CHAPTER-3

METFORMIN HCl,
GLIPIZIDE & PREPAGLINIDE
IN PHARMACEUTICAL DRUG PRODUCTS
3.1. INTRODUCTION

This chapter covers the development and validation of RP-HPLC method for the determination of Metformin Hydrochloride, Glipizide and Repaglinide in bulk drugs and dosage forms.

3.2. METFORMIN HCL

Metformin is an oral medication that lowers blood glucose (sugar) and is used for treating type-II diabetes. Insulin is a hormone produced by the pancreas that controls glucose levels in blood by reducing the amount of glucose made by the liver and by increasing the removal of glucose from the blood by muscle and fat tissues. As a result, blood glucose levels fall. Diabetes caused by a decrease in production of insulin that causes increased production of glucose by the liver and reduced uptake (and effects) of insulin on fat and muscle tissues. Metformin acts by increasing the sensitivity of liver, muscle, fat and other tissues to the uptake and effects of insulin. These actions lower the level of sugar in the blood.

Metformin is an oral diabetes medicine that helps control blood sugar levels. Metformin is for people with type-II (non-insulin-dependent) diabetes. Metformin is sometimes used in combination with insulin or other medications, but it is not for treating type-1 diabetes. The structure of Metformin represented in figure-3.1.

![Chemical structure of Metformin](image)

**Fig-3.1: Structure of Metformin**

**Chemical details:**

- **Class**: Anti-diabetic drug in the biguanide
- **Chemical name**: Metformin
- **IUPAC name**: 1-carbamimidamido-N, N-dimethylmethanimidamide
- **Molecular formula**: C₄H₁₁N₅
- **Molecular weight**: 129.16
- **CAS NO.**: 657-24-9
Chemistry

The usual synthesis of Metformin, originally described in 1922 and reproduced in multiple later patents and publications, involves the reaction of dimethylamine hydrochloride and 2-cyanoguanidine (dicyandiamide) with heating. According to the procedure described in the 1975 Aron patent and the Pharmaceutical Manufacturing Encyclopedia, equimolar amounts of dimethylamine and 2-cyanoguanidine are dissolved in toluene with cooling to make a concentrated solution and an equimolar amount of hydrogen chloride is slowly added. The mixture begins to boil on its own, and after cooling, Metformin hydrochloride precipitates with a 96% yield.

Interactions

The H$_2$-receptor antagonist cimetidine causes an increase in the plasma concentration of Metformin, by reducing clearance of Metformin by the kidneys; http://en.wikipedia.org/wiki/Metformin - cite_note-80 both Metformin and cimetidine are cleared from the body by tubular secretion, and both, particularly the cationic (positively charged) form of cimetidine, may compete for the same transport mechanism. A small double-blind, randomized study found the anti-biotic Cefalexin to also increase Metformin concentrations by a similar mechanism; http://en.wikipedia.org/wiki/Metformin - cite_note-82 theoretically, other cationic medications may produce the same effect (16-25).

Mechanism of action

Metformin improves hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis). The "average" person with type-II diabetes has three times the normal rate of gluconeogenesis; Metformin treatment reduces this by over one third. Metformin activates AMP-activated protein kinase (AMPK), an enzyme that plays an important role in insulin signaling, whole body energy balance and the metabolism of glucose and fats; activation of AMPK is required for Metformin's inhibitory effect on the production of glucose by liver cells. Research published in 2008 further elucidated Metformin's mechanism of action, showing activation of AMPK is required for an increase in the expression of SHP,
which in turn inhibits the expression of the hepatic gluconeogenic genes PEPCK and Glc-6-Pase. Metformin is frequently used in research along with AICAR as an AMPK agonist. The mechanism by which biguanides increase the activity of AMPK remains uncertain; however, research suggests that Metformin increases the amount of cytosolic AMP (as opposed to a change in total AMP or total AMP/ATP).

In addition to suppressing hepatic glucose production, Metformin increases insulin sensitivity, enhances peripheral glucose uptake (by phosphorylating GLUT-4 enhancer factor), increases fatty acid oxidation and decreases absorption of glucose from the gastrointestinal tract. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors. AMPK probably also plays a role, as Metformin administration increases AMPK activity in skeletal muscle. AMPK is known to cause GLUT4 deployment to the plasma membrane, resulting in insulin-independent glucose uptake. Some metabolic actions of Metformin do appear to occur by AMPK-independent mechanisms; a 2008 study found "the metabolic actions of Metformin in the heart muscle can occur independent of changes in AMPK activity and may be mediated by p38 MAPK- and PKC-dependent mechanisms.

**Overdose**

A review of intentional and accidental Metformin overdoses reported to poison control centers over a five-year period found serious adverse events were rare, though the elderly appeared to be at greater risk. A similar study where cases were reported to Texas poison control centers between the years 2000 and 2006 found ingested doses of more than 5,000 mg were more likely to involve serious medical outcomes in adults. Survival following intentional overdoses with up to 63,000 mg (63 g) of Metformin has been reported in the medical literature. Fatalities following overdose are rare, but do occur. In healthy children, unintentional doses of less than 1,700 mg are unlikely to cause any significant toxic effects.

The most common symptoms following overdose appear to include vomiting, diarrhea, abdominal pain, tachycardia, drowsiness, and, rarely, hypoglycemia or hyperglycemia. The major potentially life-threatening complication of Metformin
overdose is lactic acidosis, which is due to lactate accumulation. Treatment of Metformin overdose is generally supportive, as there is no specific antidote. Lactic acidosis is initially treated with sodium bicarbonate, although high doses are not recommended, as this may increase intracellular acidosis. Acidosis that does not respond to administration of sodium bicarbonate may require further management with standard hemodialysis or continuous veno-venous hemofiltration. In addition, due to Metformin's low molecular weight and lack of plasma protein binding, these techniques also have the benefit of efficiently removing Metformin from blood plasma, preventing further lactate overproduction (26-42).

Metformin may be quantitated in blood, plasma, or serum to monitor therapy, confirm a diagnosis of poisoning, or assist in a medicolegal death investigation. Blood or plasma Metformin concentrations are usually in a range of 1–4 mg/L in persons receiving the drug therapeutically, 40–120 mg/L in victims of acute overdosage, and 80–200 mg/L in fatalities. Chromatographic techniques are commonly employed.

**Combinations with other drugs**

When used for type-II diabetes, Metformin is often prescribed in combination with other drugs. Several are available as fixed-dose combinations, also with the purpose of reducing pill burden and making administration simpler and more convenient.

As of 2009, the most popular brand-name combination was Metformin with rosiglitazone, sold as avandamet by GlaxoSmithKline since 2002. Rosiglitazone actively makes cells more sensitive to insulin, complementing the action of the Metformin. In 2005, all current stock of avandamet was seized by the FDA and removed from the market, after inspections showed the factory where it was produced was violating good manufacturing practices. The drug pair continued to be prescribed separately in the absence of avandamet, which was available again by the end of that year.

In the United States, Metformin is also available in combination with pioglitazone (trade name Actoplus Met), the sulfonylureas Glipizide (trade name
Metaglip) and glibenclamide (known as glyburide in the United States, trade name Glucovance), the dipeptidyl peptidase-4 inhibitor sitagliptin (with the combination sold under the trade name Janumet) and the meglitinide Repaglinide (Prandimet). Generic formulations of Metformin/Glipizide and Metformin/glibenclamide are available (the latter being more popular). A generic formulation of Metformin/rosiglitazone from Teva has received tentative approval from the FDA, and is expected to reach the market in early 2012.

Unlike glucose-lowering drugs of the sulfonylurea class, for example glyburide (Micronase; DiaB) or Glipizide (Glucotrol), Metformin does not increase the concentration of insulin in the blood and, therefore, does not cause excessively low blood glucose levels (Hypoglycemia) when used alone. In scientific studies, Metformin reduced the complications of diabetes such as heart disease, blindness and kidney disease. Metformin was approved by the FDA in December 1994.

**Preparations:**

- Tablets: 500, 850 and 1000 mg. Tablets (extended release): 500, 750 and 1000 mg. Solution: 500 mg/5 mL

**Storage:**

Metformin should be stored at room temperature between 20-25°C (68-77°F).

**Prescription:**

Metformin is used for treating type-II diabetes in adults and children. It may be used alone or in combination with other diabetic medications. Metformin also has been used to prevent the development of diabetes in people at risk for diabetes, treatment of polycystic ovaries, and weight gain due to medications used for treating psychoses.

**Dosage:**

For treating type-II diabetes in adults, Metformin (immediate release) usually is begun at a dose of 500 mg twice a day or 850 mg once daily. The dose is gradually increased by 500 mg weekly or 850 mg every two weeks as tolerated and based on the response of the levels of glucose in the blood. The maximum daily dose
is 2550 mg given in three divided doses. If extended tablets are used, the starting
dose is 500 mg or 1000 mg daily with the evening meal. The dose can be increased
by 500 mg weekly up to a maximum dose of 2000 mg (2500 mg of Fortamet) once
daily or in two divided doses. Glumetza tablets are given once daily. Metformin
should be taken with meals.

For pediatric patients 10-16 years of age, the starting dose is 500 mg twice a
day. The dose can be increased by 500 mg weekly up to a maximum dose of 2000
mg. Glucophage XR has not been studied in children.

**Drug interactions:**

Cimetidine (Tagamet), by decreasing the elimination of Metformin from the
body, can increase the amount of Metformin in the blood by 40%. This may increase
the frequency of side effects from Metformin.

**Pregnancy:**

There are no adequate studies in pregnant women. Most experts agree that
insulin is the best treatment for pregnant women with diabetes.

**Nursing mothers:**

Metformin is excreted into breast milk and can therefore be transferred to
the nursing infant. Nursing mothers should not use Metformin.

**Side effects:**

The most common side effects with Metformin are nausea, vomiting, gas,
bloating, diarrhea and loss of appetite. These symptoms occur in one out of every
three patients. These side effects may be severe enough to cause therapy to be
discontinued in one out of every 20 patients. These side effects are related to the
dose of the medication and may decrease if the dose is reduced.

A serious but rare side effect of Metformin is lactic acidosis. Lactic acidosis
occurs in one out of every 30,000 patients and is fatal in 50% of cases. The
symptoms of lactic acidosis are weakness, trouble breathing, abnormal heartbeats,
unusual muscle pain, stomach discomfort, light-headedness and feeling cold.
Patients at risk for lactic acidosis include those with reduced function of the kidneys
or liver, congestive heart failure, severe acute illnesses, and dehydration.
Metformin is an oral diabetes medicine that helps control blood sugar levels. Metformin is for people with type-II (non-insulin-dependent) diabetes. Metformin is sometimes used in combination with insulin or other medications, but it is not for treating type-I diabetes.

**Before taking Metformin**

Some people develop a life-threatening condition called lactic acidosis while taking Metformin. You may be more likely to develop lactic acidosis if you have liver or kidney disease, congestive heart failure, a severe infection, if you are dehydrated, or if you drink large amounts of alcohol. Talk with your doctor about your individual risk. You should not use this medication if you are allergic to Metformin or if you are in a state of diabetic ketoacidosis.

If you need to have any type of X-ray or CT scan using a dye that is injected into your veins, you will need to temporarily stop taking Metformin.

### 3.3. GLIPIZIDE

Glipizide is an oral medium-to-long acting anti-diabetic drug from the sulfonylurea class. It is classified as a second generation sulfonylurea, which means that it undergoes enterohepatic circulation. Mechanism of action is produced by blocking potassium channels in the β cells of the islets of Langerhans. By partially blocking the potassium channels, it will increase the time the cell spends in the calcium release stage of cell signaling leading to an increase in calcium. The increase in calcium will initiate more insulin release from each β-cell.

Glipizide is used along with diet and exercise and sometimes with other medications, to treat type-II diabetes (condition in which the body does not use insulin normally and therefore, cannot control the amount of sugar in the blood). Glipizide is in a class of medications called sulfonylureas. Glipizide lowers blood sugar by causing the pancreas to produce insulin (a natural substance that is needed to break down sugar in the body) and helping the body use insulin efficiently. This medication will only help lower blood sugar in people whose bodies produce insulin naturally. Glipizide is not used to treat type-I diabetes (condition in which the body does not produce insulin and therefore, cannot control the amount of sugar in the body).
blood) or diabetic ketoacidosis (a serious condition that may occur if high blood sugar is not treated) the structural formula is shown below figure. Glipizide chemical structure has shown in figure-3.2.

![Structure of Glipizide](image)

**Fig-3.2:** Structure of Glipizide

**Chemical details:**

<table>
<thead>
<tr>
<th>Class</th>
<th>Anti-diabetic drug from the sulfonylurea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical name</td>
<td>Glipizide</td>
</tr>
<tr>
<td>IUPAC name</td>
<td>N-(4-[N-(cyclohexylcarbamoyl) sulfamoyl] phenethyl)-5-methylpyrazine-2-carboxamide</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>$C_{21}H_{27}N_5O_4S$</td>
</tr>
<tr>
<td>Formula weight</td>
<td>445.54</td>
</tr>
<tr>
<td>CAS NO.</td>
<td>29094-61-9</td>
</tr>
</tbody>
</table>

**Drug interactions:**

Alcohol may prolong the action of Glipizide by delaying the absorption and elimination of Glipizide. Patients taking Glipizide should keep alcohol consumption to a minimum. Cholestyramine may reduce the absorption and consequently the effect of Glipizide. Therefore, Glipizide should be administered 1-2 hours before cholestyramine is administered. Fluconazole may increase the absorption and therefore increase the effect of Glipizide.

Many drugs can potentially increase or decrease glucose levels thus increasing or decreasing the effect of Glipizide. Drug interactions which cause low blood glucose (Hypoglycemia) can occur with nonsteroidal anti-inflammatory drugs (eg., ibuprofen), sulfa drugs, warfarin, miconazole, and β-blockers (eg., propranolol). Drug interactions which cause high blood glucose (Hyperglycemia) can occur with
thiazide diuretics, corticosteroids, thyroid medicines, estrogens, niacin, phenytoin, and calcium channel blocking drugs (eg., diltiazem). Patients should be monitored closely for loss of glucose control when such drugs are administered.

**Glipizide dosing information**

**Usual adult Glipizide dose for diabetes mellitus type-II:**

Initial dose: 5mg (immediate or sustained-release) orally once a day, 30 minutes before breakfast. Maintenance dose: 2.5-30mg (immediate-release) orally in 1 or 2 divided doses or 5-20mg (sustained-release) orally in 1 or 2 divided doses.

**Usual geriatric Glipizide dose for diabetes mellitus type-II:**

Initial dose: 2.5-5mg orally once a day. Doses may be increased in 1 or 2 week intervals in 2.5-5 mg/day increments.

**Patients receiving insulin**

As with other sulfonylurea-class Hypoglycemics, many stable non-insulin-dependent diabetic patients receiving insulin may be safely placed on Glipizide. When transferring patients from insulin to Glipizide, the following general guidelines should be considered:

For patients whose daily insulin requirement is 20 units or less, insulin may be discontinued and Glipizide therapy may begin at usual dosages. Several days should elapse between Glipizide titration steps.

For patients whose daily insulin requirement is greater than 20 units, the insulin dose should be reduced by 50% and Glipizide therapy may begin at usual dosages. Subsequent reductions in insulin dosage should depend on individual patient response. Several days should elapse between Glipizide titration steps.

During the insulin withdrawal period, the patient should test urine samples for sugar and ketone bodies at least three times daily. Patients should be instructed to contact the prescriber immediately if these tests are abnormal. In some cases, especially when patient has been receiving greater than 40 units of insulin daily, it may be advisable to consider hospitalization during the transition period.
**Patients receiving other oral hypoglycemic agents**

As with other sulfonylurea-class Hypoglycemics, no transition period is necessary when transferring patients to Glipizide. Patients should be observed carefully (1-2 weeks) for hypoglycemia when being transferred from longer half-life sulfonylureas (eg. chlorpropamide) to Glipizide due to potential overlapping of drug effect.

Glipizide comes as tablets and extended-release (long-acting) tablets to take by mouth. The regular tablet is usually taken one or more times a day, 30 minutes before breakfast or meals. The extended-release tablet is usually taken once a day with breakfast. To help you remember to take Glipizide, take it around the same time(s) every day. Follow the directions on your prescription label carefully, and ask your doctor or pharmacist to explain any part you do not understand. Take Glipizide exactly as directed.

**Side effects:**

Main side effects are difficulty breathing; swelling of your face, lips, tongue or throat. Hypoglycemia, or low blood sugar, is the most common side effect of Glipizide. Symptoms of low blood sugar may include headache, nausea, hunger, confusion, drowsiness, weakness, dizziness, blurred vision, fast heartbeat, sweating, tremor, trouble concentrating, confusion or seizure (convulsions). Watch for signs of low blood sugar. Side effects are,

- Diarrhea
- Gas
- Feeling jittery
- Dizziness
- Uncontrollable shaking of a part of the body
- Red or itchy skin
- Rash
- Hives
- Blisters
Some side effects can be serious.

- Yellowing of the skin or eyes
- Light-colored stools
- Dark urine
- Pain in the upper right part of the stomach
- Unusual bruising or bleeding
- Fever
- Sore throat

3.4 REPAGLINIDE

Repaglinide is for the treatment of type-II diabetes. Repaglinide belongs to the meglitinide class of blood glucose-lowering drugs. Repaglinide lowers blood glucose by stimulating the release of insulin from the pancreas. It achieves this by closing ATP-dependent potassium channels in the membrane of the β cells. Chemical structure of Repaglinide has shown in figure-3.3.

![Structure of Repaglinide](image-url)

**Fig-3.3: Structure of Repaglinide**

**Chemical details:**
- **Class**: Type-II diabetic
- **Chemical name**: Repaglinide
- **IUPAC name**: (S)-(+) -2-ethoxy-4-[2-(3-methyl-1-[2-(piperidin-1-yl)phenyl]butylamino)-2-oxoethyl]benzoic acid
- **Molecular weight**: 452.59
- **Molecular formula**: C$_{27}$H$_{38}$N$_2$O$_4$
- **CAS NO.**: 135062-02-1
Drug class and mechanism:

Repaglinide is an oral medication for lowering blood sugar (glucose) in individuals with diabetes. It is in a class of drugs for treating diabetes type-II called meglitinides and is chemically unlike other anti-diabetic medication.

Approximately 90% of patients with diabetes have type-II or non-insulin dependent diabetes mellitus. (Type-II diabetes usually occurs in adulthood and is associated with obesity and a strong family history of diabetes.) Glucose intolerance in diabetes type-II is caused by reduced insulin secretion from the pancreas after meals and resistance of the body's cells to insulin's effect which is to stimulate the cells to remove glucose from the blood. This leads to high levels of blood glucose.

Like Sulfonylureas, for example, glyburide (Glynase; Micronase), Glipizide, Glimepiride, Tolbutamide and Tolazamide, Repaglinide Stimulates cells in the pancreas to produce insulin. Glyburide may be more potent than Repaglinide at increasing insulin release in persons with low or high blood glucose levels, whereas Repaglinide may be more potent in persons with moderate blood glucose levels. Repaglinide is unusual in that it has a rapid onset of action and a short duration of action. When taken just prior to meals, it promotes the release of insulin that normally occurs with meals and is responsible for preventing blood glucose levels from becoming high. It has been shown to lower hemoglobin A1c levels by 1.6% to 1.9%. (Hemoglobin A1c is a blood test which measures the effectiveness of a drug in controlling high blood glucose levels. The lower the hemoglobin A1c, the better the control.) Repaglinide was approved by the FDA in 1997. It can be used alone (monotherapy) or combined with Metformin.

Dosage:

Repaglinide is taken immediately before a meal or 15 to 30 minutes before a meal. It should be taken with every meal up to 4 times a day. Doses are adjusted by the physician to achieve the best effect.

Drug interactions:

Repaglinide is metabolized (eliminated) in the liver by an enzyme called CYP3A4. Drugs that affect this enzyme may affect the blood levels of Repaglinide
and thus alter its glucose lowering effect. The metabolism of Repaglinide may be prevented by Ketoconazole, Itraconazole, Fluconazole, erythromycin and Clarithromycin. As a result, blood levels of Repaglinide rise and there is an enhanced glucose-lowering effect. Dangerous Hypoglycemic (very low blood glucose) reactions could occur. On the other hand, the elimination of Repaglinide may be increased with drugs that increase levels of CYP3A4 in the liver, such as barbiturates, carbamazepine and rifampin. This can result in lower blood levels of Repaglinide and hyperglycemia (high blood glucose).

Anabolic steroids or androgens can increase the risk of developing hypoglycemia in diabetic patients taking glucose-lowering medications.

Some drugs increase blood sugar and therefore reverse the effects of Repaglinide. Such drugs include amphetamines, glucorticoids such as prednisone, estrogens, isoniazid, phenothiazines such as chlorpromazine, phenytoin, decongestants and thyroid drugs. β-blockers, for example, propranolol, atenolol can cause hypoglycemia or hyperglycemia. Also, β-blockers can blunt some of the body's responses to hypoglycemia such as rapid heart rate, thus making it difficult for patients to recognize (and treat) hypoglycemic reactions. This not withstanding, β-blockers have been used successfully in diabetic patients. (Treatment with β-blockers is associated with improved survival in diabetics who are treated with the β-blocker because of high blood pressure.)

Gemfibrozil (Lopid) should not be combined with Repaglinide because gemfibrozil may significantly increase blood levels of Repaglinide leading to side effects.

Pregnancy:

No adequate human studies on the effects of Repaglinide on the fetus have been done; however, there have been no effects in animal studies in which the mother has received Repaglinide during pregnancy. Nevertheless, animals given Repaglinide during both lactation (nursing) and gestation have developed skeletal defects. Therefore, physicians must weight the potential benefits and risks of this medication when considering its use in pregnant women.
Nursing mothers:

It is not known whether Repaglinide accumulates in breast milk. However, animals given Repaglinide during pregnancy and lactation have developed skeletal defects. Because of the possibility of Hypoglycemia in nursing infants and the skeletal effects in nursing animals, it is recommended that Repaglinide not be used in women who are breastfeeding.

Side effects:

Hypoglycemia (low blood glucose) occurs somewhat less frequently with Repaglinide (1 in 6 persons) than with sulfonylureas such as Glyburide and Glipizide (1 in 5 persons). Some symptoms of Hypoglycemia include hunger, nausea, tiredness, perspiration, headache, heart palpitations, numbness around the mouth, tingling in the fingers, tremors, muscle weakness, blurred vision, cold temperature, excessive yawning, irritability, confusion, or loss of consciousness. Headache is reported in 1 in 9 persons. Other possible side effects include nausea, vomiting, diarrhea, constipation, stomach pain, back pain and chest pain.

Pharmacology:

Repaglinide is a novel oral Hypoglycaemic agent chemically unrelated to sulphonylureas, Metformin or acarbose. Repaglinide stimulates the release of insulin from pancreatic β-cells by inhibition of potassium efflux resulting in closure of ATP regulated K+ channels. This results in depolarisation of the cell and subsequent opening of calcium channels, leading to influx of calcium into the cells which cause release of insulin. It is suggested that Repaglinide and glibenclamide probably regulate these ATP-sensitive K+ channels via different binding sites on the β-cell.

Pharmacokinetics:

Repaglinide is rapidly absorbed from the GI tract and reaches maximum plasma concentration approximately 1 hour after ingestion when given as a tablet. The bioavailability of the oral formulation was found to be 63% in a study comparing 2mg (as a tablet) with the same dose given IV to healthy volunteers. Repaglinide is at least 98% protein bound. The elimination half life is short, about 1
hour. Repaglinide is extensively metabolised in the liver with only a very small proportion of the unchanged drug appearing in the urine. Its metabolites do not contribute to the glucose-lowering effect of the drug. Age does not appear to have a significant effect on Repaglinide pharmacokinetics in normal subjects however clearance is reduced in elderly NIDDM patients. Pharmacokinetic studies in patients with mild/moderate and severe renal impairment, those on haemodialysis as well as patients with hepatic impairment indicate an increased exposure to the drug in these patient groups. Caution is advised when titrating patients with renal impairment as total plasma clearance is decreased.

**Efficacy:**

Most of the studies assessing the efficacy of Repaglinide in type-II diabetes have involved relatively small patient numbers and been published in abstract form only. These abstracts have lacked detailed results and other data including dose titration details and target treatment levels. As a result interpretation and assessment of the data is difficult. One placebo controlled trial; one comparative trial with glibenclamide and one trial comparing Repaglinide alone with the combination of Metformin and Repaglinide have been reported.

Metformin, Glipizide and Repaglinide were available in individual and combination dosage forms in market. The available dosage forms were tabulated in table-3.1.

**Table-3.1: Available tablet dosage forms**

<table>
<thead>
<tr>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin HCl-250mg,500mg and 1000mg (SR)</td>
</tr>
<tr>
<td>Metformin HCl-250mg and Glipizide-2.5mg</td>
</tr>
<tr>
<td>Metformin HCl-500mg and Glipizide-5mg</td>
</tr>
<tr>
<td>Metformin HCl-400mg and Glipizide-2.5mg</td>
</tr>
<tr>
<td>Repaglinide-0.5mg, 1mg and 2mg</td>
</tr>
<tr>
<td>Metformin HCl-500mg and Repaglinide-1mg/2mg</td>
</tr>
</tbody>
</table>

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3.5. REVIEW OF LITERATURE

Hakan Emilsson (1987) developed a sensitive and selective high-performance liquid chromatographic method for determining intact Glipizide in human plasma or urine. The plasma and urine samples were acid-buffered, before tolbutamide was added as the internal standard. The samples were extracted with benzene, and the organic layer was evaporated to dryness. The residue was dissolved in equilibrated mobile phase (acetonitrile-0.01 M phosphate buffer pH 3.5, 35:65), and an aliquot of 20 μl was chromatographed on a Spherisorb ODS reversed-phase column. Quantitation was achieved by monitoring the ultraviolet absorbance at 275 nm. The response was linear (0–1000 ng/mL) and the detection limit was 5–10 ng/mL in plasma or urine. The within-assay variation was ≤ 10%. No interferences from metabolites or endogenous constituents were observed. The assay was demonstrated by determining Glipizide in samples from a diabetic subject receiving a therapeutic dose of 5 mg.

Gandhimathi M Renu S Ket al. (2003) developed a simple, precise and rapid RP-HPLC method for the determination of Repaglinide in pharmaceutical dosage forms. The method was carried out on a Shim-pack, RP-C18 column using a mixture of methanol: 0.1% v/v triethylamine (pH adjusted to 7 with orthophosphoric acid) and detection was done at 235 nm using nimesulide as the internal standard. The linearity range was 0.1 to 0.5 ppm. The intra-day and inter-day precision were in the range of 0.48 to 1.01 and 0.15 to 1.15, respectively.

Goal A et al. (2006) have developed a simple, economical, precise, convenient and reproducible visible spectrophotometric methods for the estimation of Repaglinide in tablet formulation. The developed methods are based on the formation of chloroform extractable complex of Repaglinide with chlorophenol red and bromophenol blue in acidic medium. The extracted complex with chlorophenol red shows absorbance maxima at 406.4 nm and the extracted complex with bromophenol blue shows absorbance maxima at 407.0 nm. Both the developed methods show linearity in the concentration range 10–50 mg/mL.
Results of analysis for both the methods were validated statistically and by recovery studies.

Arayne MS et al.,(713) (2006) reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed to quantify Metformin hydrochloride (MfCl) in raw material and pharmaceutical formulations using C18 analytical reverse-phase column. Diazepam was used as an internal standard. Mobile phase consisted of methanol-water (30:70 v/v), pumped at a flow rate of 0.5 mL/min at ambient temperature and the retention time was about 4.4 min with symmetrical peaks. (MfCl) was detected by ultraviolet absorbance at 233 nm with no interference of commonly used excipients. The method was linear over the concentration range 0.312-5 ppm (R2=0.9995). The limit of detection of Metformin was 0.1 mppm and the limit of quantitation was 0.3 ppm. The results obtained showed a good agreement with the declared contents in case of pharmaceutical formulations. The proposed method is rapid, accurate, economical and selective and it may be used for the quantitative analysis of Metformin in Neodipar tablets because of its sensitivity and reproducibility.

Florentin Tache et al.,(723) (2007) have developed a proposing an algorithm for demonstrating the specificity of the analytical method for an analyte of interest against its degradation product by applying physical (thermal degradation and photolysis) and chemical (photolytic-hydrolysis, acidic- and alkaline- hydrolysis and acidic-, neutral- and alkaline-oxidation) stress conditions. The authors define specific conditions for testing capacity of the analytical method to distinguish between the analyte and its by-products resulting in different environmental conditions. As an effective example, it is used the case of Metformin hydrochloride HPLC assay method. It is shown that by photolysis with white light or UV radiation the Metformin recovery is 99.98%. By thermal degradation it can be observed a moderate difference in between mild and high temperature application (99.98% in the first case and 86.00% in the second case, respectively). Photolytic-hydrolysis induces a slightly difference depending on the wavelength (recovery of 99.98% for white light and 99.90% for UV radiation, respectively). In turn, pH dependent
hydrolysis is inducing large differences (99.93% recovery in acidic range and 89.52% in the alkaline range, respectively). Oxidation of Metformin is also conducting to significant differences in accordance with the pH (99.90% recovery in the acidic range, 87.98% in the neutral range and 71.09% in the alkaline range respectively).

Bhavesh D et al.,(73) (2007) have developed a simple and rapid HPLC assay method for the estimation of Metformin in human plasma was developed and validated. The method totally eliminates the extraction procedure. The plasma proteins were precipitated using perchloric acid: acetonitrile (50%v/v) mixture and the supernatant liquid was removed, dried under nitrogen, reconstituted in mobile phase and injected into the HPLC system. The separation was achieved with a cationic exchange column (Hihchrom, 250X4.6mm) with mobile phase of methanol: potassium di-hydrogen orthophosphate buffer (0.1M, pH 3.5) mixture 46: 54 % v/v and elevated temperature of 40 0 C. Detection was by UV detector at 236nm. The retention time (RT) observed are at around 13 and 16 minutes for Metformin and phenformin respectively. The response was linear over a range of 30-5000 ng ml-1. The same method was used for the bioequivalence study of two Metformin formulations in healthy, human, Indian, male volunteers.

AbuBakar Ruzilawati etal.,(74)(2007) http://www.science-direct.com/science/article/pii/S0731708506007990-aff1 have development and validation a highperformance liquid chromatography (HPLC) assay for determination of Repaglinide concentration in human plasma for pharmacokinetic studies. Plasma samples containing Repaglinide and an internal standard, indomethacin were extracted with ethylacetate at pH 7.4. The recovery of Repaglinide was 92% ± 55.31. Chromatographic separations were performed on Purospher STAR C18 analytical column (4.8 mm × 150 mm; 5 μ particle size). The mobile phase composed of acetonitrile–ammonium formate (pH 2.7; 0.01 M) (60:40, v/v). The flow rate was 1 mL/min. The retention time for Repaglinide and indomethacin were approximately 6.2 and 5.3 min, respectively. Calibration curves of Repaglinide were linear in the concentration range of 20–200 ng/mL in plasma. The limits of
detection and quantification were 10 ng/mL and 20 ng/mL, respectively. The inter-day precision was from 5.21 to 11.84% and the intra-day precision ranged from 3.90 to 6.67%. The inter-day accuracy ranged 89.95 to 105.75% and intra-day accuracy ranged from 92.37 to 104.66%. This method was applied to determine Repaglinide concentration in human plasma samples for a pharmacokinetic study.

Meeta A Jiladia et al.,(75) (2009) have been a simple and sensitive, HPTLC method developed for the quantitative estimation of Repaglinide in its single component tablet formulations (2 mg). Repaglinide was chromatographed on silica Gel 60 F254 TLC plate using chloroform: methanol: ammonia (4.5:0.8:0.05 v/v) as mobile phase. Repaglinide showed Rf value 0.55±0.03 and scanned at 288 nm using Camag TLC Scanner 3. The method was validated in terms of linearity (400-2400 ng/spot), precision (intra-day variation 0.7 to 2.6%, inter-day variation 0.8 to 3.2%), accuracy (97.0 to 99.0%) and specificity. The limit of detection and limit of quantification for Repaglinide were found to be 50 ng/spot and 300 ng/spot, respectively. The developed method was successfully used for the assay of Repaglinide tablet formulations. The method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

Prameela Rani. A et al.,(76) (2009) a new Reversed phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of repaglinide in pharmaceutical formulations. Optimum separation was achieved in 12 minutes using C18 column (250 mm × 4.6 mm, i.d., particle size 5 mm), and elution was accomplished using a mobile phase (1 mL/min). Detection was carried out using a UV detector set at 245 nm. A linear relationship between mean peak area and concentration of Repaglinide was observed in the range 0.5-5 ppm, with a detection limit of 0.275 ppm and a quantification limit of 0.833 ppm. Intra-day and Inter-day precision, and accuracy of the method have been established according to the current ICH guidelines. The developed method was successfully applied to the determination of Repaglinide in pharmaceutical formulations. The results were statistically compared with the reference method (UV method) by applying Student's t-test and F-test. Accuracy, evaluated by means
of the recovery method, was in the range 99.75 ± 0.55 to 100.73 ± 0.34, with precision (RSD) 0.88%. No interference was observed from the coformulated substances. The proposed method was successfully employed for the determination of Repaglinide in various pharmaceutical preparations.

Mousumi Kar et al., (2009) a simple, accurate, economical and reproducible HPLC method has been developed for quantitative estimation of Metformin Hydrochloride from tablet dosage form and formulated microspheres. The developed HPLC method is a reverse phase chromatographic method using phenomex C_18 column and acetonitrile: phosphate buffer (65:35 v/v) pH adjusted to 5.75 with O-phosphoric acid as mobile phase and Glipizide as internal standard. The linearity was observed in concentration range of 0-25 ppm for Metformin Hydrochloride. Results of analysis were validated statistically and by recovery studies.

Sheikh Rahila, (2010) a rapid and sensitive RP-HPLC method depicted for the qualitative and quantitative assay of Glipizide in pharmaceutical dosage forms. Glipizide was chromatographed on reverse phase C18 column with mobile phase consisting of 0.05M Potassium di-hydrogen orthophosphate: methanol (15:85%v/v pH=7.0 adjusted with 1% triethylamine. The mobile phase was pumped at a flow rate 1ml per min. quantification was achieved by monitoring the UV absorbance at 225nm. The average retention time for Glipizide was found to be 3.21 with this method linearity was observed in the range of 10-2000 ng/mL the LOD and LOQ were found to be 5ng/mL and 15ng/mL respectively. The method was applicable for the analysis of drug in tablet formulation. The results of analysis were validated stastically.

N Kaushalet al., (2010) Spectrofluorimetric and high-performance liquid chromatography methods for estimation of Repaglinide were developed and validated for estimation of Repaglinide in tablets as well as in receptor fluid obtained during in vitro permeation studies. Repaglinide was observed to exhibit emission and excitation wavelengths, respectively, at 379nm and 282nm with linearity in the concentration range of 5-80 g/mL. High-performance liquid
chromatography analysis of Repaglinide yielded retention time of 6.14 min with linearity ranging from 0.1-1.2 g/mL concentration. Spectrofluorimetric analysis of Repaglinide in tablets yielded results comparable to high performance liquid chromatography.

Meeta a. Jiladia\(^{(80)}\) et al., (2010) have developed a simple and sensitive, HPLC method and validated for the quantitative estimation of Repaglinide in its single component tablet formulations (2 mg). Determination was performed using a Thermo BDS C\(_{18}\) column with mobile phase phosphate buffer: acetonitrile (pH 6.0; 45:55, v/v), and ultraviolet (UV) detection at 288 nm. The method was validated in terms of linearity (10–60 ppm), precision (intra-day variation below 0.6, inter-day variation below 0.2), accuracy (97.9 to 100.1), ruggedness (within 0.5%) and specificity. The limit of detection and limit of quantification for Repaglinide were found to be 1 ppm and 3 ppm, respectively. The developed method was successfully used for the assay of Repaglinide tablet formulations. The method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

Mukesh Chandra Sharma\(^{(81)}\) (2011) has developed a simple, rapid and accurate and stability indicating RP-HPLC method for the determination of Repaglinide in pure and tablet forms. The drug was found almost stable to neutral and photolytic condition. Resolution of drug and the degradation products formed under different stress conditions were successfully achieved on a Luna C\(_{18}\) (5μm~25cm~4.6mm) column utilizing mixture of Acetonitrile and potassium dihydrogen phosphate buffer (pH 4.5 adjusted with orthophosphoric acid) in the ratio of 60:40 (v/v) as mobile phase at a flow rate of 1.0 ml min\(^{-1}\) and detection was performed at 278 nm. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, forced degradation, solution stability and selectivity. Quantitative and recovery studies of the dosage form were also carried out and analyzed; the % RSD from recovery studies was found to be less than 1. Due to simplicity, rapidity and accuracy of the method, we believe that the method will be useful for routine quality control analysis.
Madhukar A et al. (2011) developed a simple, specific, accurate and isocratic reversed phase-HPLC method for the determination of Metformin Hydrochloride. Separation was achieved with an Inertsil - Extend - C18 HPLC column 250mm in length and having an internal diameter of 4.6mm. A mobile phase comprising 10m.mol 1-Octane sulfonic acid: Acetonitrile in the volume ratio of (80:20) was developed. The detection was carried out using a PDA detector set at a wavelength of 232nm. Validation experiments were performed to demonstrate System suitability, specificity, precision, linearity and range, accuracy study, stability of analytical solution and robustness. The method was linear over the concentration range of 1-250ppm and got the correlation Regression (r2) 0.9995, showed good recoveries (100.25 - 101.13%), the relative standard deviations of intra and inter-day assay were 99.4% and 99.94% respectively. The method can be used for quality control assay of Metformin.

S.D. Jadhav (2011) has developed a simple, specific, accurate and precise reverse phase high performance liquid chromatographic method for the estimation of Glipizide and Metformin hydrochloride in tablet dosage form. A Grace smart RP18, 250x4.6mm, 5μ in isocratic mode, with mobile phase containing Phosphate buffer & acetonitrile in proportion of (60:40v/v) pH 5.72 adjusted with potassium hydroxide were used. The flow rate was 1 mL/min. The retention time of Metformin hydrochloride and Glipizide were 2.529 and 4.739 min respectively, and the resolution factor was 5.37. The proposed method is accurate, precise, specific and rapid for estimation of Glipizide and Metformin hydrochloride in tablet dosage form.

3.6. OBJECTIVE

Literature survey reveals that Spectrophotometric and RP-HPLC methods are available for individual and other combination products determination of Metformin, Glipizide and Repaglinide in pharmaceutical preparations and biological formulation so far, no single method has been reported for estimation of three actives in combined dosage forms, hence single RP-HPLC have been developed for simultaneous estimation.
3.7. MATERIALS AND METHODS

Reagents and chemicals:
AR grade Potassium di-hydrogen orthophosphate and orthophosphoric acid were purchased from Rankem, RFCL limited, New Delhi. Hydrogen peroxide 30% (w/v), Hydrochloric acid (35%) reagents were obtained from Merck, Merck specialties Ltd., Mumbai. Gradient grade acetonitrile was obtained from Merck.

Drug substances:
Metformin HCl, Repaglinide and Glipizide were obtained from reputed pharma company, Bangalore.

Glassware:
Borosil, 'A'grade glassware like pipette, volumetric flask, conical flask, glass bottle, test tubes, measuring cylinder and glass beaker were used in the present study.

Methods
Method development and validation of related compounds of Metformin HCl, Repaglinide and Glipizide were carried out by high performance liquid chromatography (HPLC).

Chromatographic conditions
Column : Zodiac C18, 150mm x 4.6mm, 3.5μ
Buffer : Weighed accurately 1gm of Potassium di-hydrogen orthophosphate in to 1L of water and adjusted the pH to 3.0 with orthophosphoric acid.
Mobile phase : Sol-A: Buffer; Sol-B Acetonitrile and gradient program: (0-4min, sol-A: 45-45; 4-8min- sol-A:45-30; 8-10min- sol-A:30-20; 10-12min- sol-A:20-45 and 12-16min- sol-A:45-45)
Detection : wave length 210 nm
Sample size : 10μL
Temperature : 35°C
Runtime : 16min
Gradient program

<table>
<thead>
<tr>
<th>Time (minute)</th>
<th>Solution-A(%)</th>
<th>Solution-B(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>45.0</td>
<td>55.0</td>
</tr>
<tr>
<td>4.0</td>
<td>45.0</td>
<td>55.0</td>
</tr>
<tr>
<td>8.0</td>
<td>30.0</td>
<td>70.0</td>
</tr>
<tr>
<td>10.0</td>
<td>20.0</td>
<td>80.0</td>
</tr>
<tr>
<td>12.0</td>
<td>45.0</td>
<td>55.0</td>
</tr>
<tr>
<td>16.0</td>
<td>45.0</td>
<td>55.0</td>
</tr>
</tbody>
</table>

Standard solution: Weighed and transferred 40mg of each active ingredient standard in to 100ml of class-A volumetric flask and 70ml of diluent added and sonicated to dissolve the contents and diluted to volume with diluent. Resulting solution further diluted to 5ml of into 50ml with diluent (each active 40ppm).

Marketed formulation: Prepared the marketed all dosage forms to get the known concentration of 40ppm for all three ingredients.

System suitability parameters: Tailing factor of three active peaks in standard solution is not more than 2.0; Resolution between three actives is not less than 3.0 and % of RSD of five replicate standard solutions area is not more than 2.0%.

Retention time of active peaks

<table>
<thead>
<tr>
<th>Name of the Analyte</th>
<th>~ RT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin HCl</td>
<td>1.4</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>9.8</td>
</tr>
<tr>
<td>Glipizide</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Calculation:

\[
\% \text{ of content} = \frac{\text{Area of test soln} \times \text{Std. Concen} \times \text{average} \times \text{Potency of standard}}{\text{Area of standard soln} \times \text{sample concen} \times \text{Label claim}}
\]
3.8. RESULTS AND DISCUSSION

In the present work a new method development and validation was carried out for the estimation of Metformin hydrochloride, Repaglinide and Glipizide by RP-HPLC technique. The wavelength selection was made at 210nm since all the selected drugs reported in various literatures were having a maximum absorbance at around 210 to 225 nm. Hence the wavelength was selected at 210 nm for the detection of the three compounds with better baseline.

METHOD DEVELOPMENT:

Method development several trials were carried out and reported. These lead to the optimized chromatographic conditions for the separation and estimation of Metformin hydrochloride, Repaglinide and Glipizide in the bulk and marketed formulation. Preliminary studies involved trying ODS-silica columns and several mobile phase compositions for the effective separation of these three drugs. By Zodiac C18, 3.5μ, 150x4.6mm column eluted with solvent-A: Phosphate buffer pH-2.3, solvent-B: acetonitrile by gradient elution pattern at a flow rate of 1.0 mL/ min and a detection wavelength of 210 nm with injection volume of 20 μl at ambient(30°C) temperature.

Results of trial-1: RT’s were observed at 11.236(MFT), 15.568(GPZ) and the third peak is not eluted in 20 min runtime. Due to assymetry in peaks and longer RT’s another trial is made with change in mobile phase-buffer pH.

Results of trial-2: RT’s were observed at 10.236(MFT), 13.568(GPZ) and the third peak is not eluted in 20 min runtime. Due to tailing in peaks and to further reduce RT’s another trial is made with change in mobile phase-buffer pH, flow rate and elution pattern.
Results of trial-3 (Final optimized method): RT's were observed at 1.4(MFT), 3.7(GPZ) and 9.0(RPT). The peaks are sharply resolved with less tailing and hence the trial-3 method is optimized for analysis.

METHOD VALIDATION:

After method development, the validation of the current method has been performed in accordance with USP requirements for assay determination (Category-I: analytical methods for quantitation of active ingredients in finished pharmaceutical products) which include accuracy, precision, selectivity, linearity and range, robustness and ruggedness.

System suitability testing:

System suitability testing is an integral part of many analytical procedures. System suitability test parameters like tailing factor, retention time, theoretical plates per unit, resolution factor are determined and the results are tabulated and are as shown in Table-3.2. Diluent, standard solution chromatograms were represented in figures-3.4 and 3.5. Diluent and standard overlaid chromatograms were shown in figures-3.6 and 3.7.

![Fig-3.4: Diluent chromatogram.](image)
Fig-3.5: Standard solution.

Fig-3.6: Diluent and standard solution overlaid chromatogram.
Table 3.2: System suitability (Area %RSD)

<table>
<thead>
<tr>
<th>Active Ingredient Name</th>
<th>Inj-1</th>
<th>Inj-2</th>
<th>Inj-3</th>
<th>Inj-4</th>
<th>Inj-5</th>
<th>Average</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin HCl</td>
<td>1033033</td>
<td>1029947</td>
<td>1025052</td>
<td>1032345</td>
<td>1036856</td>
<td>1031447</td>
<td>0.42</td>
</tr>
<tr>
<td>Glipizide</td>
<td>1197777</td>
<td>1201447</td>
<td>1201358</td>
<td>1209026</td>
<td>1210038</td>
<td>1203929</td>
<td>0.44</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>2995081</td>
<td>3000014</td>
<td>3000085</td>
<td>3016736</td>
<td>3022019</td>
<td>3006787</td>
<td>0.39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Inj-1</th>
<th>Inj-2</th>
<th>Inj-3</th>
<th>Inj-4</th>
<th>Inj-5</th>
<th>Average</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin HCl</td>
<td>1.48</td>
<td>1.47</td>
<td>1.48</td>
<td>1.48</td>
<td>1.48</td>
<td>1.48</td>
<td>0.30</td>
</tr>
<tr>
<td>Glipizide</td>
<td>3.71</td>
<td>3.7</td>
<td>3.7</td>
<td>3.7</td>
<td>3.71</td>
<td>3.70</td>
<td>0.15</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>9.82</td>
<td>9.81</td>
<td>9.81</td>
<td>9.81</td>
<td>9.82</td>
<td>9.81</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Fig-3.7: Diluent and standard solution overlaid chromatogram.
### Tailing factor

<table>
<thead>
<tr>
<th>Active Ingredient Name</th>
<th>Inj-1</th>
<th>Inj-2</th>
<th>Inj-3</th>
<th>Inj-4</th>
<th>Inj-5</th>
<th>Average</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin HCl</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td>Glipizide</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Repaglinide</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

**Linearity:** The linearity of the response of three actives was verified at six concentration level ranging from 10ppm to 60ppm. The calibration curve was constructed by plotting mean area response $A$ against concentration $C$. The result shows that an excellent correlation existed between peak area and concentration of three active ingredients. Results were tabulated in tables 3.3 to 3.5 and chromatograms were represented in figure 3.8 and overlaid chromatograms were represented in figures 3.9 to 3.11. Linearity graphs were represented in figures 3.12 to 3.14.

**Table-3.3:** Metformin hydrochloride linearity results.

<table>
<thead>
<tr>
<th>Metformin hydrochloride linearity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity level</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Level-1</td>
</tr>
<tr>
<td>Level-2</td>
</tr>
<tr>
<td>Level-3</td>
</tr>
<tr>
<td>Level-4</td>
</tr>
<tr>
<td>Level-5</td>
</tr>
<tr>
<td>Level-6</td>
</tr>
<tr>
<td>Co-relation Coefficient</td>
</tr>
</tbody>
</table>
Table-3.4: Glipizide linearity results.

<table>
<thead>
<tr>
<th>Linearity level</th>
<th>Concentration (ppm)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level-1</td>
<td>10</td>
<td>242672</td>
</tr>
<tr>
<td>Level-2</td>
<td>20</td>
<td>577587</td>
</tr>
<tr>
<td>Level-3</td>
<td>30</td>
<td>906218</td>
</tr>
<tr>
<td>Level-4</td>
<td>40</td>
<td>1222364</td>
</tr>
<tr>
<td>Level-5</td>
<td>50</td>
<td>1535436</td>
</tr>
<tr>
<td>Level-6</td>
<td>60</td>
<td>1851679</td>
</tr>
</tbody>
</table>

Co-relation Coefficient: 0.9999

Table-3.5: Repaglinide linearity results.

<table>
<thead>
<tr>
<th>Linearity level</th>
<th>Concentration (ppm)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level-1</td>
<td>10</td>
<td>607178</td>
</tr>
<tr>
<td>Level-2</td>
<td>20</td>
<td>1443294</td>
</tr>
<tr>
<td>Level-3</td>
<td>30</td>
<td>2290334</td>
</tr>
<tr>
<td>Level-4</td>
<td>40</td>
<td>3050207</td>
</tr>
<tr>
<td>Level-5</td>
<td>50</td>
<td>3844783</td>
</tr>
<tr>
<td>Level-6</td>
<td>60</td>
<td>4645485</td>
</tr>
</tbody>
</table>

Co-relation Coefficient: 0.9999
Fig-3.8: Linearity chromatograms
Fig-3.9: Overlaid linearity chromatograms.

Fig-3.10: Overlaid linearity chromatograms.
Fig-3.11: Overlaid linearity chromatograms.

Fig-3.12: Metformin hydrochloride linearity graph.
**Fig-3.13:** Metformin hydrochloride linearity graph.

**Fig-3.14:** Metformin hydrochloride linearity graph.
**Precision:** Method repeatability (intra-day precision) was evaluated by market samples which were prepared as described in the sample preparation. The mean % assay and percentage RSD. for assay values were found to be 99.0% and 0.7%, respectively, which is well within the acceptance criteria that is, assay value should be between 97.0 and 103.0% and RSD. should be not more than 2.0%. The intermediate precision (inter-day precision) was performed by different analyst, different HPLC system and different HPLC column in different days as described in the sample preparation. The assay values were found to be satisfactory. The results indicated the good precision of the developed method and results were tabulated in table-3.6.

**Table-3.6:** Precision results.

<table>
<thead>
<tr>
<th>Active ingredient name</th>
<th>Sample preparations</th>
<th>Average (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prep-1</td>
<td>Prep-2</td>
</tr>
<tr>
<td>Metformin HCl</td>
<td>98.77</td>
<td>100.10</td>
</tr>
<tr>
<td>Glipizide</td>
<td>101.10</td>
<td>99.10</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>100.10</td>
<td>99.27</td>
</tr>
</tbody>
</table>

**Accuracy:** Accuracy was determined by applying the developed method to synthetic mixtures of excipients to which known amounts of each drug 25% to 150% of std concentration. The accuracy was then calculated as the percentage of analyte recovered from the formulation matrix. Results found to be satisfactory and results tabulated in table-3.7.

**Table-3.7:** Accuracy results.

<table>
<thead>
<tr>
<th>Active Ingredient name</th>
<th>Spike level</th>
<th>Average % recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25%</td>
<td>50%</td>
</tr>
<tr>
<td>Metformin HCl</td>
<td>100.16</td>
<td>100.03</td>
</tr>
<tr>
<td>Glipizide</td>
<td>99.89</td>
<td>99.86</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>99.66</td>
<td>98.91</td>
</tr>
</tbody>
</table>
Robustness: To determine the robustness of the developed method, experimental conditions were purposely altered. One factor at a time was changed to estimate the effect. Thus, five replicate injections of standard solution were injected under each parameter and observed the change on the tailing factor for three active peaks and the RSD. for peak area also within the limit. The flow rate of mobile phase was changed by ± 10% that is 0.9 to 1.1 mL/min. The effect of column temperature was studied at 33 and 37°C instead of 35°C. System suitability results were within the limit. Results were tabulated in table 3.8 and 3.9.

**Table-3.8: Flow variation Results.**

<table>
<thead>
<tr>
<th></th>
<th>System suitability</th>
<th>Observed value</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.9mL/min</td>
<td>1.0mL/min</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>Metformin Hydrochloride</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Glipizide</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Repaglinide</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>% Relative standard deviation</td>
<td>Metformin Hydrochloride</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Glipizide</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Repaglinide</td>
<td>0.9</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Table-3.9: Column oven temperature variation results.**

<table>
<thead>
<tr>
<th></th>
<th>System suitability</th>
<th>Observed value</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>33°C</td>
<td>35°C</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>Metformin Hydrochloride</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Glipizide</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Repaglinide</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>% Relative standard deviation</td>
<td>Metformin Hydrochloride</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Glipizide</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Repaglinide</td>
<td>0.9</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Solution stability and mobile phase stability:

The % of RSD. of assay for Metformin, Glipizide and Repaglinide during solution stability and mobile phase stability experiments was within 1%. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay determination was stable up to 48 hours at room temperature.

3.9. SUMMARY AND CONCLUSION

The developed method is selective, rapid, precise and accurate. The retention time of Metformin hydrochloride is 1.4 min, Glipizide is 3.7 min and Repaglinide is 9.8 min, respectively. Method validation results reveal the method is precise, accurate and rugged. This indicates that the proposed method could be used as a stability-indicating method for the determination of Metformin, Glipizide and Repaglinide in bulk powder and pharmaceutical formulations.
3.10. REFERENCES


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