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Research Article

FORMULATION AND EVALUATION OF CYCLODEXTRIN INCLUSION COMPLEX TABLETS OF WATER INSOLUBLE DRUG-GLIMIPIRIDE

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ABSTRACT

Glimepiride (GMP) is a Third Generation Sulphonylureas used for treatment of type 2 diabetes. Poor water solubility is the main constraint for its oral bioavailability. The rationale of this study was to enhance the solubility & dissolution of the drug by preparing its complex with β-CD and HP-β-CD. In the present study attempt has been made to prepare, formulate and characterize inclusion complexes of Glimepiride with β-CD and HP-β-CD. The inclusion complexes were prepared by three different methods viz. Physical, Kneading and Co-precipitation method. The inclusion complex containing GMP: β-CD and HP-β-CD was further formulated into Tablets by Direct Compression Technique using Super-disintegrants like Crospovidone and icrocrystalline cellulose. The prepared Tablets were characterized using FT-IR and DSC and finally the prepared Tablets were Evaluated for various pharmaceutical characteristics viz. Hardness, % Friability, Weight Variation, Drug Content and *In-vitro* Dissolution profiles. The results of stability studies revealed no change in physical appearance, hardness, drug content and *in vitro* dissolution profiles, thus indicating that formulation was stable.

Keywords: Glimepiride, β-CD& HP-β-CD, Inclusion Complexation, Direct compressed Tablets, Super-disintegrants.

INTRODUCTION

Drug release is a crucial and limiting step for oral drug bioavailability, particularly for drugs with low gastrointestinal solubility and high permeability. By improving the drug release profile of these drugs, it is possible to enhance their bioavailability and reduce their side effects. Solid dispersions are one of the most successful strategies to improve the drug release of poorly soluble drugs[1]. Presenting the compound as the molecular dispersion combining the benefits of a local increase in the solubility (within the solid solution) and maximizing the surface area of the compound that comes in contact with the dissolution medium as the carrier dissolves[2]. The large surface area of the resulting suspension should result in an enhanced dissolution rate and thereby improved bioavailability[3]. The advantage of solid dispersions over other approaches is that many of the carriers that can be applied are already extensively used in the pharmaceutical industry as excipients, so additional toxicity studies above and beyond what is required for the drug itself should not be required. The possibility of combining several carriers to produce an optimized product further extends the range of possibilities for formulation[4].

Glimepiride, 1-(p-(2-(3-ethyle-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl) phenyl) sulfonyl)-3-(trans-4-methylcyclohexyl) urea is a third-generation sulfonylurea used for oral treatment of type 2 diabetes[5,6]. It causes an intensification of insulin secretion by the β-cells of the pancreas by closing the potassium channels and depolarizing the cell membrane; this leads to the initiation of metabolic processes which result in a release of insulin[7]. Glimepiride is a white or off white crystalline

powder, relatively insoluble in water, but the predicated water solubility is (1.6µg/ml) (pKa=6.2).Which causes large variations in its bioavailability[8]. Also, during storage, the excipients may interact with the drug and affect its dissolution characteristics. There are several reports showing marked changes due to aging which adversely affect dissolution and, hence, the bioavailability of oral sulphonylurea drugs[9,10]. To overcome these difficulties, several approaches have been used, namely, the formation of a complex between Glimepiride and β-CD, Hydroxylpropyl- β-CD or sulfobutylether-β-CD in presence and absence of different water soluble polymers [8,11,12].

Cyclodextrins are cyclic oligosaccharides, containing six, seven or eight glucopyranose units (α, β or γ respectively) obtained by the enzymatic degradation of starch[13]. These are torus shaped molecules with a hydrophilic outer surface and lipophilic central cavity, which can accommodate a variety of lipophilic drugs. Cyclodextrins are able to form inclusion complexes with poorly water-soluble drugs and have been shown to improve pharmaceutical properties like solubility, dissolution rate, bioavailability, stability and even palatability without affecting their intrinsic lipophilicity or pharmacological properties. Out of the three parent cyclodextrins, β-cyclodextrin (β-CD) appears most useful as a pharmaceutical complexing agent because of its complexing ability, low cost and other properties[13,14]. Natural cyclodextrins have limited water solubility. However, a significant increase in water solubility has been obtained by alkylation of the free hydroxyl groups of the cyclodextrins resulting in hydroxyalkyl, methyl and sulfobutyl derivatives. The ability of cyclodextrins to form inclusion complexes may also be enhanced by substitution on the hydroxyl group.

The objective of present study is to prepare inclusion complexes of Glimepiride with cyclodextrins in different molar ratios by different methods such as physical, kneading and co-precipitation method the inclusion complexs was further formulated into Tablets by Direct Compression Technique using Superdisintegrants in order to increase the solubility of Glimepiride for improvement of dissolution rate and bioavailability of the drug in the prepared Tablet formulations.

EXPERIMENTAL

Materials

Glimepiride was a gift sample obtained from M/s. Amsal Chem Pvt. Ltd. Mumbai, India. And all other excipients such as β-Cyclodextrin, Hyroxypropyl-β-Cyclodextrin, Crospovidone, Microcrystalline cellulose (Avicel pH-102),Talc Magnesium Stearate andwere procured from M/s. Yarrow Chem Products., Mumbai, India.

Methods

Phase Solubility Studies

Phase solubility studies were carried out according to the method reported by Higuchi and Connors[15]. An excess of Glimepiride (200 mg) was added to 15 ml portions of distilled water, each containing variable amount of β -CD or HP- β -CD such as 0, 1, 3, 6, 9, 12, and 15 x 10-3 moles/liter. All the above solutions with variable amount of β -CD or HP- β -CD were shaken for 72 hours. After shaking, the solutions were filtered and their absorbance was noted at 228 nm [16]. The solubility of the Glimepiride in every β -CD or HP- β -CD solution was calculated and phase solubility diagram was drawn between the solubility of Glimepiride and different concentrations of β -CD or HP- β –CD as shown in Figure 1.

Preparation of Cyclodextrin Inclusion Complexes

Physical mixture[17]

GMP with β -CD in different molar ratios (i.e. 1:1M, 1:2M) and with HP- β -CD in ratio (i.e., 1:1M) were mixed in a mortar for about one hour with constant trituration, passed through sieve No. 80 and stored in a desiccators over fused calcium chloride.

Kneading method[17]

GMP with β -CD in different molar ratios (i.e. 1:1M, 1:2M) and with HP- β -CD in ratios (i.e.1:1M) were taken. First cyclodextrin is added to the mortar, small quantity of 50% ethanol is added while triturating to get slurry like consistency. Then slowly drug is incorporated into the slurry and trituration is further continued for one hour. Slurry is then air dried at 25C for 24 hours, pulverized and passed through sieve No. 80 and stored in desiccators over fused calcium chloride.

Co-precipitate method[17]

GMP was dissolved in ethanol at room temperature and β -CD & HP- β -CD was dissolved in distilled water. Different molar ratios of GMP and β -CD (1:1M and 1:2 M) and GMP and HP- β -CD (1:1 M) were taken. The mixture was stirred at room temperature, for one hour and then slowly evaporated on a boiling water bath. The inclusion complex precipitated as a crystalline powder was pulverized and passed through sieve No. 80 and stored in a desiccator till free from any traces of the organic solvent.

Drug Content Estimation[18]

50 mg of complex was accurately weighed and transferred to 50 ml volumetric flask and volume was made up to the mark with methanol. From this 1ml was taken in 10 ml volumetric flask and the volume is adjusted up to the mark with same solvent. The absorbance of the solution was measured at 228 nm using appropriate blank. The drug content of GMP was calculated using calibration curve.

IR Spectroscopy

The IR spectra of GMP and their complexes were obtained by KBr pellet method by JASCO FT/IR-5300 spectrometer.

Differential Scanning Calorimetry (DSC)

The samples were analyzed by DSC using a Mettler Toledo SR System. The samples were placed into pierced aluminum container.

Formulation of GMP –Cyclodextrin Inclusion Complex Tablets

The complex of GMP -HP- β -CD was prepared into tablet by direct compression method containing 100 mg of GMP. The Complex, Crospovidone and Microcrystalline cellulose were passed through sieve # 80 & # 24 respectively. All the above ingredients were properly mixed together. Finally Talc and Magnesium Stearate were added and mixed for 5 minutes. The mixed blend of drug and excipients was then compressed in to tablet by using single punch tablet compression machine. The composition of different tablet formulations are shown in table 2.

Evaluation of Tablet[19]

The prepared tablets were evaluated for weight variation, hardness and friability. The USP weight variation test is done by weighing 20 tablets individually, calculating the average weight and comparing the individual weights to the average. The hardness of each batch of tablet was checked by using Monsanto hardness tester. The hardness was measured in terms of kg/cm2. The hardness of 6 tablets was determined using the Monsanto hardness tester. Friability was determined by first weighing 10 tablets after dusting and placing them in a friability tester (Roche friabilator), which was rotated for 4 min at 25 rpm. After dusting, the total remaining mass of tablet was recorded and the percent friability was calculated. The evaluation results of the different tablet formulations are shown in table 4.

***In vitro* dissolution studies for Glimepiride Cyclodextrin Inclusion Complex Tablets**[20]

In-vitro dissolution of Tablet was studied in USP XXIV dissolution apparatus (Electrolab) employing a paddle stirrer. 900 ml of phosphate buffer of pH 7.4 was used as dissolution medium. The stirrer was adjusted rotate at 75 rpm. The temperature of dissolution media was previously warmed to 37 ± 0.5°C and was maintained throughout the experiment. 1 ml of sample of dissolution medium were withdrawn by means of syringe fitted with pre-filter at known intervals of time and analyzed for drug release by measuring the absorbance at 228 nm after suitable dilution with phosphate buffer pH 7.4. The volume withdrawn at each time interval was replaced with fresh quantity of dissolution medium. Percentage amount of GMP released was calculated and plotted against time. The comparative graphs of the i*n- vitro* dissolution studies of Glimepiride Cyclodextrin Inclusion Complex Tablets are shown in Figure 4 & 5 and 6. For comparison, the dissolution studies of marketed tablet was studied. The comparison results of the tablets are shown in table 7 and the comparative graph is shown in Figure 7.

Stability Studies

The optimized formulation was subjected for two month stability study according to ICH guidelines**.** The selected formulations were packed in aluminium foils, which were in wide mouth bottles closed tightly. They were then stored at Room Temperature 40ºC / 75% RH for 2 months and evaluated for their permeation study.

RESULTS AND DISCUSSION

Phase Solubility Studies

The complexation of GMP with β -CD and HP-β -CD was investigated by Phase Solubility Studies. The phase solubility diagram for complex formation is shown in fig.1.The aqueous solubility of GMP was increased linearly as a function of concentration of CD. The phase solubility diagram can be classified as type AL according to Higuchi and Connors. It is assumed that the increase in solubility observed was due to the formation of a 1:1 M inclusion complex. The solubility constant (Kc) was calculated from the slope of the linear plot of the phase solubility diagram according to equation,

$$K_{a:b} = \frac{\text{slope}}{S_0\,(1\text{-slope})}$$

Where **So** is the solubility of the drug in absence of CD. The calculated Kc value was 32.95 M^{-1}
and = 42.57 M^{-1} with β -CD and HP- β -CD respectively.(Table 3, fig:1)

Evaluation of tablet
Hardness, % friability, weight variation and drug content of tablets is given in table 4. The hardness of tablets was in the range 4 - 4.5 kg/sq.cm. The percent weight loss in the friability test was less than 1 %. The tablets were found to contain the GMP within 100 ± 2% of the label claim. Dissolution of **TF_6** tablet shows higher dissolution (97.41%) than the marketed tablet (**MT**) (85.06 %) shown in Fig. 7.

Drug Content Estimation
The inclusion complexes prepared by physical mixture and kneading method showed nearly 100 % drug content. But the inclusion complexes prepared by Co-precipitate method were found to be slightly less.

IR Spectroscopy
IR Spectra of pure drug and inclusion complexes tablets of GMP with β -CD and HP- β -CD prepared by different methods are given in Fig. 6. As clearly seen from the spectra, the characteristic peaks of GMP at 709, 1082, 1444, 1674, 1705, 2360 and 3369 were modified significantly as a result of complex formation.

Differential Scanning Calorimetry (DSC)
The thermal behavior GMP -HP-β-CD complex was studied using DSC in order to confirm the formation of complex. DSC thermogram of GMP, HP-β-CD and **TF_6** Tablets are shown in Fig. 7. The DSC thermogram of GMP showed an endothermic peak at 214°C corresponding to its melting point. The thermogram of **TF_6** showed endothermic peak at 237°C which is different from the pure drug, which gives clear evidence that there is formation of the complex.

***In vitro* dissolution study**
The dissolution characteristics of GMP (pure drug) and inclusion complex tablets are shown in Fig. 4, 5 and 6. The inclusion complexe tablets produces pronounced enhancement in its dissolution rate than pure drug. The inclusion complexes prepared with HP-β-CD shows higher dissolution rate than the inclusion complexe tablets prepared with β-CD. Among these the complex tablets prepared with HP-β-CD i.e. formulation **TF_6** shows higher dissolution rate than the other methods.

Stability Studies
The selected formulation **TF_6** was subjected to accelerated stability studies for 60 days at Room Temperature 40^0C / 75% RH, *in vitro* permeation study was performed on every week and showed negligible change in permeation profile. The formulation subjected for stability studies was found to have no change in the physical appearance and drug content

CONCLUSION
Cyclodextrins like β -CD and HP- β -CD can be used to prepare inclusion complex tablets of GMP with improved solubility of the drug. GMP formed inclusion complex tablets with β -CD and HP- β -CD in 1:1 M ratio. All inclusion complex tablets showed increase in dissolution rate than pure drug. The inclusion complex tablets prepared with HP- β -CD by kneading method **TF_6** Tablet showed higher dissolution rate than marketed GMP tablet.

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Table 1: Different Formulations of Glimepiride with β-Cyclodcxtrin and Hydroxypropyl-β-Cyclodextrin in Molar Ratio

Method	Drug to carrier Complex	Drug to Carrier Ratio	Code for Complex
Physical Mixture	GMP:β-CD	1:1	TF_1
	GMP:β-CD	1:2	TF_2
	GMP:HP-β-CD	1:1	TF_3
Kneading Method	GMP:β-CD	1:1	TF_4
	GMP:β-CD	1:2	TF_5
	GMP:HP-β-CD	1:1	TF_6
Co-Precipitation Method	GMP:β-CD	1:1	TF_7
	GMP:β-CD	1:2	TF_8
	GMP:HP-β-CD	1:1	TF_9
Pure Drug Glimepiride			TF_0

Table 2: Formula for Preparation of GMP: β-CD (1:1 M) & (1:2) Tablets and GMP: HP-β-CD (1:1 M)

SL. No.	Ingredients	Quantity (For 10 Tablets) (1:1)	Quantity (For 10 Tablets) (1:2)	Quantity (For 10 Tablets) (1:1)
1	GMP : β-CD	830.00 mg	1410.00 mg	-
2	GMP : HP-β-CD	-	-	1040.00 mg
3	Crospovidone	100.00 mg	100.00 mg	100.00 mg
4	MCC (Avicel pH-102)	930.00 mg	350.00 mg	720.00 mg
5	Talc	20.00 mg	20.00 mg	20.00 mg
6	Magnesium Stearate	20.00 mg	20.00 mg	20.00 mg

Table 3: Phase Solubility Studies of Glimepiride: β- Cyclodextrin Complexes and Glimepiride: HP-β-Cyclodextrin Complexes

Concentration of β-CD (mM)	Concentration of GMP (mM)	Concentration of HP-β-CD (mM)	Concentration of GMP (mM)
0	0.04	0	0.04
3	0.043	3	0.045
6	0.047	6	0.05
9	0.051	9	0.055
12	0.055	12	0.06
15	0.059	15	0.065

Table 4: Evaluation of Tablets Containing Glimepiride with β-CD (l: l M) (1: 2 M) and HP-β-CD (l: l M) Inclusion Complexes

Formulation Code	Hardness	% Friability	Weight Variation (%)	Drug Content (%)
TF_1 Tablet	3.2 ± 0.22	0.27 ± 0.11	192 ±0.07	98.01±0.11
TF_2 Tablet	3.3 ± 0.36	0.35 ± 0.17	190 ± 0.01	98.11 ± 0.12
TF_3 Tablet	3.3 ± 0.52	0.16 ± 0.13	183 ± 0.04	98.32 ± 0.15
TF_4 Tablet	3.1 ± 0.55	0.22 ± 0.11	197 ± 0.03	99.14 ± 0.20
TF_5 Tablet	3.0 ± 0.36	0.34 ± 0.l2	195 ± 0.02	99.32 ± 0.15
TF_6 Tablet	3.2 ± 0.14	0.50 ± 0.18	179 ± 0.05	99.52 ± 0.07
TF_7 Tablet	3.3 ± 0.27	0.32 ± 0.10	187 ± 0.07	97.51 ± 0.13
TF_8 Tablet	3.2 ± 0.23	0.27 ± 0.l2	195 ± 0.05	97.72±0.12
TF_9 Tablet	3.2 ± 0.25	0.22 ± 0.14	192 ± 0.05	98.15±O.16

Table 5: Comparision of TF_6Tablets Containing Glimepiride and HP-β-CD with Marketed Product

Formulation code	Hardness	% Friability	Weight Variation (%)	Drug Content (%)
TF6	3.2 ± 0.14	0.50 ± 0.18	190 ± 0.05	99.52 ± 0.07
Marketed Tablet	3.5 ± 0.25	0.37 ± 0.14	200 ± 0.07	97.46 ± 0.41

Table 6: In- vitro Drug Dissolution Profile of different formulation in Phosphate Buffer pH 7.4

TIME (Min)	PERCENT DRUG RELEASE								
	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8	TF9
0	0	0	0	0	0	0	0	0	0
30	21.11	23.21	26.07	23.39	26.09	31.25	22.11	24.27	27.91
60	43.27	49.11	51.21	46.21	51.11	56.27	43.19	49.15	54.27
120	64.19	67.09	69.14	68.49	71.29	78.41	65.55	68.27	72.54
180	72.15	77.43	80.79	76.13	82.21	85.23	73.29	81.39	83.51
240	83.31	85.44	93.36	88.25	92.44	98.14	84.44	88.72	95.15

Table 7: Comparison of Drug Release Profile of TF_6 Tablets (Best Release) with Marketed Tablets (Glimy)

	PERCENT DRUG RELEASE	
Time (Min)	TF_6 Tablets	Marketed Tablets (Glimy)
0	0	0
30	31.34	21.27
60	56.32	38.32
120	78.45	53.41
180	85.36	68.25
240	98.21	85.06

Table 8: Short Term Stability Studies of Glimepiride Complex (F_6) at RoomTemperature and at 40°C

Time (min)	Percent Drug Content at Room Temperature	Percent Drug Content at 40°C
First day	99.57	99.57
1st week	99.52	99.47
3rd week	99.47	99.42
6th week	99.41	99.37

Table 9: Dissolution Studies of Glimepiride Complex (F_6) after Storage for Six Weeks at Room Temperature and 40°C

Sl.No	Percent Drug Release Stored at Room Temperature	Percent Drug Release Stored at 40°C
0	0	0
30	76.90	75.51
60	96.92	96.14

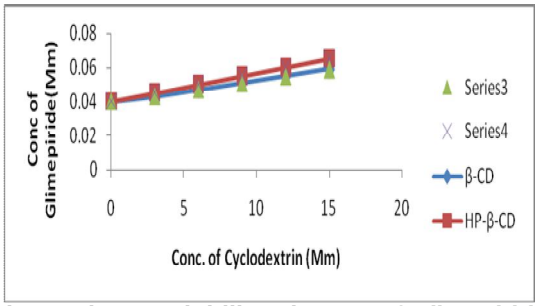

Fig. 1: Phase Solubility Diagram of Glimepiride with β-CD and HP-β-CD

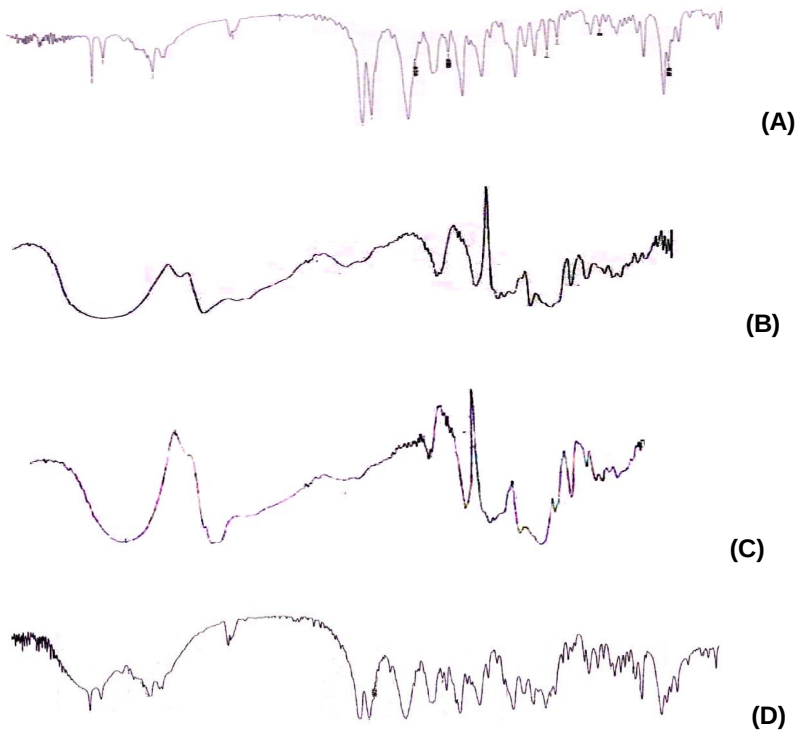

Fig. 2: F.T.I.R. Spectra of (A)GMP, (B) β-CD, (C)HP-β-CD and (D) TF6 Tablets

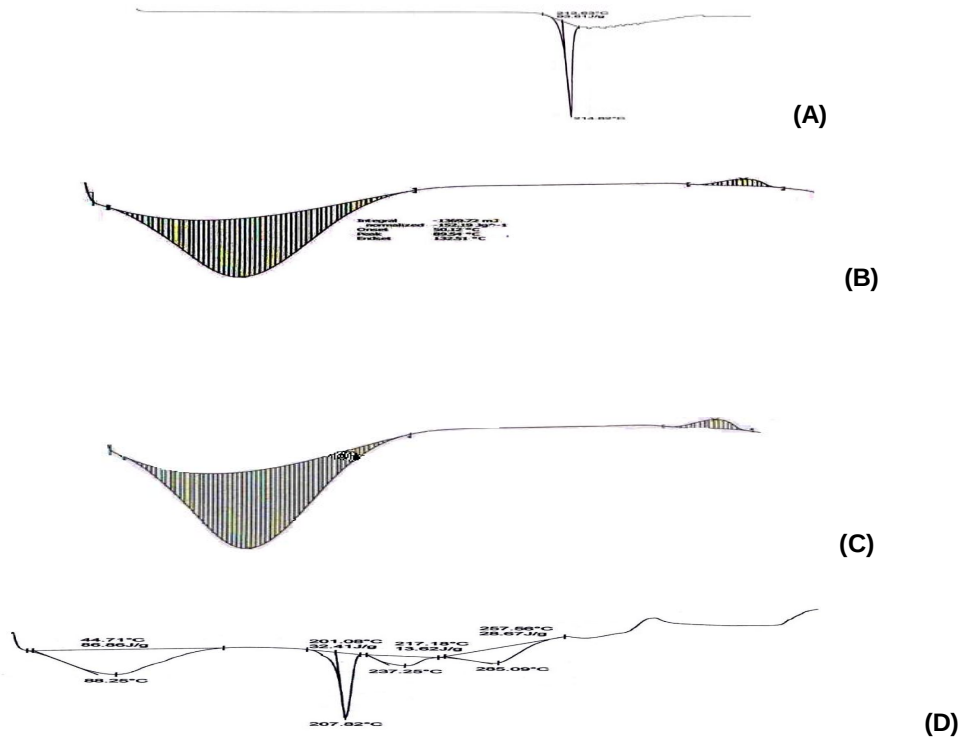

Fig. 3: DSC Thermograms of (A) GMP, (B) β-CD (C) HP-β-CD and (C) TF6 Tablets

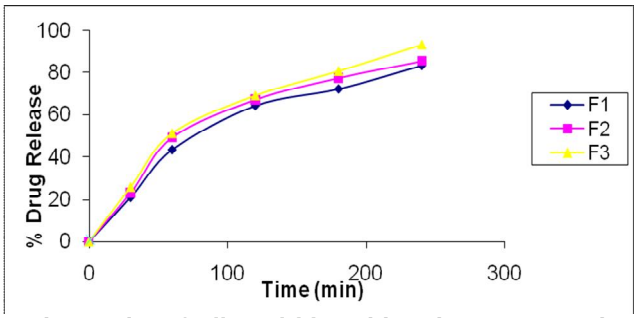

Fig. 4: Plot of Glimepiride Tablets (TF_1, TF_2 & TF_3) in Phosphate Buffer pH 7.4

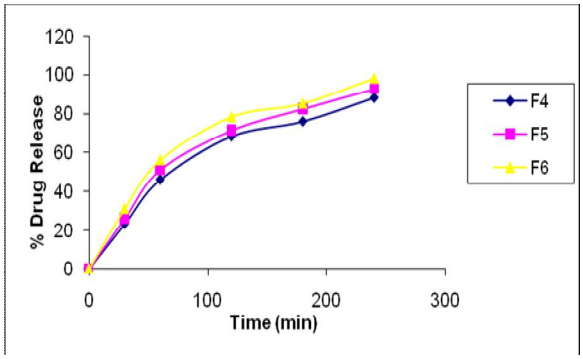

Fig. 5: Plot of Glimepiride Tablets (TF_4, TF_5 & TF_6) in Phosphate Buffer pH 7.4

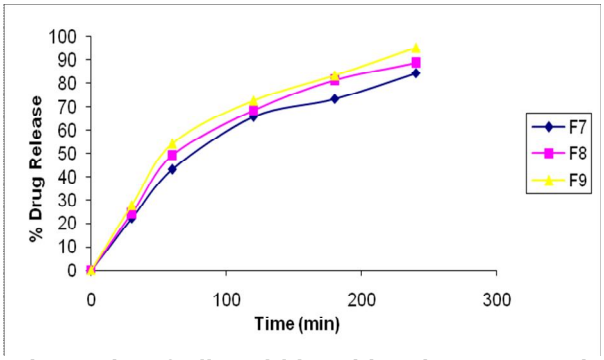

Fig. 6: Plot of Glimepiride Tablets (TF_7, TF_8& TF_9) in Phosphate Buffer pH 7.4.

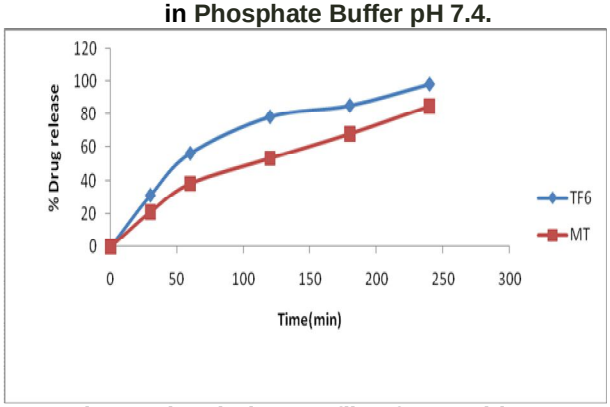

Fig. 7: Dissolution Profile of TF_6 Tablets and Marketed Tablets

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