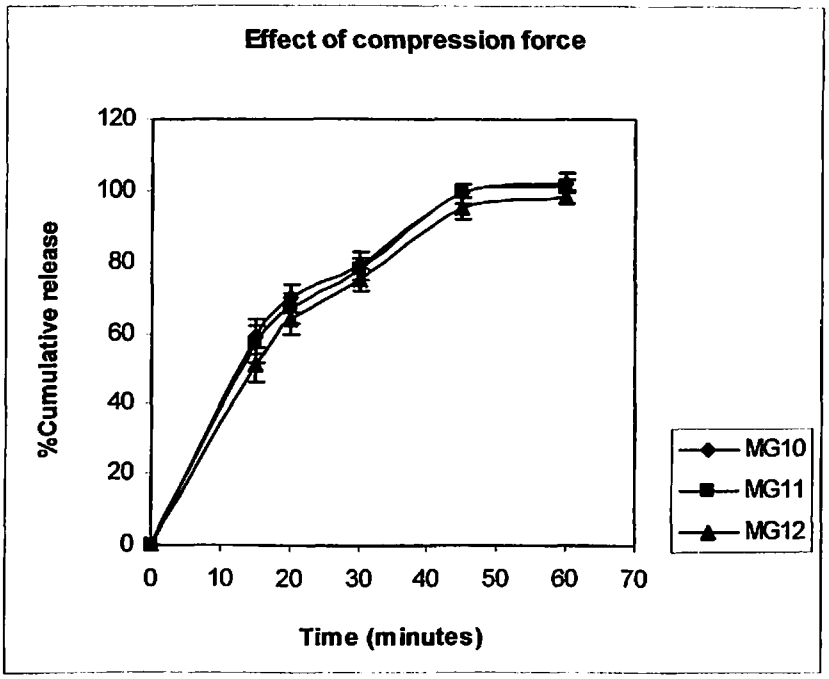


Fig 5.26: In-vitro dissolution study of effect of compression force on GPD tablets.
MG10: 40-60N; **M G11:** 60-90 N; **M G12:** 90-110 N

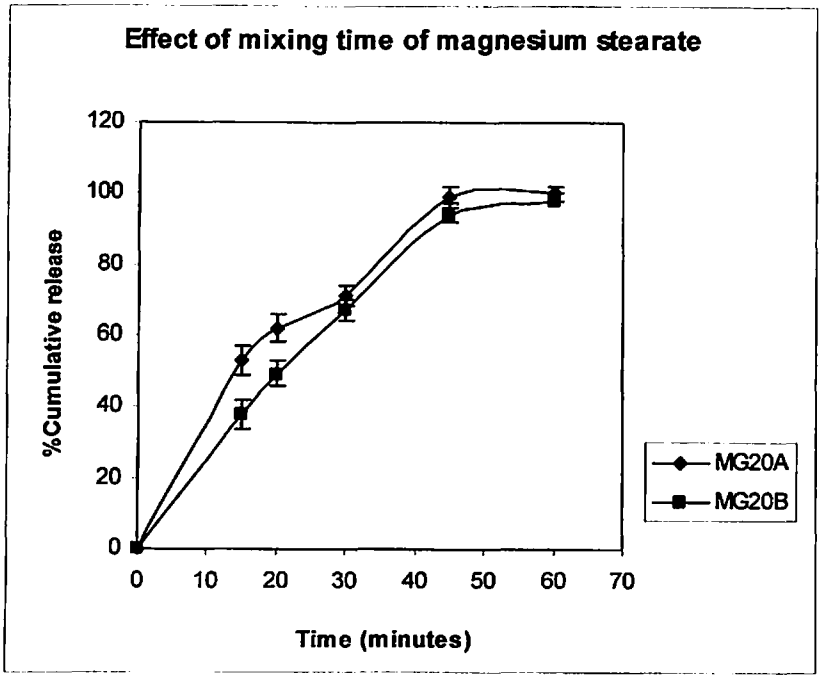


Fig 5.27: Effect of increase in mixing time of Magnesium stearate on dissolution profile of GPD tablets.

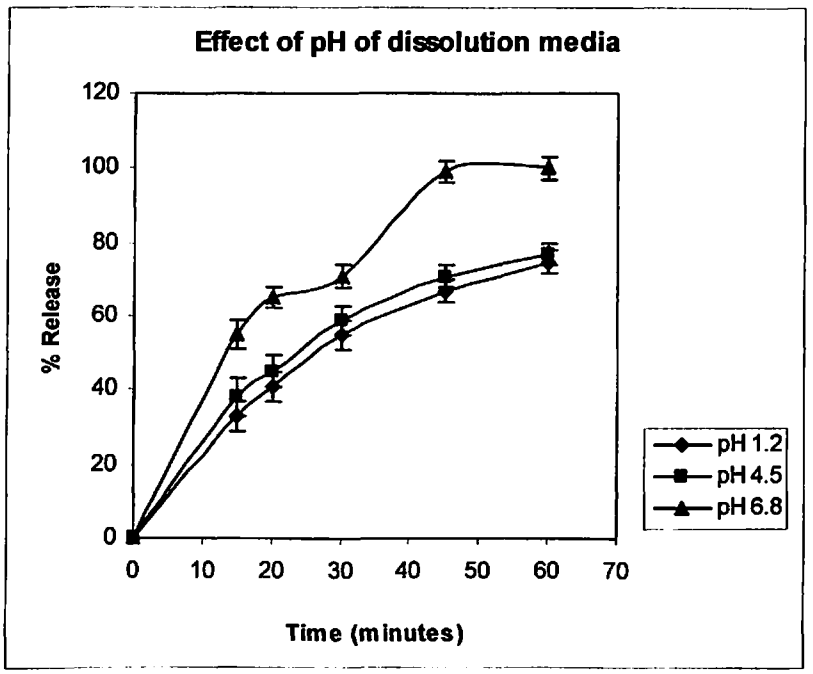


Fig 5.28: Effect of pH of dissolution media on in-vitro release profile of GPD IR tablet.

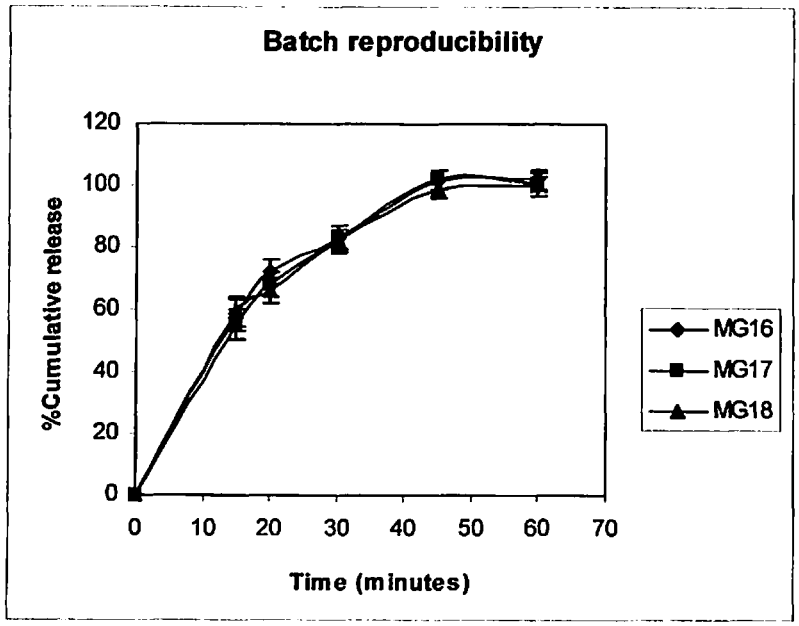


Fig 5.29: Comparative in vitro dissolution profile of reproducible batches of GPD tablet.
MG 16: AYC4001F **MG 17:** AYC4002F **MG 18:** AYC40033F
f_2 values in comparison with dissolution profile of prototype design are as follows:
MG 16: 71.86 **MG 17:** 68.33 **MG 18:** 68.88

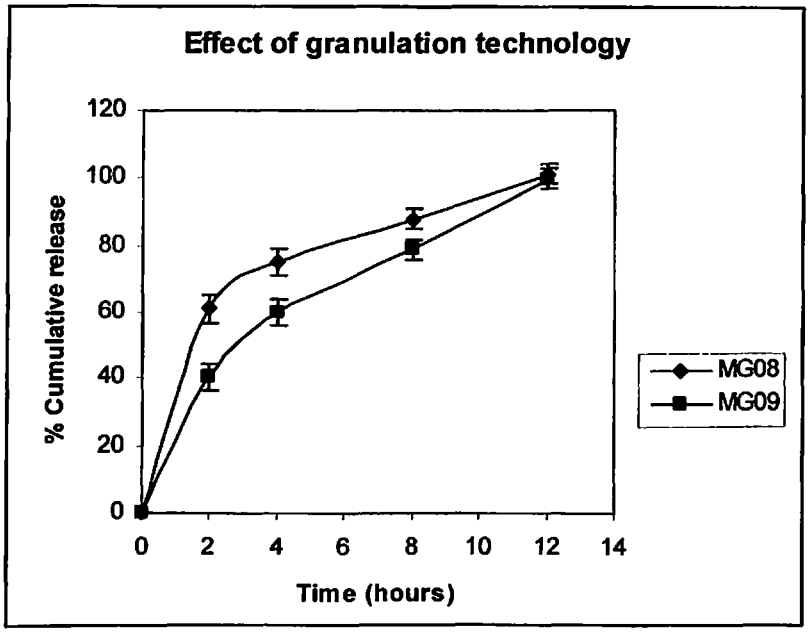


Fig.5.30: Effect of granulation technology on release profile of MFH ER layer of MFH ER and GPD IR bi-layered tablet.
MG 08: Direct compression **MG 09:** Wet granulation

Chapter 6

Pharmacokinetic and Bioavailability studies

Introduction

The ultimate therapeutic success of the dosage form is attributed to the intrinsic activity of the drug integrated with the steady and effective delivery at the site of action [122, 123]. The pharmacological response of the drug can be correlated with the concentration of drug at the site of action and its duration of stay which is always represented by drug in the systemic circulation. The goal of any drug therapy is to produce effective therapeutic concentration at the site of action to provide the desirable pharmacological effects without undesirable toxicological effects. The process of drug delivery includes the administration of the dosage form, the release of the drug from dosage form, and transport of the drug across the biological membranes to circulation and to the site of action. The drug delivery systems play a critical role in this sequence of events that provides the drug to the site of action and maintain its concentration throughout the treatment [124-126]. Thus, increasing the duration and extent of drug delivery to the site of action would improve the efficiency of the drug delivery system leading to earlier onset of action and better therapeutic effect with minimal or no toxic effects.

While, the controlled drug delivery systems provide advantages of reduce dosing frequency with techniques such as sustained, prolonged and extended release over the conventional drug delivery systems, it is always essential to investigate and establish the rate and extent of drug availability through the rational approaches like in-vivo pharmacokinetic studies [127-129]. The application of pharmacokinetic principles in the controlled drug delivery systems provides the rational for determining the time course of the drug absorption, distribution, metabolism and excretion along with the study of relationship of these unique biological processes to the intensity and time course of therapeutic and adverse effects of these drugs [130-132].

Administration of two different formulations of the same drug and of same dose does not necessarily produce the same therapeutic response. In order to establish the clinical significance of the controlled drug delivery systems over the conventional drug delivery systems, it is essential to investigate and study the comparative relationship between these two drug delivery systems in-terms of the drugs elemental pharmacokinetic parameters [133,134]. The controlled release drug delivery systems can be claimed as the clinical alternative to the existing conventional drug delivery systems, only if it is

providing better therapeutic effect or at least exhibit the therapeutic equivalence with some added advantage(s) [135-137]. The assessment of the therapeutic equivalence for different drug products is based on the fundamental bioequivalence assumption which states that "when two drug products are equivalent in the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed and becomes available at the site of drug action, it is assumed that they will be therapeutically equivalent". Application of pharmacokinetic principles is critical to the development of any drug delivery system as their clinical efficacy may significantly differ despite their same in-vitro characteristics and drug contents [138,139].

It is planned to justify the clinical significance of the designed and developed extended release drug products against the existing conventional formulations, after administration of their respective single dose in a well-planned design of a randomized two-way, two-period, two-treatment cross-over bioavailability study [140-143]. The essential pharmacokinetic parameters used for the comparison are AUC $_{(0\text{-}24)}$, C_{max} and t_{max}, of the therapeutic moiety as per FDA regulations for an in-vivo bioavailability study [144-146]. Each study was carried out under standard GCP and GLP environment after prior approval of protocols from Human Ethics Committee. Study was performed in healthy, adult, male, human volunteers and, before admission, in the study, each volunteer was informed of the nature and the risks of the study and a written informed consent was obtained (Annexure 3). The subjects were in sitting posture and were not allowed to lie down for three hours following each administration. Abnormal signs or symptoms during the study period and after were monitored and recorded in the study report.

Materials and Chemicals

The developed formulations of MFH Extended Release (ER) tablet (500mg of MFH manufactured by Ipca Laboratories Ltd., Mumbai), MFH Immediate Release tablet (IR) (500mg of MFH, Manufactured by Ipca Laboratories Ltd., Mumbai), newly developed combination formulation of MFH ER and GLZ ER tablets (500 mg of MFH, and 60 mg of GLZ manufactured by Ipca Laboratories Ltd., Mumbai), GLZ IR tablets (60 mg of GLZ, manufactured by Ipca Laboratories Ltd., Mumbai) were used for the study.

6.1 Bioavailability studies: Extended Release tablets of MFH

6.1.1 Experimental

Study title

An open label randomized two-way, two-period, two-treatment cross-over comparative bioavailability study was carried out, in twelve healthy adult human male subjects under fasting conditions, of the developed formula of extended release MFH tablets (500 mg of MFH manufactured by Ipca Laboratories Ltd., Mumbai) in comparison with existing MFH Tablets IR (500 mg of MFH, manufactured by Ipca Laboratories Ltd., Mumbai).

Aims and objectives:

The aim and the objective of the study were to evaluate the pharmacokinetic parameters and to compare the bioavailability of two preparations, and also to determine the effectiveness of the developed ER tablet.

Study center:

All the bioavailability and pharmacokinetic studies were carried out at contract research organization (CRO), Lambda Therapeutic Research Pvt. Ltd, Ahmedabad, as Ipca Laboratories Ltd. does not have such study facilities. Lambda Therapeutic Research Pvt. Ltd. has accreditation from various regulatory authorities such as DCGI (India), WHO, European authorities and USFDA.

Subjects selection:

Twelve healthy, adults, human male volunteers of the mean age 25.83 ± 4.38 years and mean weight 58.33 ± 9.07 kg were selected based on predefined selection criteria [147-151]. Volunteers were screened for inclusion in the study within 21 days before the commencement of the study. After fulfilling the selection criteria as per the protocol, they were allocated to the treatment A/B (reference or test preparation) in accordance with the generated randomization code.

Study design:

Open label, balanced, randomized, two-treatment, two-period, two-sequence, single dose, crossover, comparative oral bioavailability study in healthy, adult, male, human subjects under fasting conditions [152-154].

Investigational products:

Test product: Newly developed MFH ER tablet 500mg

Reference product: Existing MFH IR tablets 500mg

Dose administered:

It was ensured that all subjects fasted for at least 10 hours prior to the drug administration. The investigational products were administered to the subjects with 240ml of drinking water while the subjects were in sitting posture. The subjects were not allowed to lie down for three hours following the drug administration. Compliance to dosing was assessed by trained study personnel immediately after the dose administration.

Wash out period: Two weeks, between the two study periods.

Blood collection times:

Pre-dose and at 0.5, 1.0, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0 and 24.0 hours post drug administration [155-158].

Drug analysis:

Drug concentrations in plasma were analyzed using a validated HPLC-UV method as per analytical method described in Chapter 3. A calibration curve extending over the range 25.11 to 1207.95 ng/ml with a LOQ of 25.11 ng/ml was used in subject sample analysis of MFH.

Pharmacokinetic parameters:

The pharmacokinetic parameters, t_{max}, C_{max}, and AUC_{0-24}, were calculated by non-compartmental models, using WinNonlin Professional Software-Version 4.0.1 (Pharsight Corporation, USA).

Statistical methods:

Statistical analysis, to calculate C_{max}, and AUC_{0-24}, was carried out by using PROC GLM of SAS software, Version 8.2. Wilcoxon Mann Whitney one sided test was performed to assess t_{max}.

6.1.2 Results and discussion

Pharmacokinetic parameters

No serious adverse events occurred during the study. There was no drop out and all the volunteers completed the study successfully. The average values (n=12) of the pharmacokinetic parameters for both reference and test formulations have been tabulated in Table 6.1. The t_{max} value for test product was found to be higher (5.6 ± 1.4 hours) as compared with reference product (3.5 ± 0.7 hours) confirming the in vivo extended release of MFH. The C_{max} value for test was found to be on lower side (550 ± 130 ng/ml) as compared with the reference formulation (740 ± 180 ng/ml) as expected in case of extended release product. Lower C_{max} value of test formulation reiterates one of the main rationales for developing extended release formulation. The mean AUC_{0-24} value of MFH in the extended release formulation was found to be approx. 15% higher (6120 ± 1710 ng.h/ml) than its conventional release formulation (5330 ± 1400 ng.h/ml) indicating higher bioavailability of MFH from ER tablet. ER tablet also produced longer duration (Fig.6.1).

6.2 Bioavailability studies: Extended Release tablets of MFH and GLZ as a single tablet.

6.2.1 Experimental

Study title

An open label randomized two-way, two-period, two-treatment cross-over comparative bioavailability study was carried out in healthy adult human male subjects under fasting conditions, of a newly developed single combination tablet of MFH ER and GLZ ER tablets (500mg of MFH and 60 mg of GLZ, manufactured by Ipca Laboratories Ltd., Mumbai) in comparison with MFH ER tablet (500mg of MFH, manufactured by IPCA Laboratories Ltd., Mumbai) and existing GLZ IR tablet (60 mg of GLZ, manufactured by Ipca Laboratories Ltd., Mumbai).

Aims and objectives

The aim and the objective of the present study was to evaluate the pharmacokinetic parameters and to compare the bioavailability of combination tablet of MFH ER 500 mg and GLZ ER 60 mg (Ipca Laboratories Ltd., Mumbai) with previously studied MFH ER 500mg tablet (Ipca Laboratories Ltd., Mumbai) and existing GLZ IR 60 mg tablet (Ipca Laboratories Ltd., Mumbai), as unit dose administered together in twelve healthy human volunteers in a randomized, two way complete crossover design.

Subjects selection

Twelve healthy, adults, human male volunteers of the mean age 24.13 ± 3.27 years and mean weight 59.17 ± 8.64 kg were selected based on predefined selection criteria [159-163]. Volunteers were screened for inclusion in the study within 21 days before the commencement of the study. After fulfilling the selection criteria as per the protocol, they were allocated to the treatment A/B (reference or test preparation) in accordance with the generated randomization code.

Study design

Open label, randomized, two-treatment, two-period, two-sequence, single dose, crossover, comparative oral bioavailability study in healthy, adult, male, human subjects under fasting conditions [164-167].

Investigational products:

Test product: Newly developed combination tablet of MFH ER and GLZ ER (MFH 500mg and Glicalzide 60mg).

Reference products: MFH ER 500mg tablet

Conventional GLZ 60mg tablet.

Dose administered:

It was ensured that all subjects fasted for at least 10 hours prior to the drug administration. The investigational products were administered to the subjects with 240ml of drinking water while the subjects were in sitting posture. The subjects were not allowed to lie down for three hours following the drug administration. Compliance to dosing was assessed by trained study personnel immediately after the dose administration.

Wash out period: Two weeks, between the two study periods.

Blood collection times:

Pre-dose and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0 and 24.0 hours post drug administration [168-171].

Drug analysis

Drug concentrations in plasma were analyzed using a validated HPLC-UV method as per the analytical methodology described in Chapter 3. A calibration curve extending over the range 0.102 to 8.020 μg/ml with a LOQ of 0.102 μg/ml was used in subject sample

analysis of GLZ and a calibration curve extending over the range 25.11 to 1207.95 ng/ml with a LOQ of 25.11 ng/ml was used in subject sample analysis of MFH.

Pharmacokinetic parameters:

The pharmacokinetic parameters, t_{max}, C_{max} and AUC_{0-24} were calculated by non-compartmental models, using WinNonlin Professional Software-Version 4.0.1.

Statistical methods:

Statistical analysis, to calculate C_{max}, and AUC_{0-24}, was carried out by using PROC GLM of SAS software, Version 8.2. Wilcoxon Mann Whitney one sided test was performed to assess t_{max}.

6.2.2 Results and discussion

Pharmacokinetic parameters for GLZ ER:

No serious adverse events occurred during the study. There was no drop out and all the volunteers completed the study successfully. The average values (n=12) of the pharmacokinetic parameters for both reference and test formulations of GLZ ER have been tabulated in Table 6.2. The t_{max} value for GLZ of test product was found to be higher (9.10 ± 3.20 hours) as compared with that of reference product (3.61 ± 1.148 hours) confirming the in vivo extended release of GLZ. The C_{max} value for GLZ of test product was found to be on lower side (2.24 ± 0.55 µg/ml) as compared with the reference formulation (4.89 ± 1.19 µg/ml). Lower C_{max} value of test formulation reiterates one of the main rationales for developing extended release formulation.

The AUC_{0-24} value of GLZ in extended release form was found to be nearly same (50.89± 18.41µg.h/ml) to that of immediate release formulation (47.40 ± 15.90µg.h/ml).

All the three pharmacokinetic parameters (t_{max}, C_{max} and AUC_{0-24}) of MFH ER tablet remained practically unaltered, statistically insignificant as shown in Table 6.3 and Fig.6.3 when administered alone or in combination with GLZ IR or with GLZ ER. Administration of GLZ did not affect the bioavailability of MFH ER tablets. This suggests that presence of GLZ did not interfere absorption rate and bioavailability of MFH.

6.3 Conclusion

In all subjects, delivery was reproducible and prolonged release was observed from MFH ER in comparison to its IR formulation. For all subjects, C_{max} was reduced in the designed MFH ER tablets as expected from an extended release product.

Absorption of MFH is non-linear (saturable and site specific). Considering this fact, the improvement in relative bioavailability of MFH ER can be explained by its availability in gastrointestinal tract for prolonged period of time.

The pharmacokinetic parameters of MFH ER remain unchanged when administered alone or in combination with GLZ ER (co-administration) and from single unit dosage tablet.

In summary the use of MFH ER tablet to restrict MFH delivery to the small intestine resulted in reproducibly enhanced bioavailability and extended plasma concentration-time profiles relative to those of IR formulation of MFH.

Similar results were also achieved in GLZ ER tablet wherein there was an increase in t_{max}, reduction in C_{max} and without much increase in bioavailability. In summary it can be concluded that the designed formulation found to be useful for the purpose it is designed.

Table 6.1: Pharmacokinetic parameters of MFH IR (Reference) tablet and MFH ER tablet (Test) formulations.

Parameters	Units	Mean ± SD	
		MFH ER (Test)	MFH IR (Reference)
t_{max}	hours	5.6±1.4	3.5±0.7
C_{max}	ng/ml	550±130	740±180
AUC_{0-t}	ng.h/ml	6120±1710	5330±1400

Table 6.2: Pharmacokinetic parameters of GLZ IR (Reference) tablet and GLZ ER (Test) tablet.

Parameters	Units	Mean ± SD	
		GLZ ER (Test)	GLZ IR (Reference)
t_{max}	hours	9.10±3.2	3.61 ± 1.148
C_{max}	ng/ml	2240.1±551	4891.5 ± 119.02
AUC_{0-t}	ng.h/ml	47401.0±15901.0	50890.25 ± 18410.1

Table 6.3: Pharmacokinetic parameters of

a) MFH ER tablet when administered separately

b) In combination with GLZ IR and

c) Single dosage unit of MFH ER + GLZ ER tablet.

Parameters	Units	Mean ± SD		
		MFH ER (Met ER 1)	MFH ER + GLZ IR (Met ER 2)	MFH ER + GLZ ER (Met ER 3)
t_{max}	hours	5.6±1.4	5.1±0.53	5.4±0.9
C_{max}	ng/ml	550±130	596±154	551±159
AUC_{0-t}	ng.h/ml	6120±1710	6095±1250	6080±1510

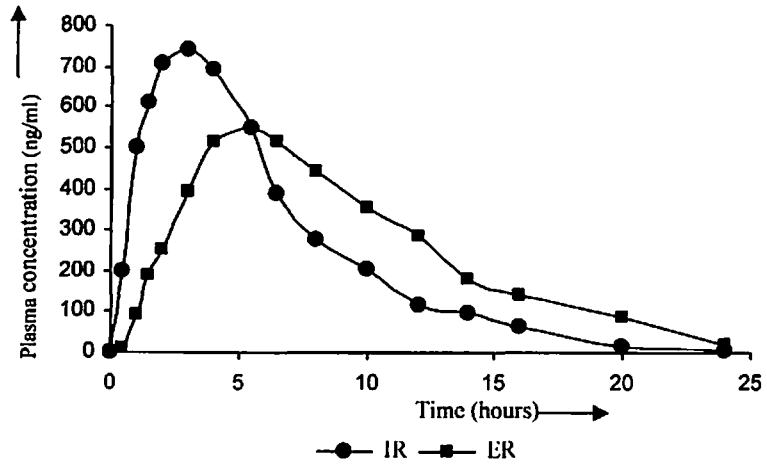


Fig 6.1: MFH concentration vs. time curves after administration of MFH IR and MFH ER.

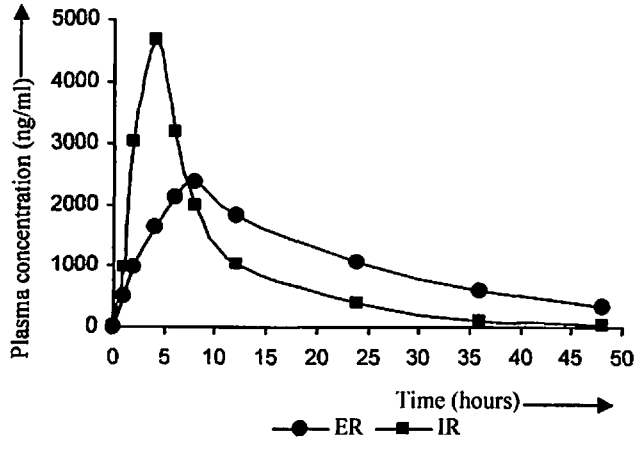


Fig 6.2: GLZ concentration vs. time curves after administration of GLZ IR and GLZ ER.

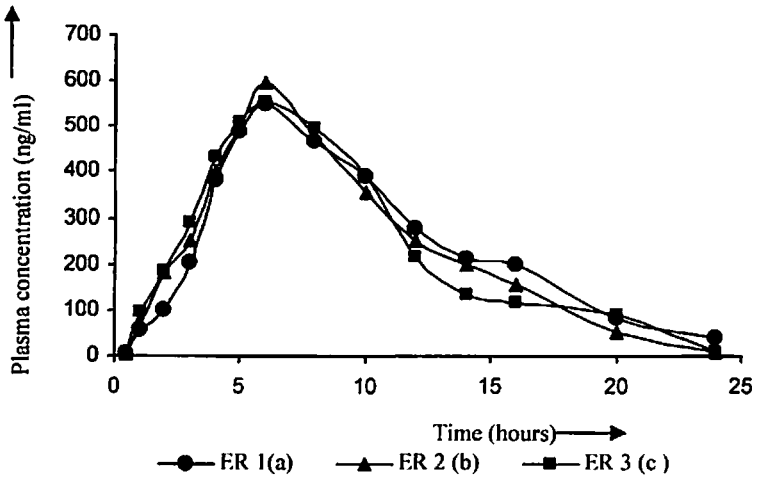


Fig 6.3: MFH concentration vs. time curves when,

a) MFH ER tablet administered separately

b) In combination with GLZ IR and

c) Single dosage unit of MFH ER + GLZ ER tablet.

Chapter 7

Conclusion and Future Plan

Conclusion

Rapid change in the life style has lead to phenomenal increase in occurrence of life style related diseases. Diabetes has become fourth leading cause of death with type 2 diabetes, the most prevalent type. As occurrence of diabetes, particularly type 2 diabetes, is expected to increase enormously this research work was aimed at designing and developing a stable and efficacious platform technology for metformin (MFH) extended release tablet, as well as its combination with other anti-diabetic drugs like gliclazide (GLZ) and glimepiride (GPD) in a single dosage unit for better patient compliance.

Oral controlled release delivery systems were developed for MFH alone and in combination for improved therapeutic performance, which can help in better glycemic control and can also offer various advantages like maximized drug therapeutic indices, reduced side effects and adverse effects, reduced dosing frequency, reduced cost of treatment, improved stability, better availability of drug, along with better patient compliance and improved quality of life.

As required for various purposes, HPLC analytical methods, for estimation of the drugs individually and in a combination were developed. All the methods were validated in accordance with ICH guidelines and were found to be simple, accurate, precise, sensitive and economic. Developed methods were suitable for conducting various pre-formulation, formulation and in-vivo pharmacokinetic studies.

The preformulation studies showed that all the three drugs were stable with most commonly used excipients and release retarding polymers used for the project work. The drugs also exhibited good stability in usual experimental and manufacturing conditions. All the three drugs showed pH-dependant stability. MFH showed good stability in acidic media whereas GLZ and GPD showed better stability in slightly alkaline medium. MFH was found to be highly water soluble, whereas, GLZ and GPD were practically insoluble in water and had high pH dependant solubility. The solubility study suggested that all the three drug substances have maximum solubility in alkaline media. GPD was found to precipitate in acidic pH due to its poor solubility in acidic media.

The platform technology was developed for MFH extended release tablet and was used as a stepping stone for preparing a single dosage unit tablet for rational use of various anti-diabetic drug combinations, either with immediate release GPD or extended release GLZ.

The study of release profile indicated that designed formulations of both MFH ER tablets and GLZ ER tablets showed first order release except, GLZ ER tablets with 25% HPMC K15M showed nearly zero order release profile. Bi-layered tablet technology proved to be the most feasible and efficient platform technology as compared with other various technologies like, drug loading and tab-in-tab technology, etc. The accelerated stability studies carried out on the developed formulations proved that the formulations are stable with respect to physical parameters, drug content and dissolution profile. There was no interference of one drug on the stability and release profile of the other.

The pharmacokinetic and bioavailability study was carried out at Contract Research Organization (CRO), Lambda Therapeutic Research Centre., Ahmedabad. The single dose pharmacokinetics of MFH ER tablet was compared with the currently marketed immediate release MFH tablet, using cross over design. It can be concluded from the studies that mean bioavailability from MFH ER tablet was sufficiently higher, relative to immediate release product. Lower C_{max} value and extended t_{max} values indicated the sustained release character of MFH ER formulation. It was also concluded that GLZ ER tablet has good sustained release property when combined with MFH ER tablet in a single unit dosage tablet. Moreover there was no interference in the pharmacokinetics parameters when drugs were administered alone and in combination.

On the basis of above study commercial formulations were also developed.

Future plan

In future, the developed platform technology can be combined with other identified rational anti-diabetic drugs (one or two more) in multiple-layered tablets (bi-layered or tri-layered tablets). The same platform technology can be used for combining other strengths of the same drugs or combining drugs of other therapeutic categories e.g. anti-hypertensive drugs. Tri-layered technology can be used for combining more than two drugs.

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Appendices

Patents and Presentations made on the Doctoral Research Work

As the work taken up under this project was for commercial purpose, emphasis was given on patent filing as priority instead of publication. Number of patents has been applied at concept level or at midway of the work to protect the products, process and its technological aspects. In some case, provisional applications were made at the stage of idea generation to take advantage of priority date of filing and then complete application was filed after completion of the experiment work. Following patent applications were made related to research work.

[1] Process for preparation of controlled release anti-diabetic formulations. Indian Patent No. 194218; Bansal Y.K. and others. Work is on a new process for Gliclazide Novel Drug Release system (extended release formulation) (Process patent).

[2] Process for multiple release anti-diabetic drugs. Indian Patent No. 202763: Bansal Y.K. and others. Work is related to a process of dual release formulation for Metformin and Glimepiride with extended release of Metformin and immediate release of Glimepiride (Process patent).

[3] A controlled release anti-diabetic formulation. Indian Patent Application No. 890/MUM/2004: Bansal Y.K. and others. Work is on design of controlled release formulation of Gliclazide (Product patent).

[4] A multiple release anti-diabetic pharmaceutical tablet composition. Indian Patent Application No. 889/MUM/2004: Bansal Y.K. and others. Work is related to novel formulation containing Metformin and Glimepiride with controlled release of Metformin and immediate release of Glimepiride (Product patent).

[5] A dual release pharmaceutical formulation. Indian Patent Application No. 895/MUM/2004: Bansal Y.K. and others. This work relates to a dual release pharmaceutical formulation comprising extended release of Metformin and immediate release of Thiozolidinedione (Product patent).

Papers presented at Workshop/Conferences:

Following presentations are made in various conferences on the research topic:

[1] "Technological Advancements on Anti-diabetic Drugs"; World Health Organization (WHO) at Geneva, October 2006.

[2] "Design and Development of Extended Release Formulations of Anti-diabetic Drugs"; IBC Life sciences conference at Tokyo, February 2006.

[3] "Industrial Approach to Develop Novel Anti-diabetic Drug Formulation"; UNICEF at Denmark, December 2005.

Products introduced in the Market

Following products, based on the outcome of research work, have been introduced in the market, by Ipca Laboratories Ltd., Mumbai, after necessary approvals from Drug Controller General of India (DCGI and FDA authorities).

S. N.	Brand Name	Generic Name	Label Claim
1	Glyree M2	Glimepiride & extended release metformin hydrochloride tablets	**Each uncoated tablet contains:** Glimepiride USP.....2mg Metformin Hydrochloride IP..... 500mg (in extended release form) Colour: Lake of sunset Yellow FCF
2	Emnorm CR 500	Metformin hydrochloride extended release tablets	**Each extended-release tablet contains:** Metformin Hydrochloride USP... 500mg
3	Glycinorm – M 60 MR	Gliclazide modified release and metformin hydrochloride extended release	**Each uncoated bilayered tablet contains:** Gliclazide BP.....60mg (as extended release form) Metformin Hydrochloride IP..... 500mg (as extended release form)

BIOGRAPHY OF YATISH KUMAR

Mr. Yatish Kumar completed his M.Pharm from Panjab University, Chandigarh. Formulation scientist by training, he is presently heading R&D (Formulation), Ipca Laboratories Ltd. He is carrying a profound research experience of more than 22 years from the top-notch Pharmaceutical Industries in India like Ranbaxy Laboratories Ltd., Dabur pharmaceuticals and Cadila group of companies. He has worked on almost all dosage forms like, Parentral drug delivery system, Liquid orals and Solid oral dosage forms. He carries a keen interest and expertise for development of novel drug delivery systems for solid oral dosage forms. He has acquired more than 15 national and international patents during his career. He is also an honorary and adjunct faculty member of BITS, Pilani and guided several M.Pharm and B.Pharm students for their research work during Practice School and continuing to do so.

BIOGRAPHY OF Prof. RANENDRA N. SAHA

Dr. R.N. Saha is Professor of Pharmacy and Dean, Faculty Division III and Educational Development Division, BITS, Pilani. He obtained his B.Pharm and M.Pharm (Pharmaceutics) degrees from Jadavpur University, Kolkata and Ph.D. from BITS, Pilani. He has more than 25 years of teaching and research experience and guided several doctoral students, M.Pharm and B.Pharm students. He has many publications in international and national journals of repute and presented papers in international and national conferences in India and abroad. He has successfully completed several government and industry sponsored projects and continuing so. Dr.Saha has developed commercial products for industries and transferred technologies of production to industries. He is expert member to various committees of UGC and other agencies and selection committee members of CSIR laboratories and several universities / colleges. He is also a member of Board of Studies of several universities / colleges and Visiting Professor to a few universities.

ANNEXURE 1

Common controlled, modified, delayed and extended release coating polymers

Generic name	Soluble in	Properties
Non –enteric coats		
Ethyl cellulose	Ethanol, IPA, organic solvents.	Low viscosity aqueous films.
Hydroxy ethyl cellulose	GI fluids, Water.	Low viscosity aqueous films giving clear solutions.
Hydroxy Propyl Methyl Cellulose	GI fluids, Water, Ethanol, Methylene chloride.	Component in swelling or eroding polymers/matrixes.
Carbopol	GI fluids, Water, organic solvents 971 P.	Adjuvant as film modifiers / coat plasticizers.
Enteric coats		
Cellulose acetate	IPA, Acetone, Ethyl acetate, alkalies.	Dissolves in distal end of duodenum.
Hydroxy Propyl Methyl Cellulose Phtalate	IPA, Acetone and Alkalies with pH>4.5.	Dissolves in proximal end of duodenum.
Methacrylic acid co-polymer	pH>6 and pH>7.	Solubilizes in alkali media. Combinations used as enteric coating plus sustained release.
Polyvinyl acetate phthalate	IPA, Acetone, alkalies pH>5.	Dissolves in full length duodenum.

ANNEXURE 2

Different grades of Eudragit

Grade of Eudragit	Solubility	Application
Eudragit E-	Soluble in gastric fluid below pH 5	insulating film former
Eudragit L 100	Soluble at pH > 6	used as enteric coating agents
Eudragit S 100	Soluble at pH > 7	used as enteric coating agents
Eudragit RL, RS,* and *NE 30 D	Swellable polymer	water-insoluble film coats for sustained-release products

ANNEXURE 3

Volunteer Consent Form

Lambda *ICF*
Project No. 012-03

DECLARATION

- I have read, attended the presentation of the Informed Consent Form and understood the information provided above about the drug and procedures to be used in this study.
- I have been explained, all the relevant matters of the study, to my satisfaction.
- I have been informed about the nature and the purpose of the procedure, the benefits and the risks that are involved in their performance and the risk and potential side effects associated with the drug to be used in this study.
- I understand that it is my responsibility to ask questions to clarify any points, which I do not clearly understand.
- I have been provided with contact numbers of the persons to be contacted in this regard.
- I have not hidden any information regarding my medical history and have stated my correct date of birth.
- I am 18 year old or more and am eligible for giving consent on my own.
- I understand that the study I am participating is for research purposes only.
- I hereby authorise the designated staff of Lambda Therapeutic Research Pvt. Ltd. to perform the procedures of the study.
- I hereby authorise Lambda Therapeutic Research Pvt. Ltd. to check my eligibility for participation in the study.
- I also authorise the release, for any lawful purpose, of any information or data obtained or generated in connection with this study except personal information.
- I authorise Lambda Therapeutic Research Pvt. Ltd. to disclose the matters pertaining to my identity only in case of legal necessities.
- I shall not indulge myself in any unlawful activities or shall not cause any harm or damage to the property of Lambda Therapeutic Research Pvt. Ltd. I am aware that I am liable for suitable action in case of any misconduct on my part.
- I am aware that this participation will not fetch me any medical benefit.
- I am also aware that my blood samples will have to be withdrawn for the study and I know that this is meant for research purposes only.
- I am aware that I can opt out from the study at any time during the course of the study even without giving any reason for doing so, and without affecting my right to project related medical care, and future voluntary participation in research studies.

M:\clinical\ABA-DE STUDIES\PROJECTS-CLINICAL\Projects
Subject's signature:

I, the undersigned, Mr., :

(Your full name)
Residing at (address) :

after applying my free power of choice, give my consent for my inclusion as a subject in the Bioavailability study of metformin hydrochloride tablets. I have received a copy of this consent form and have understood its contents.

Signature of the subject :

Date :

Full Name of the witness :

Address :

Signature of witness :

Date :

(For Lambda's use only)

Signature of the study person involved in informed consent discussion :

ALLOTTED SUBJECT NO.: ☐

☐ Principal investigator
or
☐ Medical Expert
(Please tick (√) as applicable)

Date