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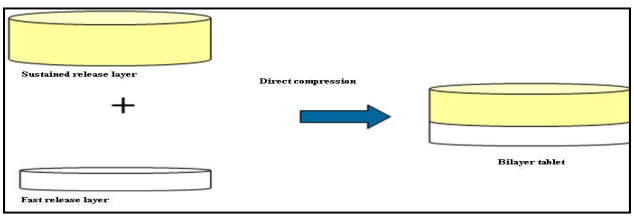
Preparation and evaluation of bilayer tablets containing metformin hydrocloride, glimepiride and pioglitazone hydrocloride

Deepak Kumar Gupta, Manohar Chouhan and Dr. Arun Kumar Gupta

Abstract
In proposed work, Bilayer tablets of an antidiabetes agent: Metformin, Glimepiride and Pioglitazone hydrochloride is to formulate, and evaluate for oral sustained drug release, in pharmaceutical system to enhance its oral bioavailability, Reduction in drug toxicity, Reduction in dosing frequency of drug. Biphasic release is characterized by rapid initial release of the drug, followed by sustained rate of release. The drug released by the initial pulse, quickly attains the therapeutic plasma drug levels and ameliorates the slow onset of action of sustained release layer (approx. 60 min). This increases patient compliance as the patient is quickly relief. Such type of drug delivery systems is designed to deliver the drug in such a way that the drug level is maintained within the therapeutic window for a period as long as the system continues to deliver the drug and to avoid fluctuations in plasma drug level.
The two principal defects in type 2 diabetes are insulin deficiency and insulin resistance. Therefore, combining an insulin-providing agent with an insulin sensitizing agent will augment the efficacy of current anti- hyperglycaemic agents. This is the rational for the development of Glimepiride, Metformin and Pioglitazone combination. The ultimate or primary goal of therapy for type 2 diabetes is to prevent the mortality and morbidity related to the microvascular and macrovascular complications. It is increasingly obvious that to achieve this on a global perspective will have to identify better and more effective treatment strategies to maintain tight glycemic control.
Administration of Metformin, Glimepiride and Pioglitazone combination is used for reducing the Oxidative stress-induce nuclear damage & Reproductive toxicity.

Keywords: Metformin, Glimeperide, pioglitagone, immediate release, sustained release, Bilayer tablet, Crospovidone, crosscarmellose sodium, microcrystalline cellulose

Introduction
The term bilayered tablets refers to tablet containing subunits that may be either the same (homogeneous) or different (heterogeneous). Bilayer tablets allows for designing and modulating the dissolution and release characteristics. Bilayer tablets are prepared with one layer of drug for immediate release while second layer designed to release drug, later, either as second dose or in an extended release manner. Bilayer tablet is suitable for sequential release of two drugs in combination, separate two incompatible substances. Bilayer tablets are preferred when the release profiles of the drugs are different from one another.
*CYP2C19*3* arises from a G→ A transition at position 636 in exon 4 of *CYP2C19*, which produces a truncated protein, that results in a premature termination codon at amino acid 212 [15] changes the tryptophan codon to the termination codon, which leads to protein synthesis stopping earlier and the protein become functional defect [16]. The *CYP2C19*3* allele frequencies in most populations are below 1%; however, it is more prevalent among Asians (2–9%) [3].
The aim of this study was to genotype healthy Palestinian people for cytochrome *P4502C19*, to identify the variant allele of *CYP2C19* (*CYP2C19*3*) at position 636 in exon 4 of



Preparation of bilayer tablet

Advantages of Bilayer tablets

- Bilayer tablet in FDCs: Fixed dose combination with two or more ingredients to be formulated together inspite of actives having different physico–chemical characteristics and active-active incompatibility.
- Bilayer tablet can be manufactured in such a way that one layer provides sustained release and second later provides immediate release of the medicament. This approach is beneficial for providing initial loading dose and then maintenance dose within therapeutic window so it avoids frequent dosing of the drug.
- Bilayer tablet can be formulated as buoyant dosage form (floating bilayer tablet) which is helpful to increase residence time in the stomach that is a need for a drug whose absorption occurs from stomach and also to enhance the therapeutic effect.

Applications of bilayered tablets-

1. Bilayer tablets are mainly used in the combination therapy.
2. Bilayer tablets are used to deliver the loading dose and sustained dose of the same or different drugs.
3. Bilayer tablets are used for bilayer floating tablets in which one layer is floating layer another one is immediate release layer of the drug.
4. Bilayer tablets are used to deliver the two different drugs having different release profiles.

Advantages of bilayered tablets

1. They are used as an extension of a conventional technology.
2. Potential use of single entity feed granules.
3. Separation of incompatible components.
4. Patient compliance is enhanced leading to improved drug regimen efficacy.
5. Patient convenience is improved because fewer daily doses are required compared to traditional delivery system.
6. Maintain physical and chemical stability.
7. Retain potency and ensure dose accuracy.

Oral Hypoglycemic Agents

Insulin has the disadvantages of having to be injected, it is without question the most uniformly effective treatment of diabetes mellitus. The success of oral hypoglycemic drug therapy usually based on restoration of normal blood glucose levels and the absence of glycosuria. Traditionally the term oral hypoglycemic was used as interchangeably with sulfonylurea, but more recently the development of several new drugs has broadened this designation to include all oral medications for diabetes. Because these drugs do not have to be injected, oral agents enhance compliance in type II diabetes.

Oral Hypoglycaemic Agents

Drug Class	Examples	Principal Mode Of Action
Biguanides	Metformin	Decrease Hepatic Glucose Production
Thiazolidinediones	Rosiglitazone, Pioglitazone	Improve Peripheral Insulin Sensitivity
Alpha-Glucosidase Inhibitor	Acarbose	Delays Carbohydrate Absorbtion
Sulfonylureas	Glimepiride, Glipizide, Gliclazide, Glibenclamide	Stimulate Insulin Secreation From Pancreatic Beta Cells
Short Acting Insulinotropic Agents	Repaglinide, Netaglinide	Stimulate Insulin Secreation From Pancreatic Beta Cells

Diabetes Mellitus

Diabetes mellitus is a chronic disorder characterized by impaired metabolism of glucose. Diabetes mellitus is a group of disorders involving distinct pathogenic mechanisms with hyperglycemia as the common denominator. Regardless of the cause, the disease is associated with insulin deficiency, which may be total, partial or relative when viewed in respect of co-existing insulin resistance.

Diabetes mellitus has reached epidemic proportions and affects more than 170 million individuals worldwide. In more developed societies, the prevalence of diabetes mellitus has reached about 6% and even more alarmingly, among obese white adolescents 4% had diabetes and 25% had abnormal glucose tolerance. Some 90% of diabetic individuals have Type-2 (Non-Insulin-dependent) diabetes mellitus, and within this category no more than 10% can be accounted for monogenic forms such as maturity-onset diabetes of the young and mitochondrial diabetes or late-onset autoimmune diabetes of the adult, which is actually late-onset Type 1 diabetes. Thus, most diabetes in the world is accounted for by "common" Type 2 diabetes, which has a multifactorial pathogenesis caused by alterations in several gene products.

Causes

Insulin is a hormone produced by the pancreas to control blood sugar. Diabetes can be caused by deficiency of insulin, resistance to insulin or both. People with diabetes have high blood sugar. This is because:

1. Their pancreas does not make enough insulin
2. Their muscle, fat, and liver cells do not respond to insulin due to insulin resistance.

Classification of Diabetes Mellitus

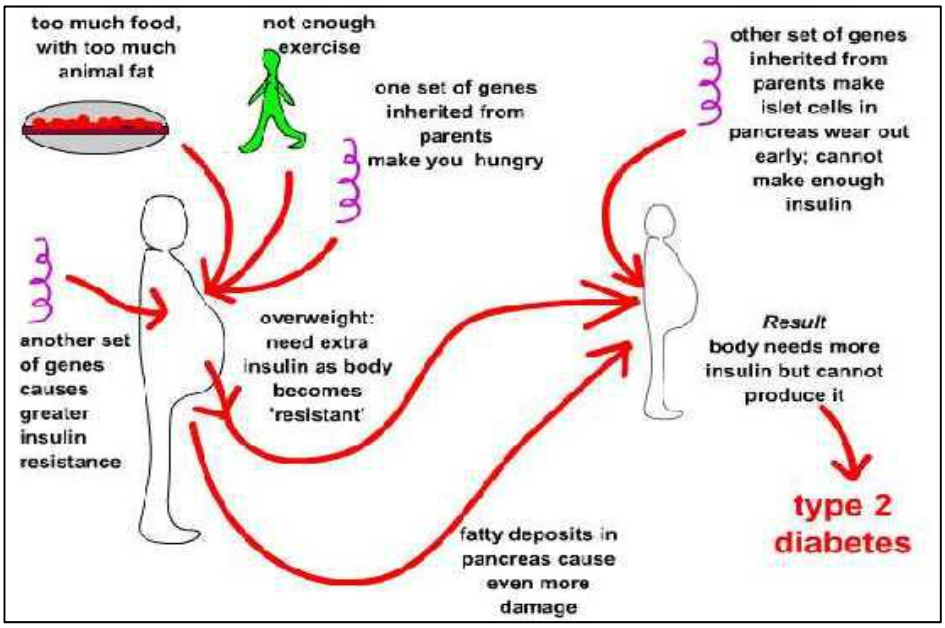
1. Type 1 diabetes (beta cell destruction, usually leading to absolute insulin deficiency)

a) Immune Mediated b) Idiopathic

2. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretary defect with relative insulin resistance).
3. Other Specific Types

a. Genetic defects of beta cell function and insulin acticn
b. Disease of the exocrine pancreas
c. Endocrinopathies
d. Drug induced
e. Infections
f. Gestational Diabetes

Metabolic Causes of Type 2 Diabetes



Symptoms of Diabetes Mellitus

The classical symptoms of diabetes mellitus are:

- Polydipsia (Increased intake of water due to increased thirst)
- Polyuria (Increased formation of urine)
- Polyphagia (Increased ingestion of food).

Diagnosis of Diabetes Mellitus

Two kinds of blood estimations are done to estimate the normal plasma glucose levels. The first one is known as Random Plasma Glucose (RPG) in which a sample is drawn at any "random" time during the day without consideration to the "fed" state of the patient. The other are samples known as Fasting Plasma Glucose (FPG) followed by Post–prandial Plasma Glucose (PPG). The patient is advised not to eat anything after dinner till the blood sample for FPG is withdrawn the next morning.

There are three ways to diagnose diabetes each must be confirmed on a subsequent day, by any one of the three methods.

1. FPG > 126 mg/dl (0.7 mmol/l), fasting is defined as no caloric intake for at least 8hours.
2. 2-h PPG > 200 mg/dl (11.1 mmol/1) during an Oral glucose Tolerance Test (OGTT).

The Expert Committee recognizes an intermediate group of subjects whose (FPG> 110 mg/dl (6.1 mmol/l) but < 126 mg/d; (7.0 mmol/l) or 2-h values in the OGTT of > 140 mg/dl (7.8 mmol/l) but <200 mg/dl (11.1 mmol/l). Thus, the categories of FPG values are as follows,

1. FPG< 110 mg/dl (6.1 mmol/l) = normal fasting glucose
2. FPG> 110 mg/dl (6.1 mmol/l) and < mg/dl (7.0 mmol/l) = FPG
   FPG> 126 mg/dl (7.0 mg/dl) = provisional diagnosis of diabetes (the diagnosis must be confirmed, as described above)

Experimental Materials: Glimepiride, Pioglitazone hydrochloride, metformin hydrochloride, sodium starch glycolate/cross carmelose sodium as a superdisintegrants, Microcrystalline cellulose as a direct compressible agent, Mannitol as a diluents, talc and stearic acid, HPMC K-4M/HPMC K-100M as a sustained release polymer, xanthan gum, mannitol as a diluents and PVP K-30 used as a binder. All other materials used were of pharmacopoeial grade.

Methods

1) Preparation of Immediate Release Layer

The Immediate release layer were prepared by Direct Compression technique by blending Glimepiride, Pioglitazone hydrochloride uniformly with sodium starch glycolate/cross carmelose sodium as a superdisintegrants, Microcrystalline cellulose as a direct compressible agent, Mannitol as a diluents as per the formula. The blend obtained was passed through a 40# sieve. The powder blend was mixed with talc and stearic acid.

2) Preparation of Sustained Release Layer: The sustained release granules were prepared by wet granulation technique by blending metformin hydrochloride uniformly with HPMC K-4M/HPMC K-100M as a sustained release polymer, xanthan gum, mannitol as a diluents and PVP K-30 used as a binder, as per the formula. The cohesive mass obtained was passed through a 40# sieve, dried at 60°C. The granules were mixed with talc and magnesium stearate.

Preparation of Bi Layered Tablet: For the purpose of bilayer tablet punching, single punch hand driven tableting machine will be utilized. Bilayer tablets will be punched on 10 mm concave die and punches set by the following method:

1. Fast releasing powder blend of glimepiride, pioglitazone hydrochloride will be fed to the die cavity.
2. The powder will be punched for half compression cycle, with hardness as minimum as 1-1.5 kg/cm^2 so as to form a weak compact. The weak compacts are use in the bilayer tablet punching because they prevent the intermixing of the tablet layers.

Upper punch of the machine will be raised, with lower punch at lowest position and sustained release powder blend will be progressively filled by hand inside the die cavity, which already contains weak compact of solid dispersion.
Finally, both layers will be compressed together, resulting in bilayer tablet with diameter of 10 mm. The layers could be seen distinctively without any intermixing between the two layers.

Pre compression Properties
The granules of all sustained release formulations were evaluated for powder flow properties. The fixed funnel method was employed to measure the angle of repose. Bulk and tapped densities were determined by tapped density apparatus from which compressibility index and Hausner's ratio values were calculated and the results are given in table 5.

Post compression properties for tablets
Thickness: The thickness of the tablets was determined by using vernier calipers. Randomly 10 tablets selected were used for determination of thickness that was expressed in mean ±SD and unit is mm results given in tables 6&11.

Uniformity of Weight: The individual weight of 20tablets was taken after selecting them randomly for weight variation. Then the average weight and the mean and standard deviation were calculated and compared with the standards. The weight of the tablet being made is measured to ensure that it contains predetermined amount of drug.

Hardness: Hardness of tablets was measured by selecting 5 tablets randomly and the hardness of each tablet was measured with Monsanto hardness tester.

Friability: The friability of the tablets was determined using electrolab friabilator. It is expressed in percentage (%). Ten tablets were initially weighed and transferred into the friabilator. The friabilator was operated at 25rpm for 4min. After 4min the tablets were weighed again. The friability was then calculated using the formula,

$$Friability\ (\%) = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100$$

Disintegration test: The disintegration time for the tablet was determined using the disintegration apparatus. One tablet was placed in each of six tubes placed in a beaker containing 1000 ml of purified water maintained at 37 ± 5^0 C and the apparatus was operated. The time taken for the tablets to disintegrate and pass through the mesh was noted.

Dissolution study
Dissolution test was carried out using dissolution apparatus USP Type-II using buffer pH6.8 as the dissolution medium, maintained at a temperature of 37±0.5ºC. Randomly selected three tablets from each batch were taken for the evaluation. Aliquot amount of solution was withdrawn in every 5 minutes. The filtered solution was analysed for the drug concentration by measuring absorbance at 322 nm using U.V Spectrophotometer.

Drug content uniformity
Twenty tablets were finely powdered and an amount equivalent to 500 mg of Metformin hydrochloride, 2mg of Glimepiride and 15mg of Pioglitazone hydrochloride was accurately weighed and transferred to a 100 mL volumetric flask and was shaken for 10 min. with 70 mL of methanol. Finally the volume was made up to mark with methanol This was filtered through Whatman filter paper No.41 and suitably diluted. Drug content was determined using U.V spectrophotometer at 269nm and 232nm respectively.

Results And Discussion-1 Formulation of Immediate release layer

Ingredients	IF1 (mg)	IF2 (mg)	IF3 (mg)	IF4 (mg)	IF5 (mg)	IF6 (mg)	IF7 (mg)	IF8 (mg)
Glimepiride	2	2	2	2	2	2	2	2
Pioglitazone Hydrochloride	16.71	16.71	16.71	16.71	16.71	16.71	16.71	16.71
Sodium starch glycolate	10	-	15	-	10	-	15	-
Crosscarmelose sodium	-	10	-	15	-	10	-	15
Microcrystalline cellulose	30	30	30	30	30	30	30	30
Mannitol	51.5	51.5	46.5	46.5	51.5	51.5	46.5	46.5
Stearic acid	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Talc	5	5	5	5	5	5	5	5

Formulation of Sustained release layer

Ingredients	SF1 (mg)	SF2 (mg)	SF3 (mg)	SF4 (mg)	SF5 (mg)	SF6 (mg)	SF7 (mg)	SF8 (mg)
Metformin Hydrochloride	500	500	500	500	500	500	500	500
HPMC K 4 M	60	60	80	80	-	-	-	-
HPMC K 100 M	-	-	-	-	60	60	80	80

Xanthan gum	15	15	15	15	15	15	15	15
Mannitol	36	36	16	16	36	36	16	16
PVP K 30	20	20	20	20	20	20	20	20
Magnesium Stearate	7	7	7	7	7	7	7	7
Talc	12	12	12	12	12	12	12	12

Identification study

a. Measurement of λ_{max} using U.V. Spectroscopy

The λ_{max} of Metformin hydrochloride, Glimepiride and Pioglitazone hydrochloride were found to be 233.5 nm, 229nm and 267.5nm respectively and it was as per official literature.

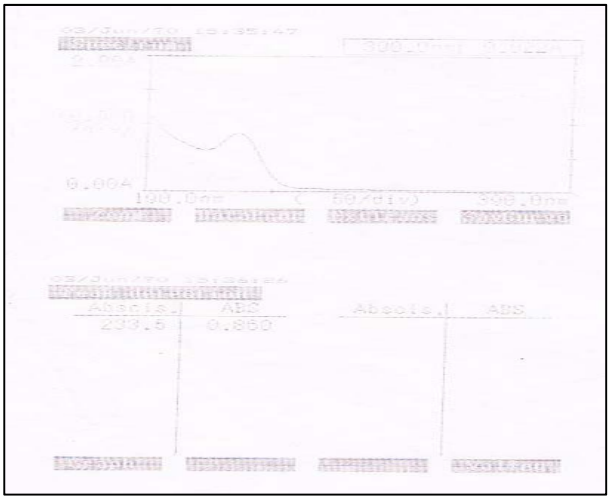


Fig 1: The spectrum of Metformin hydrochloride in distilled water using UV- spectrophotometer.

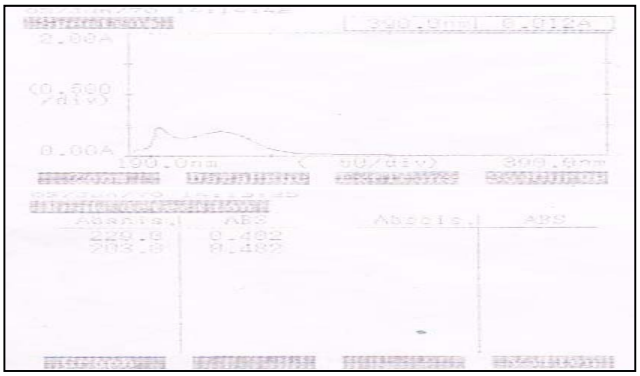


Fig 2: The spectrum of Glimepiride in methanol using UV spectrophotometer

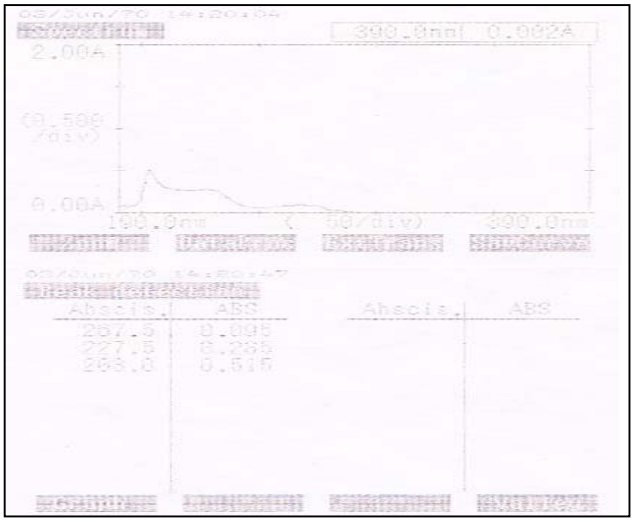


Fig 3: The spectrum of Pioglitazone hydrochloride in methanol using UV spectrophotometer.

Drug excipients interaction study using TLC-From TLC R_f value of Metformin hydrochloride, Glimepiride and Pioglitazone hydrochloride with all the excipients were found as follows. These results conclude there was no interaction that affects the drug during formulation. So these excipients were found compatible for development of formulation

R_f value of Metformin hydrochloride, Glimepiride and Pioglitazone hydrochloride alone.

S. No	Sample	Initial values	Value after 2 weeks
1.	Pure Metformin hydrochloride	0.36 ± 0.02	0.35 ± 0.02
2.	Pure Glimepiride	0.74 ± 0.03	0.75 ± 0.03
3.	Pure pioglitazone	0.48 ± 0.02	0.46 ± 0.02

* Values are expressed in Mean ± SD, n=3

Table 7.7: R_f value of Metformin hydrochloride, Glimepiride and Pioglitazone hydrochloride combined and with Excipients

S. No	Sample	Initial values			Value after 2 weeks		
		Metformin	Glimepiride	Pioglitazone	Metformin	Glimepiride	Pioglitazone
1.	Metformin: Glimepiride: Pioglitazone.	0.34±0.02	0.73 ± 0.03	0.48 ± 0.02	0.36± 0.02	0.75 ± 0.03	0.49 ± 0.02
4.	All Drug : Mg. Stearate	0.35±0.02	0.74 ± 0.03	0.48 ± 0.02	0.35± 0.02	0.73 ± 0.03	0.48 ± 0.02
5.	All Drug:PVP	0.36± 0.02	0.75 ± 0.03	0.49 ± 0.02	0.36± 0.02	0.73 ± 0.03	0.49 ± 0.02
4.	All Drug : HPMC	0.36± 0.02	0.73 ± 0.03	0.48 ± 0.02	0.3 ± 0.02	0.73 ± 0.03	0.47 ± 0.02
5.	All Drug : Guar Gum	0.31± 0.02	0.75 ± 0.03	0.49 ± 0.02	0.36± 0.02	0.73 ± 0.03	0.49 ± 0.02
6	All Drug :Lactose	0.34± 0.02	0.74 ± 0.03	0.48 ± 0.02	0.3 ± 0.02	0.72 ± 0.03	0.48 ± 0.02
7	All Drug:All Excipients	0.34± 0.02	0.75 ± 0.03	0.49 ± 0.02	0.36± 0.02	0.73 ± 0.03	0.49 ± 0.02

* Values are expressed in Mean ± SD, n=3

On the basis of above data drug R_f was not changed after addition of another drug and different excipients, hence there was no interaction observed.

Evaluation of Bilayer Tablets

Pre-compression Parameter

a) Angle of Repose

The angle of repose for the entire formulations blend was found to be in the range of 24.22° to 27.56°. This indicates excellent flow property of all formulation.

b) Compressibility Index

Compressibility Index was found to be in the range of 11.11% to 19.64%. This indicates good to fair flow property of all formulation.

c) Hausner's Ratio

Hausner's Ratio was found to be in the range of 1.125 to 1.244. This indicates good to fair flow property of all formulation.

Table 7.8: Evaluation of powder blend of Immediate release layer:

Batch Code	Bulk Density (gm/cm^3) ±SD	Tapped Density (gm/cm^3) ±SD	Angle of Repose (degree) ±SD	Compressibility Index (%) ±SD	Hausner's Ratio ± SD
IF1	0.30 ± 0.013	0.35 ± 0.023	28.14 ± 0.41	14.28 ± 0.08	1.166 ± 0.064
IF2	0.27 ± 0.025	0.29 ± 0.031	31.33 ± 0.54	6.89 ± 0.27	1.074 ± 0.075
IF3	0.32 ± 0.02	0.38 ± 0.023	27.05 ± 0.63	15.79 ± 0.08	1.187 ± 0.069
IF4	0.26 ± 0.022	0.29 ± 0.021	27.88 ± 0.44	10.34 ± 0.12	1.115 ± 0.055
IF5	0.29 ± 0.02	0.33 ± 0.015	27.66 ± 0.50	12.12 ± 0.17	1.137 ± 0.059
IF6	0.28 ± 0.012	0.31 ± 0.014	29.96 ± 0.53	9.67 ± 0.25	1.107 ± 0.068
IF7	0.33 ± 0.015	0.40 ± 0.024	26.22 ± 0.65	17.5 ± 0.14	1.212 ± 0.057
IF8	0.31 ± 0.025	0..34 ± 0.017	28.64 ± 0.49	8.82 ± 0.10	1.096 ±0.050

Table 7.9: Evaluation of powder blend of Sustained release layer

Batch Code	Bulk Density (gm/cm^3) ±SD	Tapped Density (gm/cm^3) ±SD	Angle of Repose (degree) ±SD	Compressibility Index (%) ±SD	Hausner's Ratio ± SD
IF1	0.37 ± 0.013	0.43 ± 0.016	29.34 ± 0.33	13.953 ± 0.07	1.162 ± 0.071
IF2	0.35 ± 0.025	0.40 ±0.043	26.94 ± 0.69	12.50 ± 0.092	1.143 ± 0.064
IF3	0.36 ± 0.02	0.41 ±0.022	27.48 ± 0.58	12.195 ± 0.22	1.139 ± 0.055
IF4	0.34 ± 0.022	0.38 ±0.032	28.12 ± 0.44	10.526 ± 0.19	1.117 ± 0.065
IF5	0.39 ± 0.02	0.46 ±0.025	30.08 ± 0.37	13.043 ± 0.09	1.179 ± 0.049
IF6	0.40 ± 0.012	0.45 ±0.022	29.47 ± 0.55	11.111 ± 0.23	1.125 ± 0.042
IF7	0.42 ± 0.015	0.47 ±0.026	27.38 ± 0.40	10.638 ± 0.15	1.119 ± 0.058
IF8	0.38 ± 0.025	0.44 ±0.031	28.64 ± 0.61	13.636 ± 0.10	1.157 ± 0.060

Post-compression Parameter

Physical tests of bilayer tablets

Table 7.10: Physical tests of bilayer tablets

Batch Code	Thickness (mm.)	Hardness (Kg)	Average weight (mg)	Friability (%)
F1	6.94±0.021	7.33±0.577	775.34±5.22	0.64±0.020
F2	7.15±0.024	7.66±0.577	774.35±4.49	0.57±0.025
F3	7.06±0.014	7.66±0.577	773.85±5.01	0.54±0.028
F4	6.98±0.018	8.0±1.00	774.69±4.76	0.61±0.012
F5	6.95±0.037	7.33±0.577	774.41±4.86	0.65±0.015
F6	7.07±0.038	8.33±1.155	774.42±3.72	0.57±0.025
F7	7.10±0.016	8.33±0.577	774.75±2.60	0.54±0.022
F8	7.12±0.037	7.33±1.155	774.575±4.69	0.57±0.016

* Values are expressed in Mean ± SD, n=3

All the batches of bilayer tablets were produced under similar conditions to avoid processing variables. Average weight of the optimized bilayer tablets F7 was 774.75±2.60 mg. Hardness was in the range of 8.33 kg thickness was in range of 7.10 mm. The percentage friability of all the formulations was in the range of 0.54%. Values of the hardness test and percentage friability indicate good wear and tear properties of the bilayer tablets.

Disintegration time of immediate release layer

Table 7.11: Disintegration time of Immediate release layer.

S.NO.	Batch Code	Disintegration time (sec.)
1.	F1	58.18 ± 0.20
2.	F2	61.28 ± 0.42
3.	F3	48.40 ± 0.57
4.	F4	53.57 ± 0.70
5.	F5	60.65 ± 0.62
6.	F6	61.88 ± 0.64
7.	F7	45.13 ± 0.51
8.	F8	52.07 ± 0.39

* Values are expressed in Mean ± SD, n=3

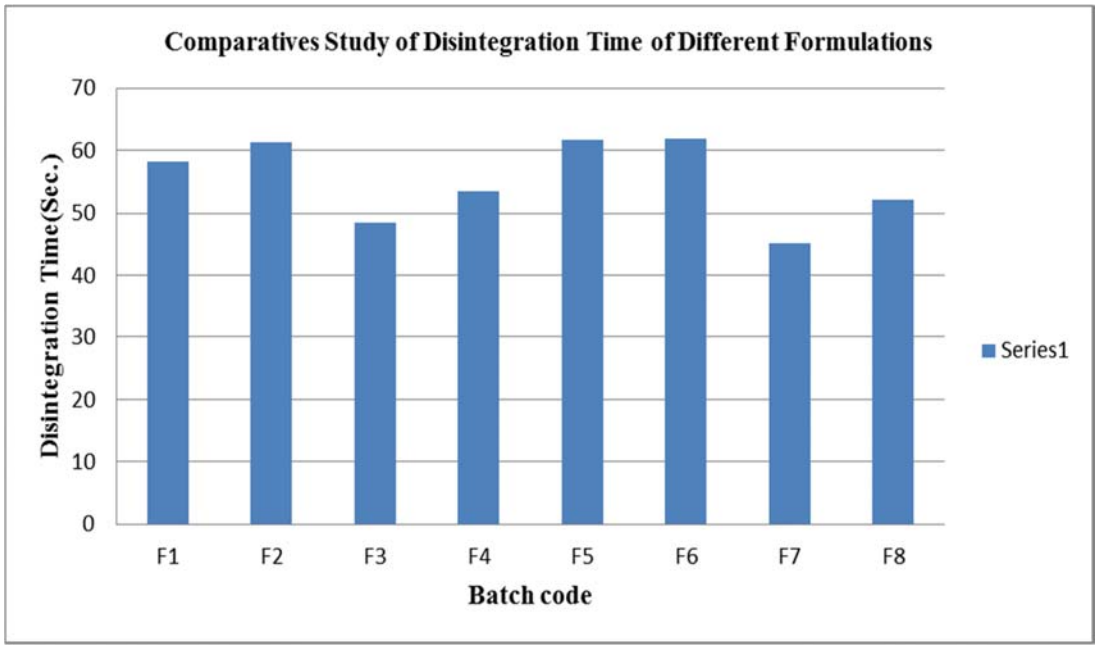


Fig 7.8: Comparatives Study of Disintegration time of Different Formulation Percent drug content of bilayer tablets

Table 7.12: Drug content of bilayer tablets

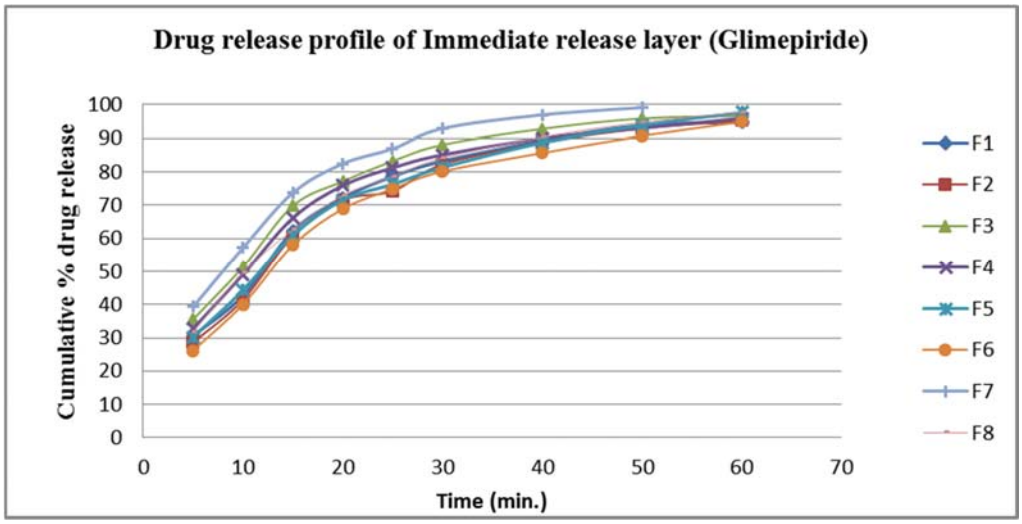
S.NO.	Batch Code	% Drug content		
		Glimepiride	Pioglitazone hydrochloride	Metformin hydrochloride
1.	F1	94.90 ± 0.024	95.06 ± 0.021	96.3 ± 0.045
2.	F2	95.31 ± 0.041	96.22 ± 0.035	98.37 ± 0.027
3.	F3	96.67 ± 0.032	96.95 ± 0.020	98.12 ± 0.038
4.	F4	96.06 ± 0.015	97.83 ± 0.031	95.88 ± 0.015
5.	F5	97.79 ± 0.031	97.13 ± 0.026	96.68 ± 0.033
6.	F6	96.92 ± 0.026	95.95 ± 0.025	97.92 ± 0.018
7.	F7	99.20 ± 0.019	99.16 ± 0.015	99.33 ± 0.026
8.	F8	97.95 ± 0.025	97.24 ± 0.032	97.82 ± 0.024

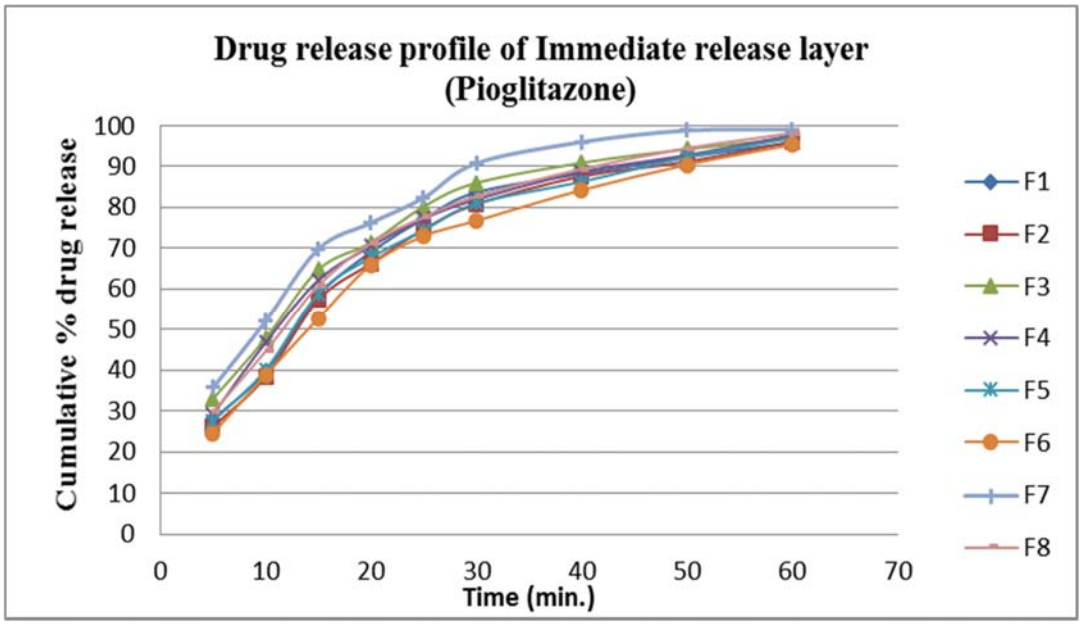
* Values are expressed in Mean ± SD, n=3.

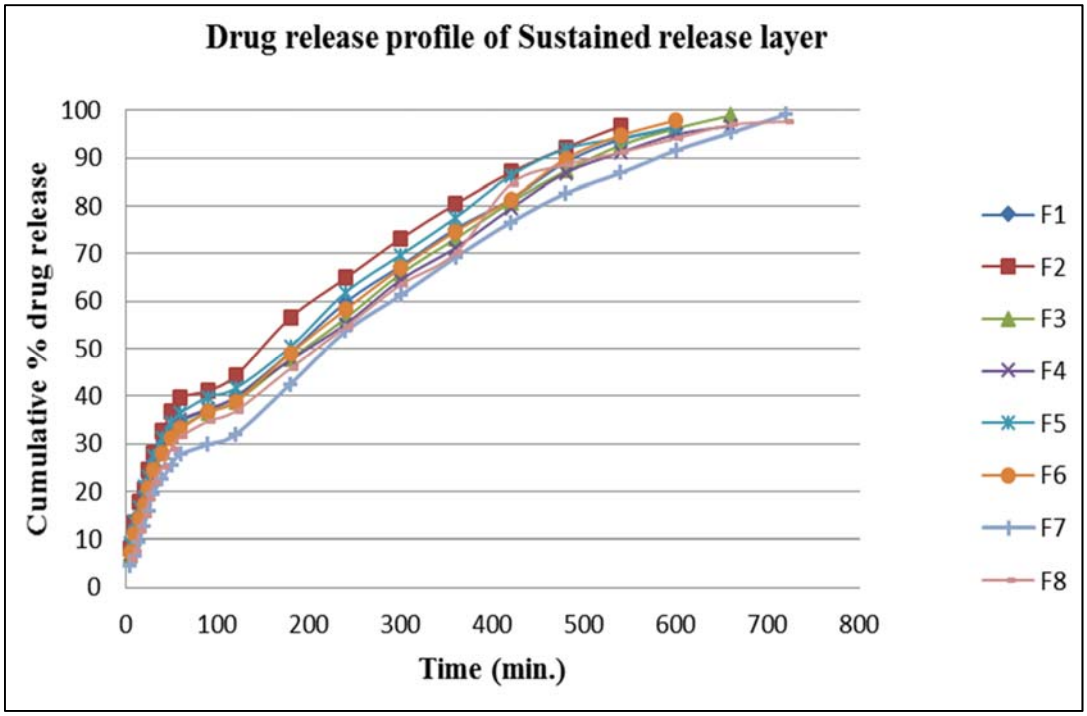
The drug content uniformity was estimated by HPLC method. The drug content uniformity in the bilayer tablets was found to be more than 95-99% in each formulation which is shown in table

***In vitro* drug release studies**

In vitro drug release studies of all formulations were carried out first 2 hours in 0.1 N hydrochloric acid using 0.1% SLS and then in phosphate buffer pH 6.8 for 12 hours as per Indian pharmacopoeia and cumulative drug release was calculated at specific time intervals and sample was analyzed using HPLC after proper dilution.







The drug release profile of Glimepiride, Pioglitazone hydrochloride and Metformin hydrochloride from the prepared formulations was analyzed by plotting the cumulative percent drug release *vs* time as shown in figure 7.9, 7.10 and 7.11. Over 45% of the Glimepiride and Pioglitazone hydrochloride was released within the first 1 hour of dissolution study. The Metformin hydrochloride was released within 12 hours. On the basis of above in-vitro drug release F7 batch was optimized and selected for further studies.

Kinetic analysis of dissolution data

In this selected formulation, the calculated regression coefficients of Glimepiride and pioglitazone for zero order, first order, Higuchi and korsmeyer-peppas models were 0.8001 and 0.7928, 0.9205 and 0.9194, 0.9091 and 0.9054, 0.9452 and 0.9421 respectively. Therefore the release pattern seems to fit the korsmeyer-peppas and First order model. To explore the release pattern, results of *in vitro* dissolution data were fitted to the Korsmeyer and Peppas equation which characterizes the transport mechanism.

Stability testing of formulated bilayer tablet of optimized batch

The formulated bilayer tablets were kept at different storage conditions. The test samples were kept at was kept at 25 ºC±2 ºC, 60% RH and at 40 ºC±2 ºC, 75% RH according to ICH guidelines. The Hardness, friability, drug content of the tablets was determined initially and then at the interval of 15 days and one month. The hardness, friability and drug content of the optimized formulation after 30 days were reported in table

Stability testing of optimized batch (F7) of bilayer tablets.

S. No	Parameters		Storage Condition					
			25°C±2°C, 60% RH			40°C±2°C, 75% RH		
			0 days	15 days	30 days	0days	15 days	30days
1.	Hardness (kg/cm^3)		8.33±0.577	8.33±0.577	8.33±0.577	8.33 ± 0.577	8.33±0.577	8.33±0.577
2.	Friability (%)		0.54±0.022	0.54±0.035	0.55±0.019	0.54 ± 0.022	0.57±0.030	0.59±0.024
3.	% Drug content	G	99.2±0.019	99.18±0.24	99.08±0.046	99.2 ± 0.019	99.12±0.033	98.98±0.028
		P	99.16±0.015	99.14±0.043	99.02±0.034	99.16±0.015	99.02±0.019	98.93±0.076
		M	99.33±0.026	99.29±0.021	99.12±0.057	99.33±0.026	99.24±0.045	99.09±0.037

* Values are expressed in Mean ± SD, n=3.

The formulated bilayer tablets (optimized batch F7) were kept at different storage conditions at 25 °C±2 °C, 60% RH and at 40°C±2°C, 75% RH according to ICH guidelines. The hardness, friability, drug content of bilayer tablets F7 was determined initially and after one months. The hardness, friability and drug content of the optimized formulation after one month were reported in table 7.21. The formulated bilayer tablet F7 was found to be stable under the conditions of 25 °C±2 °C, 60% RH and at 40 °C±2 °C, 75% RH for the period of one month. Preparation of calibration curves using HPLC-

The isocratic mobile phase consisted of methanol–phosphate buffer (pH 4.3) in the ratio of 75:25, v/v, flowing through the column at a constant flow rate of 1.0 mL/min. An Phenomenex Luna C-8 Column (25cm x 5µm x 4.6mm) was used as the stationary phase. Metformin hydrochloride, Pioglitazone, and Glimepiride hydrochloride have different λmax (235, 265, 227 nm, respectively) but considering chromatographic parameter, sensitivity, and selectivity of the method for all three drugs, 251 nm was selected as detection wavelength for UV–PDA detector.

Table. Calibration Curves of Metformin hydrochloride

Sr. No.	Concentration (µg/ml)	Peak area
1	10	1692924
2	20	2827708
3	30	3953857
4	40	5054087
5	50	6230017

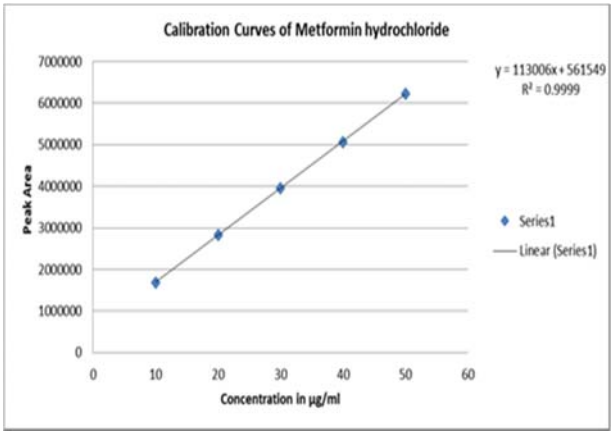
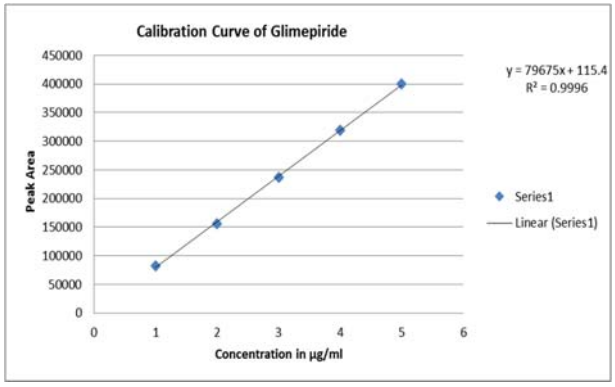


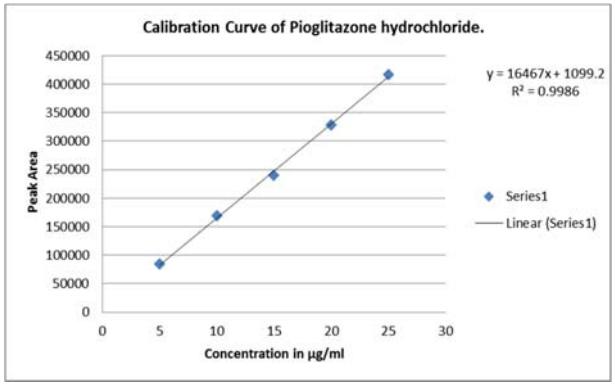
Table. Calibration Curves of Glimepiride

Sr. No.	Concentration (µg/ml)	Peak area
1	1	82773
2	2	156546
3	3	237416
4	4	319092
5	5	399875



Calibration Curves of Pioglitazone hydrochloride

Sr. No.	Concentration (µg/ml)	Peak area
1	5	85162
2	10	169244
3	15	240375
4	20	328546
5	25	417182



Determination of Solubility using HPLC

Mobile Phase - Methanol: Phosphate Buffer (pH 4.3) in ratio 75:25

Table. Determination of Solubility of Metformin hydrochloride, Glimepiride and Pioglitazone hydrochloride.

S. No	Drug	Solvents	Peak area	Dilution factor	Solubility(mg/ml)
1.	Metformin hydrochloride	a) Water	573420	10^4	308
		b) 0.1 HCL	515810	10^4	274
		c) Phosphate buffer(pH6.8)	483617	10^4	255
2	Glimepiride	a) Water	224606	_	0.00157
		b) 0.1 HCL	3838708	10	0.485
		c) Phosphate buffer(pH6.8)	2552707	10	0.318
3	Pioglitazone hydrochloride	a) Water	1834278	10	0.384
		b) 0.1 HCL	1985110	10	0.416
		c) Phosphate buffer(pH6.8)	1900267	10	0.398

Summary and Conclusion

Metformin is a biguanide hypoglycemic agent used in the treatment of non-insulin-dependent diabetes mellitus. It improves glycemic control by improving insulin sensitivity and decreasing intestinal absorption of glucose. Glimepiride is a medium-to-long acting sulfonylurea anti-diabetic drug. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors. Pioglitazone is a prescription drug of the class thiazolidinedione (TZD) with hypoglycemic action. It is a selectively stimulates nuclear receptor PPAR-gamma is used for the treatment of diabetes mellitus type 2. In the present research work, it was planned to develop a bilayer tablets of an antidiabetic agents containing Metformin, Glimepiride and Pioglitazone hydrochloride. The drug released by the initial pulse quickly attains the therapeutic plasma drug levels and the slow onset of action of sustained release layer for prolonged period of time. Dissolution rate of Glimepiride and Pioglitazone hydrochloride from fast release layer was increased using combination of superdisintegrants, sodium starch glycolate and cross carmelose sodium & sustained release action was show using sustained release polymer HPMC K4M and HPMC K100M.

Characterization of drug was carried out by employing various analytical techniques such as spectrophotometric analysis, melting point determination and thin layer chromatography. The drug sample metformin hydrochloride, glimepiride and pioglitazone hydrochloride exhibited peaks at 233.5 nm, 229nm and 267.5nm in water, methanol and methanol, respectively and it was as per official literature. Melting point of metformin hydrochloride, glimepiride and pioglitazone hydrochloride was found to be 220 ºC, 208 ºC and 195 ºC respectively. R_f value of metformin Hydrochloride, glimepiride and pioglitazone hydrochloride was found to be 0.36, 0.74, 0.48.

In this selected formulation, the calculated regression coefficients of metformin hydrochloride for zero order, first order, Higuchi and korsmeyer-peppas models were 0.9652, 0.7443, 0.9947 and 0.9846 respectively.

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