Research

FORMULATION AND EVALUATION OF METFORMIN , GLIBENCLAMIDE TABLETS AND ANALYSING ALPHA AMYLASE INHIBITION WITH IT'S DIFFERENT COMPOSITIONS OF TABLET FORM DRUG AND NANO-PARTICLED SIZED DRUGS

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1. ABSTRACT

The present investigation deals with the comparison of nano-particled and Tablet form drug combination of Metformin and Glibenclamide. From the investigation we conclude that the Nano particled combination possess significant inhibition activity when compared to that of the normal sized drug. We conclude that the work ends with the decision that the nano particled size have absorption rate was than the normal sized drug. Metformin Hcl is antihyperglycemic agent used in the treatment of type 2 Non Insulin Dependent Diabetes Mellitus. The extended release formulation of Metformin Hcl (MER), prolongs drug absorption in the upper gastrointestinal tract and permits once daily dosing in patient with Type 2 Diabetes Mellitus .This newer formulation may enhance patient compliance with oral therapy compared to conventional immediate release (MIR) Metformin Hcl in Type 2 Diabetes Mellitus Extended release formulation of Metformin Hcl presents significant challenges due to its poor inherent compressibility, high dose and high water solubility. Glibenclamide matrix sustained release tablet which can release the drug up to time of 14 hrs in predetermined rate. The formulation of Glibenclamide matrix tablet was prepared by the polymer combination in order to get required theoretical release profile. The influence of hydrophilic and hydrophobic polymer on Glibenclamide was studied. Different release models like zero order, first order, higuchi, and krosmeyer-peppas were applied to invitro drug release data in order to evaluate the drug release mechanisms and kinetics. All the tablets formulation showed acceptable pharmaco technical properties and complied with pharmacopoeial standards.

Keywords: Metformin, Glibenclamide, Nano particles, Anti-diabetic, Alpha amylase inhibition

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2.INTRODUCTION

Oral drug delivery is the largest and oldest segment of the total drug delivery market. It is the fastest growing and most preferred route for drug administration. Use of hydrophilic matrices for oral extended release of drugs is common practice in the pharmaceutical industry. However, also drugs with long half-life qualify if a reduction in steady state fluctuation is desired. With many drugs, the basic goal of therapy is to achieve a steady-state blood level or tissue level that is therapeutically effective and non toxic for an extended period of time. production and improves insulin sensitivity by increasing peripheral glucose uptake. Because of its shorter and variable biological half-life of 1.5—4.5 hr, it should be repeatedly administered (500 mg thrice a day) to maintain effective plasma concentration. In spite of its favorable clinical response and lack of significant draw backs, chronic therapy with Metformin Hcl suffers from certain problems of which the most prominent is the high dose (1.5 – 2.0 g/day)low bio-availability (60%) and high incidence of gastrointestinal tract (GIT) side effect (30% case).Therefore, there were continued efforts to improve the pharmaceutical formulation of metformin hydrochloride in order to achieve an optimal therapy.These efforts mainly focus on extended release of drug.Administration of a extended release, once-a-day Metformin Hcl dosage form could reduce the dosing frequency and improve patient compliance. Glibenclamide is a second generation Sulfonylurea compound used as an oral hypoglycemic or antidiabetic agent[6] Therapy with Glibenclamide is usually initiated with 2.5mg given once daily. The maximal recommended daily dose is 20mg. Glibenclamide is 200 times more potent than tolbutamide in evoking pancreatic secretion of insulin. It differs from other oral poglycemic drugs where in tolerance to this action apparently does not occur. It also upregulates insulin receptors in the periphery, which seems to be the primary action. It has a special status in the treatment of non-insulin-dependent diabetes mellitus because it is effective in many cases which are resistant to all other oral hypoglycemic drugs. It differs from other oral hypoglycemic drugs ie more effective during eating than during fasting. About 50% of Glibenclamide is metabolized to its inactive metabolites in liver

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3. MATERIALS AND METHODS

3.1. Materials

Metformin hydrochloride and Glibenclamide was obtained from Merck Specialities Pvt.Ltd,Mumbai,India . Povidone and ethyl cellulose was purchased from S. D. Fine Chem. Labs, (Mumbai, India). Hydroxypropyl methylcellulose K100M, PVP K-30, were obtained as a gift sample from Rolex chemical industries, Mumbai. Phosphate buffer alpha amylase, starch from Nice Chemicals Pvt. Ltd,Cochin,India

3.2. Methods

3.2.1. Formulations of Metformin matrix tablet :

INGREDIENTS	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9	F-10
Metformin HCL	500	500	500	500	500	500	500	500	500	500
PVP K-30	18	16	12	13	13	13	13	13	16	17
IPA	45	45	50	55	-	-	-	-	-	-

Water	-	-	-	-	35	35	30	30	30	30
HPMC K 100M	300	200	250	300	240	260	280	260	260	280
Carbopol 71 G	90	190	190	190	190	190	170	160	170	142
Magnesium stearate	8	8	8	8	8	8	8	8	8	8

Table no-01 : Experimental trials from Metformin Tablets F-1 to F-10

3.2.2.Preparation of Metformin matrix tablet by direct compression method:

The active pharmaceutical ingredients and all other excipients were accurately weighed & passed through 60 mesh sieve for 100 gram batch size according to the formulations .Metformin Hydrochloride, HPMC K100 M , PVP K-30 &IPA were added into poly bag and mixed for 30 minutes. Magnesium stearate and Carbopol 71 G were finally added for lubrication & mixed for 10 minutes. Finally powder blend compressed into tablets using single punch tablet compression machine.Before compression of each batch, the surfaces of the die and punch were lubricated using Magnesium stearate as lubricant. All the batches were stored in airtight containers with proper label at room temperature for further study

3.2.3. Formulations of Glibenclamide matrix tablet :

Formulation Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Glibenclamide (mg)	12	12	12	12	12	12	12	12
Ethyl Cellulose (mg)	18	18	18	18	18	18	18	18
HPMC K4M	24	30	36	42	---	---	---	---
HPMC K100M	---	---	---	---	24	30	36	42
Di basic Calcium Phosphate(mg)	93	87	81	75	93	87	81	75
Magnesium stearate (mg)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Aerosil (mg)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Table no- 02 : Experimental trials from Glibenclamide Tablets F-1 to F-08

3.2.4.Preparation of Glibenclamide matrix tablet by direct compression method:

Matrix tablets containing 12mg of Glibenclamide along with various amounts of polymerssuchas HPMC (Variousgrades) Ethylcellulose, and other excipients (such as magnesium stearate, Dibasic Calcium Phosphate, Anhydrate and Aerosil) were used and tablets were prepared by direct compression technique. Ethyl Cellulose and HPMC were passed through mesh No.40.Inthefirst step, the drug and ingredients with the exception of magnesium stearate were blended in aV-

Cone blender for 5minutes.Then magnesium stearate was added and formulation was mixed for an additional 2minutes. Desired amount of blend was directly compressed into tablets using rotary tablet compression machine. Before compression, the surfaces of the die and punch were lubricated with magnesium stearate.

3.2.5. Anti-diabetic studies inhibition of alpha amylase enzyme

3.2.5.1 Preparation of Drug Extracts

Drugs are powdered into fine particle introducing into mortar pestile. It is allowed to grind well. The Drug sample is kept into beaker powdered 5mg Glibenclamide mixed with alcohol of 50 ml and kept in stirring machine for 3hours. The other beaker contain powered 500mg of Metformin tablet mixed with alcohol of 100ml and kept in stirring machine for 4hours, in between 1 hour gap alcohol mixed with Glibenclamide is stirred well for 15 minutes. Both the alcohol dissolved drug samples are allowed to heat in a hot air oven for 1 hour at 50c temperature.(Metformin and Glibenclamide). The beaker containing drug is converted into Round bottom flask (RBF) 500mg metformin powder with alcoholic solution is poured into 250ml RBF. Both solutions are allowed to heat for 10 minutes in Reflux condenser by adding glass beads to RBF.

Then the solutions are allowed to cool and filtered ,It is allowed to transfer into standard flasks. Metformin drug containing solution in 50ml standard flask and Glibenclamide in 25ml standard flask.

3.2.5.2. Preparation of Drug Nano particles Extracts

The drug sample of 100 mg Metformin is dissolved in 30ml of alcohol in a beaker .It is allowed to stir for 3 hrs in stirring machine. Filter the drug extract.and transfer the drug solution into standard flasks of 50ml. and100mg of Glibenclamide is done by this method.

4. RESULT AND DISCUSSION

4.1.Results

4.1.1. Evaluation Of Formulated Blend for metformin tablet :

BATCH NO.	BULK DENSITY	TAPPED DENSITY	COMPRSIBILITY (%)	HOUSNER RATIO	ANGLE OF REPOSE
F1	0.614	0.789	22.2	1.28	25.0
F2	0.668	0.726	15.4	1.08	21.0
F3	0.699	0.776	9.92	1.11	20.0
F4	0.621	0.712	12.78	1.14	23.0
F5	0.656	0.722	9.14	1.10	22.0
F6	0.677	0.778	12.98	1.14	21.0
F7	0.624	0.723	13.69	1.15	23.0
F8	0.621	0.744	16.53	1.19	22.0
F9	0.658	0.734	10.34	1.11	20.0
F10	0.719	0.854	15.80	1.18	25.0

Table no- 03 : Evaluation Of the Formulation Blend of Metformin Hcl

4.1.2. Evaluation of Metformin. Hcl Matrix Tablet

FORMULATION CODE	WEIGHT VARIATION	HARDNESS Kg/cm^2	THICKNESS Mm	FRIABILITY	CONTENT UNIFORMITY
F-1	Passes	160-180N	6.20-6.30N	0.82	99.18
F-2	Passes	160-180N	6.20-6.30N	0.87	99.78
F-3	Passes	160-180N	6.20-6.30N	0.80	98.12
F-4	Passes	160-180N	6.20-6.30N	0.93	99.19
F-5	Passes	200-240N	6.55-6.70N	0.86	98.68
F-6	Passes	200-220N	6.50-6.60N	0.78	99.18
F-7	Passes	180-200N	6.60-6.70N	0.81	99.36
F-8	Passes	180-200N	6.45-6.55N	0.72	98.23
F-9	Passes	180-200N	6.45-6.55N	0.49	98.95
F-10	Passes	180-200N	6.45-6.55N	0.53	99.20

Table no-04: Evaluations of Metformin Matrix Tablet

4.1.3. Dissolution Profile Of Metformin Tablet:

- **Apparatus:** USP dissolution apparatus II
- **Medium:** 6.8 pH phosphate buffer.
- **Rpm:** 100 rpm
- **Absorption maxima:** 233 nm

4.1.4. <u>In-Vitro Drug Release:</u>

% Cummulative Drug release of Formulations

Sr.No	TIME (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1	1	37.9	35.0	27.7	24.6	37.2	32.1	35.3	33.7	25.2	29.6
2	3	60.6	58.9	48.7	50.3	64.6	55.1	49.1	53.0	44.2	53.1
3	5	72.6	73.5	62.9	62.6	77.4	68.5	62.2	66.0	62.1	71.3
4	7	78.0	82.2	74.3	7.1	87.1	78.1	72.0	76.1	72.6	81.3
5	10	91.4	90.8	83.4	82.2	100.4	88.6	81.0	85.6	81.9	9.4

Table -05: Cumulative % drug released of Metformin Hcl formulations of tablets.

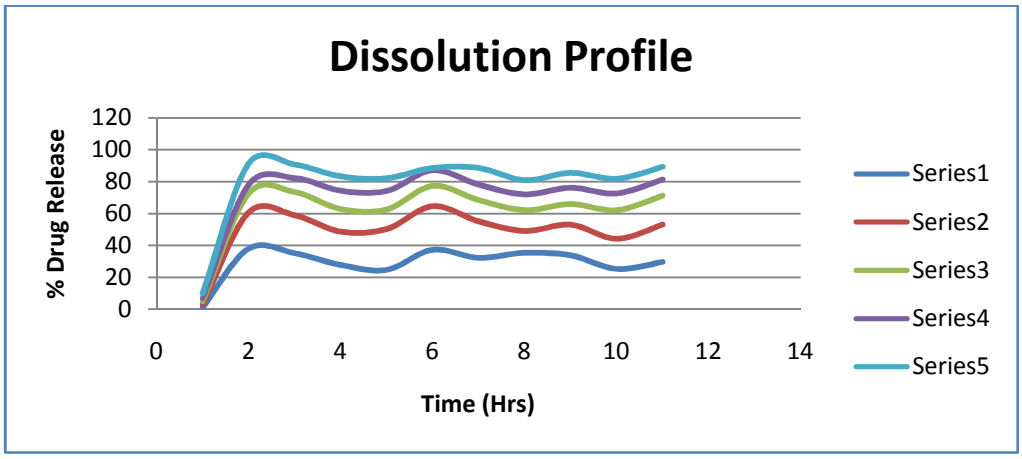


Figure -01: Dissolution profile of Metformin formulations F1 to F5

4.1.5. Release of Kinetics of Metformin :

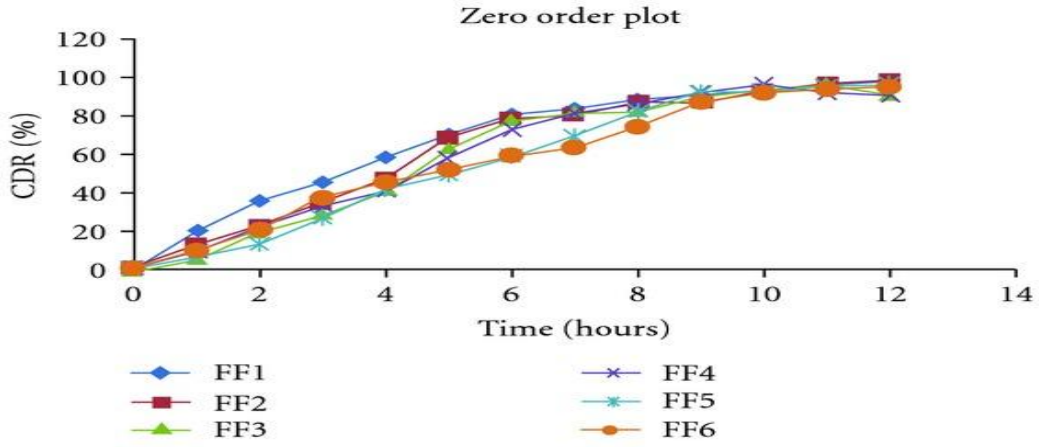


Fig-02: Zero order release plot of F1 to F6 formulations of Metformin Hcl

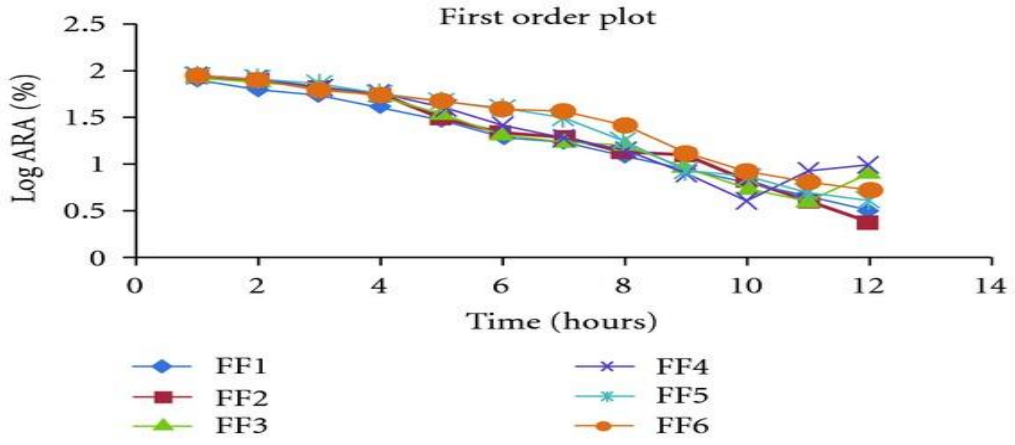


Fig -03: First order release plot of F1 to F6 formulations of Metformin Hcl

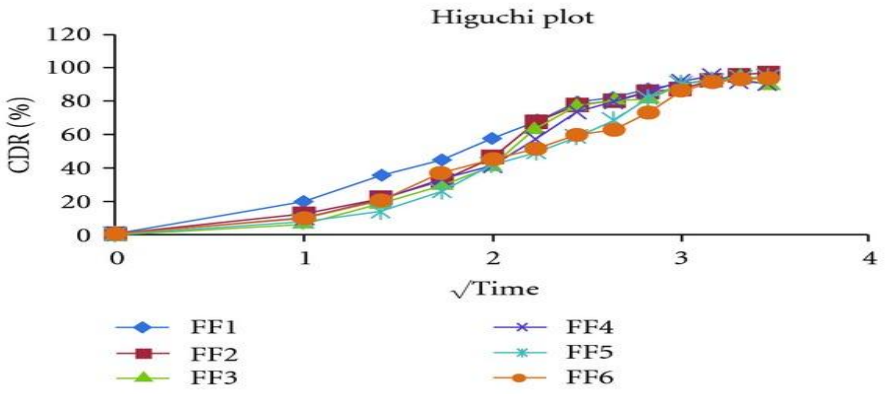


Fig - 04 : Higuchi plot of F1 to F6 formulations of Metformin Hcl

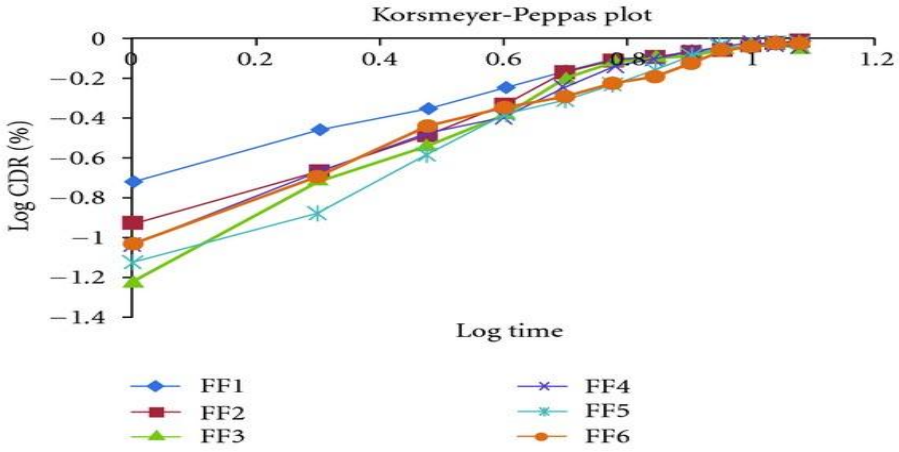


Fig - 05 :Korsmeyer peppas plot of F1 to F6 of Metformin

Formulation	Angle of repose	Loose Bulk density (g/ml)	Tapped Density (g/ml)	Hausner's ratio	Carr's Index (%)	Drug content (%)
F1	23.26	0.4457	0.5903	1.182	18.367	92.87
F2	22.36	0.4367	0.5832	1.198	16.396	90.24
F3	22.33	0.441	0.5886	1.206	21.898	94.45
F4	24.36	0.433	0.5841	1.227	20.32	89.68

F5	21.91	0.4323	0.5886	1.244	19.516	93.22
F6	23.95	0.4389	0.6019	1.186	18.257	91.87
F7	26.07	0.4345	0.5900	1.234	19.174	90.02
F8	22.91	0.4225	0.5803	1.256	20.574	94.58

4.1.6.Evaluation Of Formulated Blend for Glibenclamide :

Formulation	**Angle of repose**	**Loose Bulk density (g/ml)**	**Tapped Density (g/ml)**	**Hausner's ratio**	**Carr's Index (%)**	**Drug content (%)**
F1	23.26	0.4457	0.5903	1.182	18.367	92.87
F2	22.36	0.4367	0.5832	1.198	16.396	90.24
F3	22.33	0.441	0.5886	1.206	21.898	94.45
F4	24.36	0.433	0.5841	1.227	20.32	89.68
F5	21.91	0.4323	0.5886	1.244	19.516	93.22
F6	23.95	0.4389	0.6019	1.186	18.257	91.87
F7	26.07	0.4345	0.5900	1.234	19.174	90.02
F8	22.91	0.4225	0.5803	1.256	20.574	94.58

Table no-06 : Evaluation Of the Formulation Blend of Glibenclamide

4.1.7. Evaluation of Glibenclamide Matrix Tablet

Formulation code	**Thickness (mm)**	**Hardnss (Kg/cm^2)**	**Weight variation (%devn.)**	**Friability (%)**
F1	3.00±0.18	7.36±0.24	1.517±0.126	0.516±0.092
F2	2.97±0.22	6.74±0.23	1.478±0.216	0.384±0.046

F3	3.00±0.18	7.25±0.23	1.534±0.156	0.486±0.087
F4	3.10±0.20	7.34±0.20	1.614±0.412	0.268±0.028
F5	3.00±0.18	7.67±0.06	1.524±0.624	0.387±0.022
F6	2.98±0.11	7.08±0.12	1.486±0.267	0.579±0.05
F7	3.00±0.21	6.95±0.21	1.519±0.545	0.446±0.05
F8	3.10±0.16	7.72±0.11	1.546±0.223	0.302±0.039

Table no-07: Evaluations of Glibenclamide Matrix Tablet

4.1.8. Dissolution Profile Of Glibenclamide Tablet:

- **Apparatus:** VDA-6DR type2
- **Medium:** 6.8 pH phosphate buffer.
- **Rpm:** 100 rpm
- **Absorption maxima:** 229nm

4.1.9. <u>In-Vitro Drug Release:</u>

Sr.No	TIME (hrs)	F1	F2	F3	F4	F5	F6	F7	F8
1	1	10.67	9.94	9.34	6.24	9.94	8.45	7.89	6.12
2	3	22.98	20.56	18.45	16.27	21.89	19.43	18.87	17.47
3	5	46.87	35.28	40.63	35.72	42.16	34.26	35.72	31.28
4	7	64.66	54.78	50.38	46.53	61.36	47.25	51.76	45.09
5	10	86.89	78.65	79.41	70.46	80.28	72.42	73.36	67.15

Table-08: Cumulative % drug released of formulations of Glibenclamide tablets.

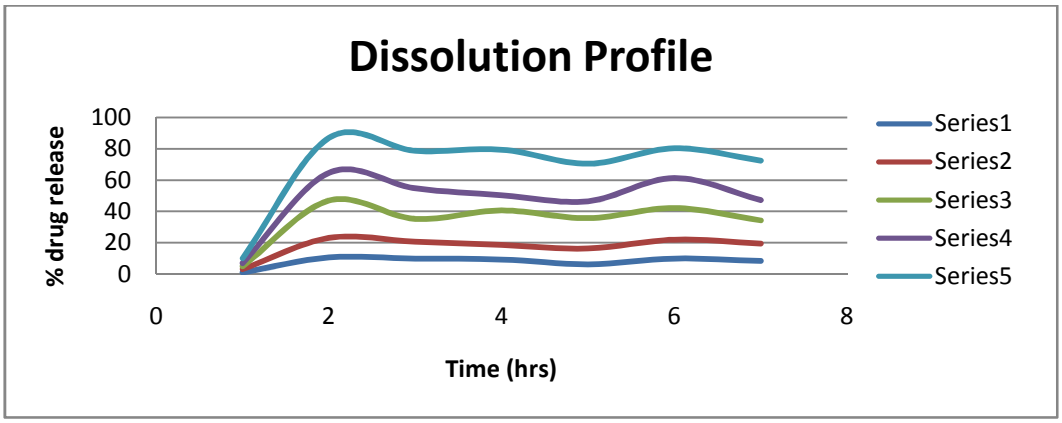


Figure -06: Dissolution profile of Glibenclamide formulations F1 to F5

4.1.10. Release of Kinetics of Glibenclamide :

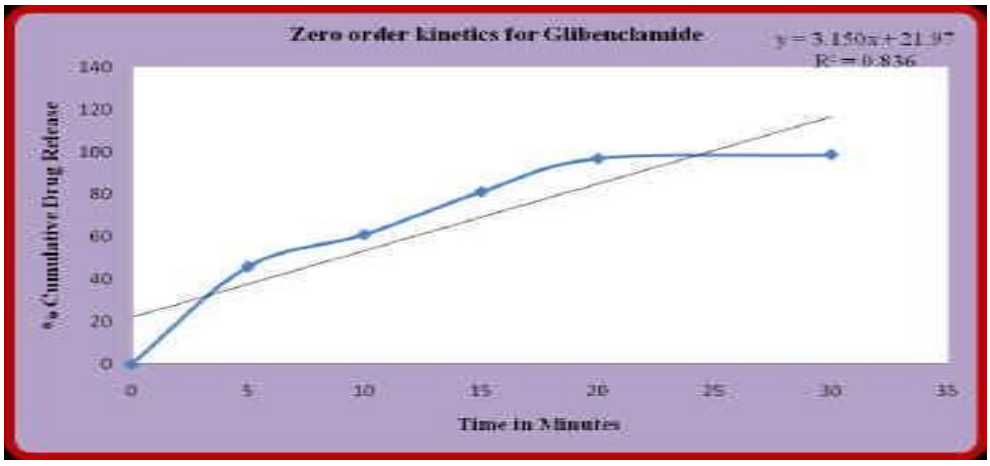


Fig-07: Zero order release plot of F1 to f6 formulations of Glibenclamide

Fig-08: First order release plot of F1 to f6 formulations of Glibenclamide

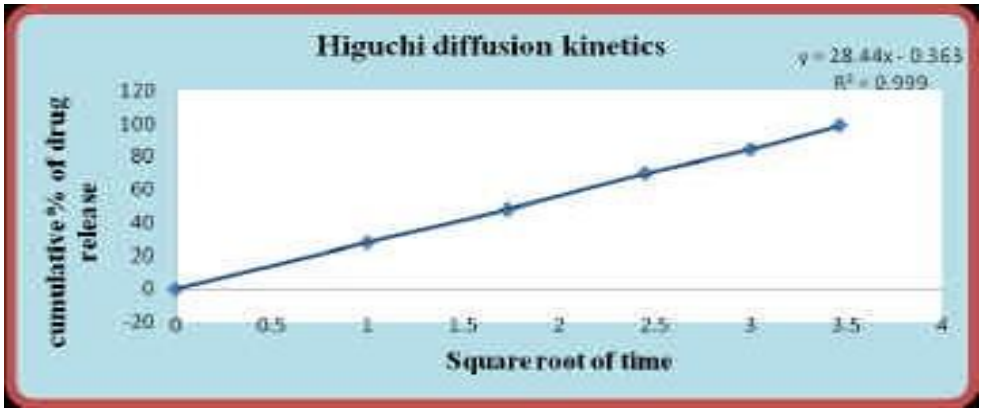


Fig - 09 : Higuchi plot of F1 to F6 formulations of glibenclamide

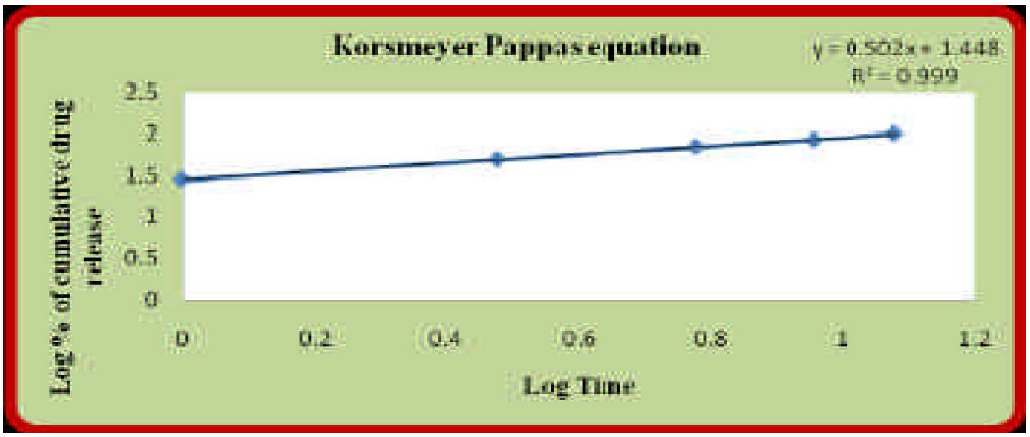


Fig -10 : Korsmeyet Pappas plot of F1 to F6 formulations of glibenclamide

4.1.11.Combinational evaluation of Nano particled sizes of Metformin and Glibenclamide with tablet form to analysis alpha amylase inhibition

At low concentration of solution high absorbance and at high concentration low absorbance showing presence of activity, from this finding percentage inhibition.

4.1.11.1 Evaluation of tablet form of drug in 10:1 ratio

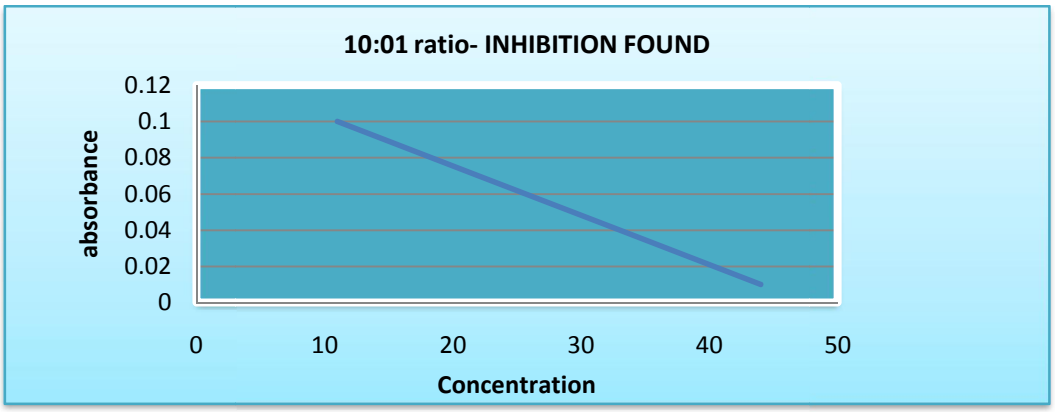


FIG-11:Combinational evaluation of 10:01ratio of tablet dosage form

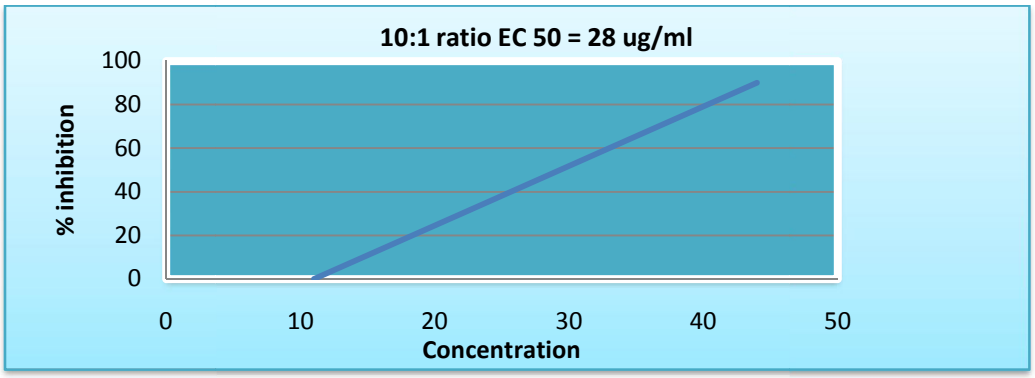
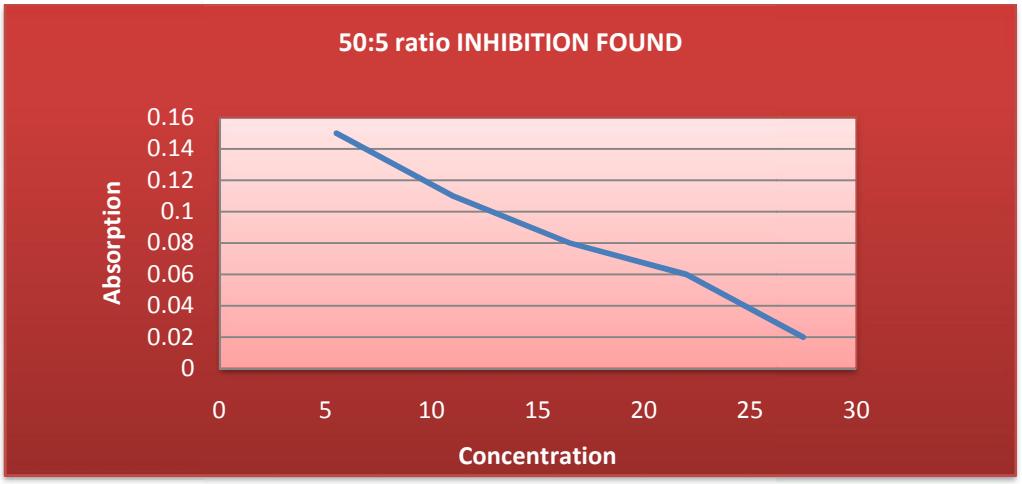


FIG-12: % inhibition of 10:01ratio of tablet dosage form



4.1.11.2. Evaluation in 50:05 Drug Nano particles with enhancers

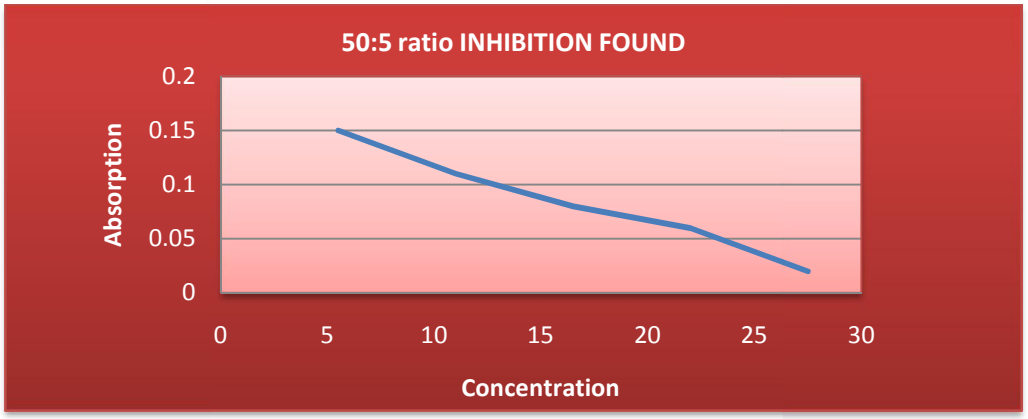


FIG-13: Evaluation in 50:05 ratio of Drug Nano particles

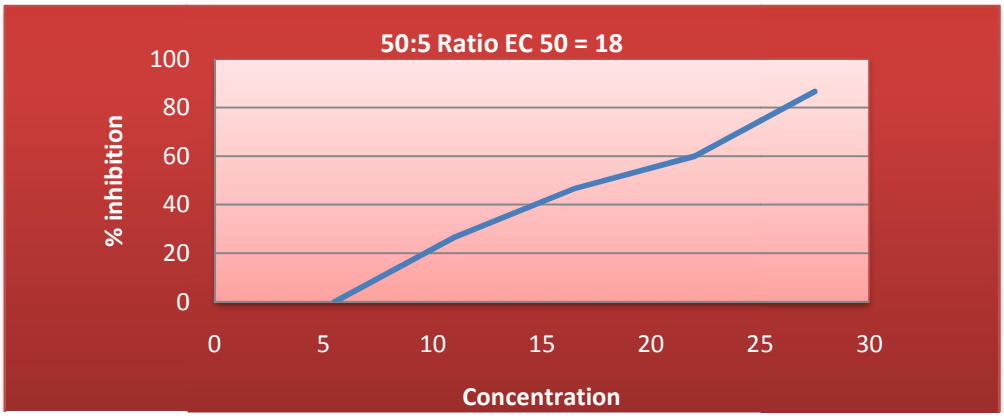


FIG-14: % inhibition of nano particled drug in different concentrations

4.2. Discussion

The objective of study to formulation and develop to pharmaceutical equivalent of controlled release Metformin and Glibenclamide tablets. To achive objective the preformulation characteristics of drug was studied API used to shown used to shown all result within specification. The selection of probable excipients were carried out by literature survey and checked for drug excipient compatibility. The result of the drug excipient compatiability shows all the excipient are compatible with the drug. To formulate pharmaceutical equivalent & bio-equivalent of Metformin and Glibenclamide controlled release Matrix tablets.

To increase the controlled extended release and binding capacity of Metformin Hcl ,in starting formulations mixing the blend with PVP K-30 and IPA to form complex. For controlled release of drug combination of PVP K-30 and IPA changing in the ratio of sample to controlled release was performed and portion of using diluents and lubricants (like magnesium striate) to controlled the drug release to formulate it as it has extended release tablet.

In Glibenclamide the starting formulations mixing the blend with Ethyl Cellulose and HPMC to form complex. For controlled release of drug combination of Ethyl Cellulose and HPMC changing in the ratio of sample to controlled release was performed and portion of using diluents and lubricants (like magnesium striate) to controlled the drug release to formulate it as it has extended release tablet.

For Metformin after comparing with the marketed Tablet instead of the F-5 formulations, it is decided F-5. it has controlled release in the 3rd hrs (64.6%),5th hrs (77.4%) and 7th hrs. (87.1%). For Glibenclamide after comparing with the marketed Tablet instead of the F-5 formulations, it is decided F-5. it has controlled release in the 3rd hrs (21.89%),5th hrs (42.16%) and 7th hrs. (61.36%). The F-5 formulation of all parameters i.e, powder characterization after lubrication, In process controlled parameters during tablet compression. In process controlled parameters & finished products testing.

The result of all the testes of powder characterization after lubrication, like tapped density, Bulk density, sieve analysis were found within limits and result of all in process checking parameters during compression

such as weight variation Thickness, diameter , hardness and friability are within limits.Assay of Metformin Hcl and Glibenclamide, content uniformity, *In-vitro* release and relative substance. Result of all substance within limits.Accelerated stability studies (25 ^{0}C and 60 ± 2^{0C} and 60±5% RH & 40C± 2^{0C} and 75±5% RH) for 3 months, which the result of parameters for all four batches with in the specification limits.

The result of batches according to the I.P. results are in the limits comparing to marketed Metformin Hcl and Glibenclamide tabletsFormulation -5 was showing good controlled release. For further development in future

IN-VIVO studies also will be conducting.

5. SUMMARY AND CONCLUSION

The aim of study was the "**Formulation and evaluation of Metformin and Glibenclamide tablets and analysis of alpha amylase inhibition with different compositions of nano-particled drugs and tablet form"** having controlled release system. This meant for treating diabetes mellitus.

In This main objective of the study was develop a stable was to develop a stable product which provides controlled drug release profiles. The optimum formulation of F-1(Metformin),F-5(Glibenclamide) was given the best. Preformulation studies and post compression studies such as Angle of repose, compressibility index, thickness, hardness, content uniformity, drug release. The matrix tablet were subjected to test for accelerated stability (studies (25 ^{0}C and 60 ± 2^{0C} and 60±5% RH & 40C± 2^{0C} and 75±5% RH) for 3 months studies.

The release kinetics profile had also given a best result. The optimum formulation of F-5 drug was following mixed-order kinetics. In this formulations, The combination of Ethyl Cellulose and HPMC(for Glbenclamide), PVP K-30 and IPA(for Metformin Hcl),was improved the % cumulative release of the drug, which was given the best release of drug within the limits. When comparing to Marketed tablet the drug release of the marketed tablet upto 7Hrs 78% (Metformin), 61.36% (Glibenclamide). Our formulation had given a good drug release upto 10Hrs 91.4%(Metformin), 80.28 %(Glbenclamide). So our formulation had produced a best result.

The identification of the best formulation using different compositions of Glibencalmide and Metformin for evaluating the anti-diabetic activity. From the present work we conclude that if the drugs are in nano-particled size the absorption will increase, thus the activity will increase. In this study we proved that the nano particled drug shows very good EC 50, when compared that of the tablet form. Even-though we added some enhancers for increasing the activity/absorption it couldn't show any further activity.

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