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Formulation Development and *In vitro* Evaluation of Combination Product of Glyburide and Metformin Hydrochloride Tablet

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Abstract

This study was attempted to formulate a combination product of Glyburide and Metformin Hydrochloride Tablets USP 2.5mg/500mg and to evaluate their physico-chemical properties. Wet granulation method was adopted for preparation of tablet using different excipients namely Microcrystalline cellulose, Povidone K-30, Copovidone, Croscarmellose sodium and Sodium stearyl fumerate in six different formulations (F1-F-6). The granules for tabletting were evaluated for angle of repose, bulk density, tapped density, compressibility index and drug content etc. The tablets were subjected to thickness, hardness, friability, disintegration and *in vitro* release studies. The results of physical parameters of tablets showed that there were capping, hardness and friability problems in formulation F-1, F-2 and F-3. Granules of formula F-4, F-5 and F-6 showed satisfactory flow properties, compressibility index and the physical parameters of tablets from these three formulations gave optimum result in comparison to innovator's brand. Disintegration time of these three formulations (7-8 min) was found similar with innovator's brand (6.30-7.30 min). Assay of formula F-6 of glyburide (97.97%) and Metformin Hydrochloride (100.2%) met the USP specification (90%-110%). It was also found that dissolution profile of Glyburide depends on particle size of Glyburide powder. When micronized and non micronized grade of Glyburide was used in a ratio of 3:1 (F-6) it gave similar dissolution profile as innovator's brand where the similarity factor (f2) was calculated as 59. On the other hand, dissolution profile of Metformin hydrochloride was found similar in all the three formulations (F-4, F-5, F-6) with reference to innovator having all f2 values above 50. Formulation F-6 possessed good stability in accelerated condition for 6 months study. By comparing the dissolution profiles with the innovator's drug glucovance® tablet, it was revealed that the formulation F-6 exhibit similar drug release profile for both Glyburide and Metformin Hydrochloride.

Key words: Glyburide, Metformin Hydrochloride, Dissolution, Micronized, Non-micronized

Introduction

The combination of Glyburide and Metformin hydrochloride is used to treat type 2 diabetes in people whose diabetes cannot be controlled by diet and exercise alone (Product information, 2000). Glyburide belongs to a class of drugs called sulfonylureas and Metformin Hydrochloride is in a class of drugs called biguanides (Rang *et al*., 2003; Goodman & Gilman, 2001). Glyburide lowers blood sugar by stimulating the pancreas, the organ that makes insulin. Insulin helps control blood sugar levels. The pancreas must produce insulin for this medication to work (Marble, 1971). Metformin hydrochloride helps body regulate the amount of glucose (sugar) in blood. It decreases the amount of glucose that we get from our diet and the amount made by our liver. It also helps body to use its own insulin more effectively. Metformin hydrochloride lowers blood sugar levels by reducing the amount of glucose the liver produces and by increasing insulin sensitivity (Zhou *et al*., 2001). Glyburide lowers glucose levels in a similar manner, but it also stimulates insulin release by the insulin-producing cells of the pancreas. This is something Metformin Hydrochloride doesn't do. Because of their different mechanisms of action, the combination of these two drugs may be more effective for lowering blood sugar levels than using either alone.

Doctors sometimes treat patients with a combination of Glyburide and Metformin hydrochloride as the first-line therapy for type 2 diabetes. They may also use this drug combination when a person with type 2 diabetes fails to

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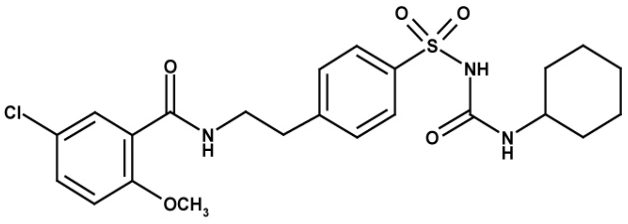
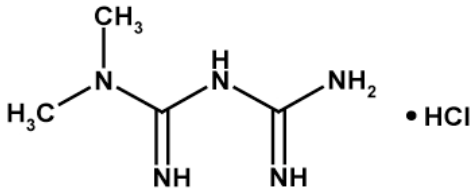
respond to Metformin hydrochloride or Glyburide alone (Howlett *et al*., 2003). In some trials, the glucose-lowering effect of Glyburide and Metformin hydrochloride tablets appeared to be slightly more than additive compared with Metformin hydrochloride and Glyburide as separate tablets. When the combination tablet is taken with or immediately before a main meal, this increases the prandial insulin response, adding particularly to the reduction in postprandial hyperglycaemia (Garber *et al*., 2002). Thus, minor adaptations in the formulation of fixed dose combinations can provide efficacy gains.



Glyburide



Metformin Hydrochloride

The number of diabetic population is increasing in Bangladesh at an alarming rate due to changes in lifestyle, obesity, lack of physical work and ageing of people. At present, the diabetic population number in the country is 8.4 million, which is expected to double by 2030, according to International Diabetes Federation. Fixed-dose combinations of oral antidiabetic agents are slowly becoming established as convenient options in the treatment of type 2 diabetes. They can simplify administration and improve compliance, especially for patients taking many different therapies. Fixed-dose combinations provide an expedience for extra medication without extra tablets. With this view in mind, this study attempted to formulate and develop a fixed dose combination of Glyburide and Metformin hydrochloride as an immediate release tablet dosage form.

Materials and Methods

Materials: Glyburide USP (Micronized) (USV Limited, India), Glyburide USP (Non-micronized) (USV Limited, India) and Metformin Hydrochloride USP (Wanburry Limited, India) were used in this investigation as an active ingredients. Microcrystalline Cellulose (Type-102) (JRS Pharma GmbH, Germany), Povidone K-30, Copovidone (Plasdone S-630) (ISP Sales, UK), Croscarmellose Sodium (FMC Biopolymer), Sodium Stearyl Fumerate (JRS Pharma GmbH, Germany), Opadry II 31F530001(Orange) (Colorcon Asia private limited, India) and Purified water USP were taken as an excipients.

Equipments: Sartorius electronic weighing balance. High speed mixer granulator, Fluid Bed dryer and processor, Multi-mill, Blender, 16 station IMA Killian (Pressima) Compression Machine, NR Cota Coating machine, Erweka electronic hardness tester, Erweka disintegration tester, Friability tester, Halogen Moisture Analyzer, Dissolution tester, Shimadzu UV spectrophotometer, Shimadju High performance liquid chromatography, and Pharma test stability chamber were used to perform this work.

Formulation of uncoated tablets: For the preparation of uncoated tablets six different formulas was proposed meeting all the USP specification. All the formulas contained 1.45 kg in batch size and each tablet contained 725 mg in weight. So, all the formulas produced 2000 tablets. Proposed formulations (given in Table 1) were designed as F-1, F-2, F-3, F-4, F-5 and F-6 and measured in mg/tablet. Formula F1-F4 contained only micronized grade Glyburide powder and formula F5-F6 contained both micronized and non-micronized grade Glyburide powder.

Evaluation of tablets: In order to assess different physic-chemical properties, the prepared tablets were subjected to hardness and thickness test, weight variation test, friability test, disintegration test, dissolution test and stability test (Aulton, 1998; Liberman *et al*., 2001; Grim, 1985). The similarity factor (f2) was computed using the formula (Yuksel *et al*., 2000; Costa *et al*., 2001; Ocana *et al*., 2009)

$$`f2= 50\times\log \{[1+ (1/n) \Sigma_{t=1}^{n} (R_t-T_t)^2]^{-0.5} \times 100\}$$

Where, n is the number of dissolution sample times, R_t and T_t are the individual or mean percent dissolved at

each time point, t, for the reference and test dissolution profiles, respectively.

Determination of active drugs: 5 tablets were dissolved in diluent by stirring with a magnetic stirring bar to obtain a solution having a glyburide concentration of approximately 0.025 mg per ml. A portion of this solution was centrifuged at 3000 rpm for 10 minutes and the clear supernatant collected as an assay sample. Separately equal volumes (about 100 µL) of the standard preparation (USP Glyburide RS) and the assay preparation were injected into the chromatographic (HPLC) system (SHIMADJU, Japan). The chromatograms were recorded for about 1.25 times the retention time of the glyburide peak and measured the responses for the major peaks. The quantity of glyburide ($C_{23}H_{28}ClN_3O_5S$) was calculated in mg per tablet by the formula:

$$\mathbf{CVD(r_U / r_S)/N}$$

in which C was the concentration in mg per mL, of USP Glyburide RS in the standard preparation; V was the volume in ml, used to prepare the assay preparation; D was the dilution factor of the assay preparation; r_U and r_S were the peak responses obtained from the assay preparation and the standard preparation respectively; and N was the number of tablets used to prepare the assay preparation.

Quantitative determination of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) was performed using the same method as directed for the assay of glyburide,

Table 1. Proposed six formulations of Glyburide and Metformin hydrochloride Tablets USP 2.5 mg/500 mg.

Name of Materials	F-1	F-2	F-3	F-4	F-5	F-6
Intragranular						
Glyburide (micronized)	2.500	2.500	2.50	2.50	1.25	1.875
Glyburide (non micronized)	-	-	-	-	1.25	0.625
Metformin hydrochloride	500.000	500.000	500.00	500.00	500.000	500.000
Microcrystalline cellulose (Type 102)	29.526	24.590	24.590	24.590	24.59	24.59
Povidone K-30	14.514	19.450	19.450	9.53	9.53	9.53
Binder Solution						
Povidone K-30	-	-	-	9.92	9.92	9.92
Purified water	qs	qs	qs	qs	qs	qs
Extragranular						
Microcrystalline cellulose (Type-102)	162.585	162.585	137.205	123.330	123.330	123.330
Copovidone (Plasdone S-630)	-	-	18.125	32.000	32.000	32.000
Croscarmellose sodium	10.875	10.875	18.130	18.130	18.130	18.130
Sodium stearyl fumarate	5.000	5.000	5.000	5.000	5.000	5.000
Total Weight (Core)	725.000	725.000	725.000	725.000	725.000	725.000

Results and Discussion

Physicochemical parameters: The results of the physicochemical properties of the investigated formulations are summarized in the tables 2 and 3.

Assay method: The average values of the content of Glyburide and Metformin Hydrochloride have been shown in the table 4.

Effect of hardness: The results of the influence of hardness range on the product characteristics were investigated and summarized in the table 5 and in the figures 1 & 2.

From the above data, it can be concluded that the DT and dissolution profile largely depends on the hardness of tablets. It was also observed that when the hardness range was 12-14 kp, the dissolution profile became closer to that of innovators.

Effect of machine speed: Lubricated blend was compressed into tablets at optimum hardness ranges (12-15 kp) at three different machine speeds, i.e., 15, 30, 45 rpm by using 16 station IMA Killian (Pressima) compression machine. These tablets were evaluated for physical parameters and the results tabulated in Table 6.

Table 2. Average value of physicochemical parameters of all the formulations (uncoated tablets).

Formulation	Average weight (mg)	Hardness (kp)	Thickness (mm)	Friability (%)	DT (min)
F-1	724.9	5.50-6.32	6.89-6.95	Capping observed	0.20
F-2	725.4	6.23-7.21	6.87-6.92	Capping observed	0.30
F-3	725.7	11.01-12.84	6.81-6.87	0.26%	1.20-2.0
F-4	726.1	13.15-14.48	6.78- 6.85	0.28%	3.30 - 4.30
F-5	725.5	11.72-12.44	6.79-6.85	0.23%	3.30 - 4.30
F-6	725.3	12.95-14.78	6.77-6.83	0.25%	4.20-4.50

Table 3. Average dissolution profile (%) of Glyburide and Metformin hydrochloride of proposed formulation F-4, F-5 and F-6.

Formulation	F-4	F-5	F-6
Dissolution profile of Glyburide :(pH 9.5 Borate buffer, 500 ml, Apparatus: Paddle, 75 rpm) Average (% Released)			
10 min	94	52	58
15 min	97	74	80
30 min	99	82	96
45 min	100	91	98
Dissolution profile of Metformin: (pH 6.8 Phosphate buffer, 1000 ml, Apparatus: Paddle, 50 rpm) Average (% Released)			
10 min	65	74	63
15 min	88	95	90
30 min	99	99	100
45 min	100	100	100

Table 4. Glyburide and Metformin hydrochloride content of tablets from formulation F-6.

Assay (%) of label claim	USP specification	Formulation F-6
Glyburide	90 - 110 % label claim	97.97
Metformin hydrochloride	90 - 110 % label claim	100.2

Table 5. Effect of hardness range on product characteristics.

	Formulation F-6			
	Thickness (mm)		Friability (%)	Disintegration time (min)
Low Hardness (10-12 kp)	6.89-6.99		0.32	1 – 2
Medium Hardness (12-14 kp)	6.77 – 6.83		0.23	4.2-4.5
High Hardness (18-24 kp)	6.45 – 6.50		0.21	8-9
Dissolution Profile of Glyburide :(pH 9.5 Borate Buffer, 500 ml, Apparatus: Paddle, 75 rpm) Average (% Released)				
Core Tablet				
Time (min)	5	10	15	30
Low Hardness (10-12 kp)	89	93	95	97
Optimun Hardness (12-14 kp)	70	91	94	96
High Hardness (18-24 kp)	31	59	83	95
Dissolution Profile of Metformin: (pH 6.8Phosphate Buffer, 1000 ml, Apparatus: Paddle, 50 rpm) Average (% Released)				
Core Tablet				
Time (min)	5	10	15	30
Low Hardness (10-12 kp)	100	103	103	104
Optimum Hardness (12-14 kp)	69	102	104	103
High Hardness (18-24 kp)	37	61	86	101

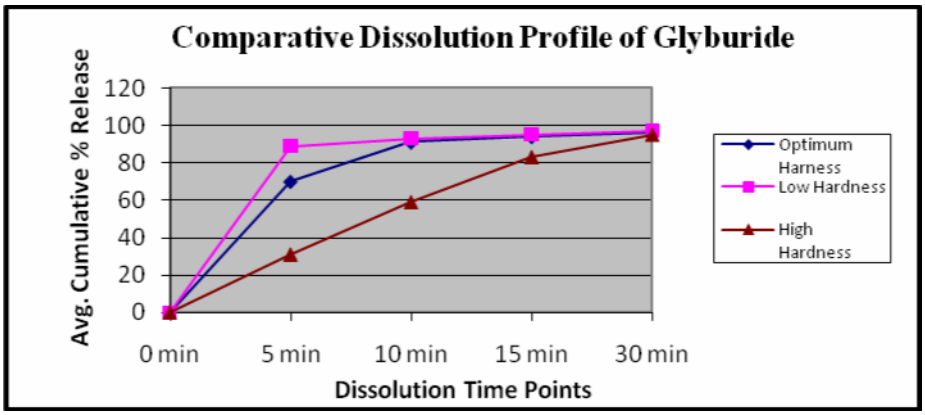


Figure 1. Comparative dissolution profile of glyburide in pH 9.5 borate buffer/75 RPM/500 ml/USP-II/37°C.

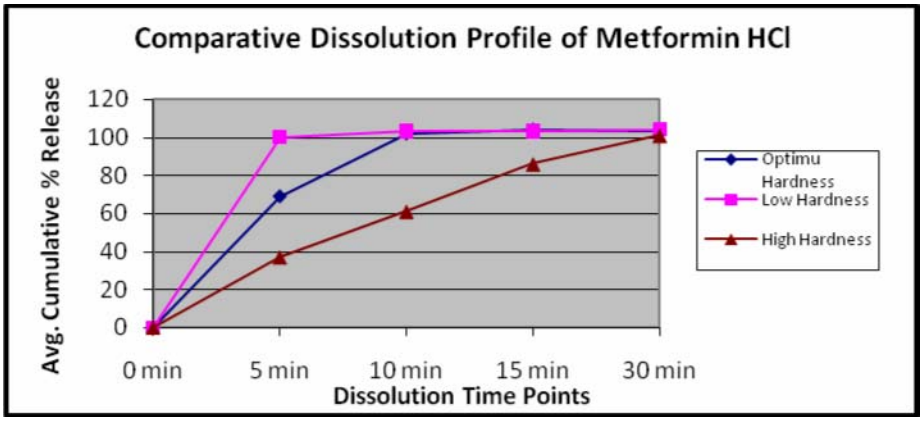


Figure 2. Comparative dissolution profile of metformin HCl in pH 6.8 phosphate buffer/50 RPM/1000 ml/USP-II/37°C.

Table 6. Effect of machine speed on product characteristics.

		Formulation F-6		
		Compression Machine Speed (RPM)		
		15	30	45
S.No.	Test	Observations/Results		
1	Weight variation			
	Average, mg	725.2	726.3	725.1
	Minimum, mg	720	722	719
	Maximum, mg	730	732	732
	%RSD	0.43	0.48	0.58
2	Thickness (mm)	6.77-6.83	6.77-6.83	6.77-6.83
3	Friability (%)	0.23	0.23	0.20
4	Disintegration time (min.)	4.30-5.00	4.20-4.50	4.30-5.10

From the above data it was concluded that the average weight, thickness, hardness, friability and DT did not deviate with machine speed.

Comparison with innovator's product: Some physicochemical properties of the formulation F-6 tablet was compared with innovator product glucovance® tablets USP 2.5 mg/ 500mg which was taken as RS (Reference Standard). The summarized data were presented below in the tables 7, 8 & 9 and in the figures 3 & 4.

Table 7. Comparison of Average weight, hardness, thickness, DT, friability, assay of tablets of F-6 and RS.

Formu-lation	Avg wt. (mg)	Hard-ness (kp)	Thick-ness (mm)	DT (min)	Tablet size	Assay of Glyburide (% label claimed)	Assay of Metformin HCl (% label claimed)
F-6	751.2	17.33-19.67	6.84-6.91	7.00-8.00	17.6 mm × 7.6 mm	97.97	100.2
RS	619.20	16.31-19.06	5.62	6.30- 7.30	16.1 mm × 8.1mm	101.2	102.8

Table 8. Comparison of dissolution profile of Glyburide tablets of F-6 and RS (in pH 9.5 Borate buffer/75 RPM/500 ml/ USP-II/37°C).

Product	Unit	% Glyburide dissolved at			
		10 min	15 min	30 min	45 min
F-6	Mean	57.50	79.79	96.17	97.72
	std. devn.	6.40	6.09	1.57	0.87
	%RSD	11.13	7.63	1.63	0.89
RS	Mean	67	89	102	102
	std. devn.	6.70	4.55	1.06	0.98
	%RSD	10.04	5.11	1.04	0.96

Table 9. Comparison of dissolution profile of Metformin hydrochloride tablets of F-6 and RS (in pH 6.8 Phosphate buffer/50 RPM/1000 ml/USP-II/37°C).

Product	Unit	% Metformin HCl dissolved at			
		10 min	15 min	30 min	45 min
F-6	Mean	63	90	101	101
	std. devn.	4.14	4.00	0.42	0.45
	%RSD	6.54	4.45	0.41	0.45
RS	Mean	68	90	99	99
	std. devn.	1.86	2.28	1.05	1.03
	%RSD	2.74	2.54	1.06	1.04

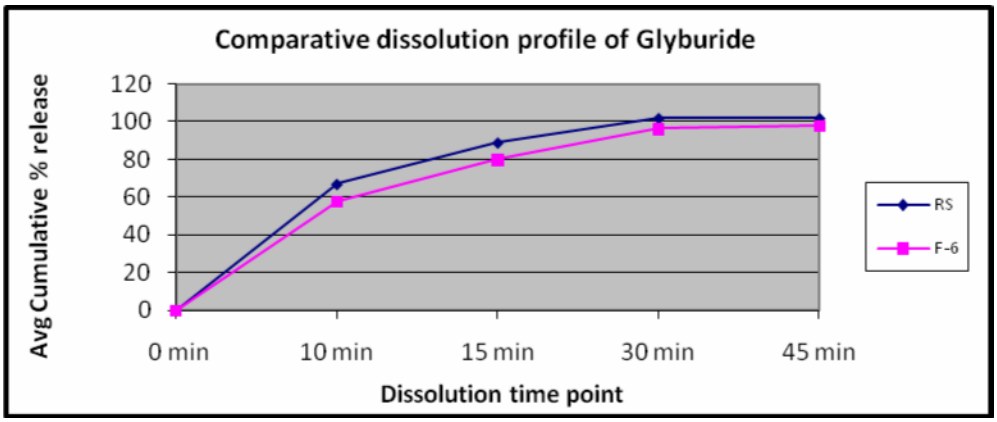


Figure 3. Comparative dissolution profile of Glyburide in pH 9.5 Borate buffer/75 RPM/500 ml/USP-II/37°C; From the above data, the similarity factor (f2) for Glyburide of F-6 Vs RS was calculated as 59.

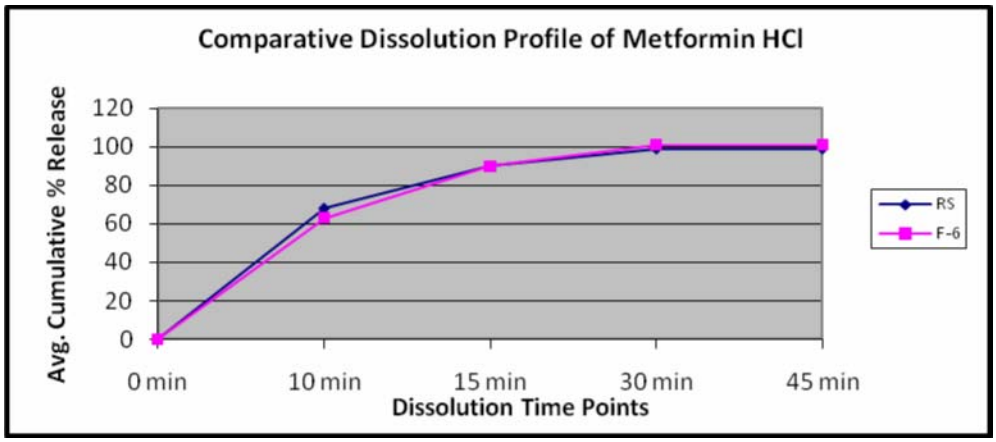


Figure. 4. Comparative dissolution profile of Metformin HCl in pH 6.8. Phosphate buffer/50 RPM/1000 ml/USP-II/37°C.

Here, the similarity factor (f2) for Metformin HCl of F-6 Vs RS was found as 78.

From the above observation, it can be concluded that in both cases Glyburide and Metformin hydrochloride of F-6 showed similar dissolution profile as reference standard.

Stability studies: The stability studies were carried out at 40°C ± 2°C and 75% RH ± 5% RH for accelerated condition in sealed 60cc HDPE container pack. The samples were tested initially and the stability test completed up to 06 months at accelerated condition. The results observed at the above mentioned condition were given in the table 10.

Table 10. Accelerated stability study of Glyburide and Metformin hydrochloride tablets USP 2.5mg/ 500mg (F-6).

	Initial	3 Month/40°C/75% RH	6 Month/40°C/75% RH
Appearance	An orange colored, oval shaped film coated tablet, debossed with "BPL" on one side and "002"on other side	An orange colored, oval shaped film coated tablet, debossed with "BPL" on one side and "002" on other side	An orange colored, oval shaped film coated tablet, debossed with "BPL" on one side and "002" on other side
Average Weight (mg)	725.3	725.6	725.5
Hardness (kp)	12.95-14.78	12.63-14.53	12.75-14.92
DT (min)	4.20-4.50	4.10-5.00	4.30-5.00
Assay (NLT 90% & NMT 110%)			
Glyburide	97.97	98.45	97.71
Metformin Hydrochloride	100.2	102.6	101.2
Dissolution (NLT 85% (Q) in 30 mins)			
For Glyburide: 9.5 Borate buffer, Paddle/500 ml/75 rpm 30 min	96	91	96
For Metformin Hydrochloride: 6.8 Posphate Buffer, Paddle/ 1000ml/50 rpm 30 min	101	99	103

Discussion

For the formulation and development of Glyburide and Metformin hydrochloride tablets USP 2.5mg/ 500 mg different formulations with various excipients and their different quantity were used in the formulations. In order to produce quality tablets, it was imperative that the blend of ingredients sent to the press be dry and of uniform particle size. In addition, it was important that the API was evenly distributed within each tablet produced (Arthur, 2001; Nachegari *et al*., 2004). As this formulation contains Glyburide in a very small quantity, so for proper distribution of API in tablet, wet granulation method

would be the first choice. The flow properties of the powder can be judged from the angle of repose and Carr's compressibility index. The powder flow depends on 3 general areas: (1) the physical properties of the particles (eg. Shape, size, compressibility, (2) the bulk powder properties (eg. Size, distribution, compaction, and (3) the processing environment (eg. storage, humidity) (Herbert *et al*.,1989; Lordi *et al*.,1987). The results revealed that the granules exhibited passable and good flow in view point of USP.

Microcrystalline cellulose was used as diluent, croscarmellose sodium as disintegrant, sodium stearyl fumerate as lubricant whereas Povidone and Copovidone were used as binders. As Metformin hydrochloride has poor compressibility property, formula with only povidone as binder produced capping problem. So to improve binding property Copovidone can be used. Copovidone is a synthetic, 60:40, linear, random copolymer of N-vinyl-2-pyrrolidone and vinyl acetate. The addition of vinyl acetate to the vinylpyrrolidone polymer chain reduces hydrophilicity and glass transition temperature (Tg) of the polymer relative to polyvinyl pyrrolidone (PVP) homopolymer (Inactive ingredient guide, 1996). As a result, Copovidone copolymer is an excellent adhesive material and a tougher, more flexible film former than PVP homopolymer. With these unique properties, Copovidone copolymer is suggested to be used in pharmaceutical formulations as a tablet binder (data as shown in Table 2).

Assay value of formula F-6 of glyburide (97.97%) and Metformin Hydrochloride (100.2%) met the USP specification (90%-110%) which were shown in table 4, where the assay value of Glyburide of innovator product was 101.2% and Metformin Hydrochloride was 102.8% . DT and dissolution profile depends on the hardness of tablet. And when the hardness range was (12-14) kp dissolution profile became closer to innovator (Table 5). Average weight, thickness, hardness, friability and DT did not deviate with machine speed (Table 6). Disintegration time of these three formulations (7-8 min) which is very important for dissolution profile was found similar with innovator product (6.30-7.30 min) (Table 7).

The dissolution profile of Glyburide was considered very important in this product as Glyburide is a class II molecule having low solubility which may have an impact in bioavailability (Rudnic,1995). From this study it was found that dissolution profile of Glyburide depended on particle size of Glyburide powder. And when micronized and non micronized grade of Glyburide was used in a ratio of 3:1 then it gave similar dissolution profile as innovator where the similarity factor (f2) was 59 (Table 8). But when only micronized grade Glyburide was used then the release pattern became faster initially. Here the similarity factor (f2) recorded as 45 which were not very similar to innovators. And when micronized and non micronized grade of Glyburide was used in a ratio of 1:1 then the release profile became slower than innovator. Here the similarity factor (f2) found to be 43 which were also not very similar with innovator. On the other hand dissolution profile of Metformin hydrochloride showed similar in all the formulations with reference to innovator having all f2 values above 50. This is because Metformin HCl has high solubility and it has been in a high dose in formulation.

Table 10 revealed that the formula F-6 showed good stability result after six month study at accelerated condition. This is perhaps due to the fact that each film coated tablet of formula F-6 were tightly packed in 60cc HDPE container with proper leak proof sealing which has protected the tablet from light and environmental degradation.

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