8.0 SUMMARY

8.1 INTRODUCTION

Diabetes can cause several metabolic disorders if it remains untreated and all this happened because of high level of blood sugar level may be due to either non production of insulin (type 1) or if produced insulin is not sufficient(type 2). Whatever the type of diabetes mellitus is, proper diabetes management is required to keep blood sugar in control. Despite the availability of numerous treatment options, patients with type 2 diabetes mellitus still have unmet medical needs. Reduction in efficacy over time with existing therapies leads to hyperglycemia, which in turn can potentiate vascular complications. Further monotherapy often fails after a period of treatment, so that multiple drugs are needed to achieve effective glycemic control (Derosa and Sibilla, 2007). Newer combination therapies are thus sought after to achieve and maintain glycemic control.

Repaglinide (RG) and Metformin HCL (MH) in conventional form was approved by FDA June, 2008 with its first and only fixed-dose combination and brand name PrandiMet® for the treatment of T2DM (PrandiMet®, Novo Nordisk. Inc). PrandiMet® is prescribed by doctors with a diet and regular work-out; it is used to control high blood sugar in patients with T2DM. Since then it has been prescribed in conventional dosage form to large amount of population for the treatment of T2DM without any adverse event or death (FDA Adverse Events Reporting System (FAERS), August - 2012; Medpagetoday, 2014). It has been proved that combining MH with RG provided a safe and effective therapeutic strategy when monotherapy with Metformin was no longer adequate in adult patients with type 2 diabetes followed in a primary setting care (Hermans and Hooge, 2009).

RG is a megitinide class or also called as glinides, increase insulin secretion similar to sulfonylureas bind with weak affinity and faster dissociate from binding site hence act for shorter time. Although the researches show that Glinides act rapidly than sulfonylurea (Shigeto et al., 2007) and has no effect on insulin release in the absence of glucose like sulfonylurea (Repaglinide Apollo, 2012). RG is BCS class II drug with with poor solubility (Raskin et al., 2003). MH a highly water-soluble anti-hyperglycaemic agent, is commonly used as first-line treatment of type 2 diabetes. It
is the most preferred biguanide class drug acts by decreasing hepatic glucose output, as well as enhancing sensitivity of the hepatic and peripheral tissues to circulating insulin and thus also referred as insulin sensitizing drug (Veltkamp et al., 2012). It also inhibits the intestinal absorption of glucose and exerts anorexic effect. MH effect reduces if there is not enough endogenous/exogenous insulin and patients cannot maintain perfect glycemic control (Hundal et al., 2000). Repaglinide and metformin target different pathophysiological components of T2DM (insulin secretion and insulin resistance, respectively) and different aspects of hyperglycemia (postprandial and fasting, respectively). In a recent study, Lund et al. have demonstrated that in nonobese T2DM patients, metformin reduced postprandial glycemia, triglycerides and free fatty acids similarly to repaglinide (Lund et al., 2008). These data support a possible synergistic effect on postprandial hyperglycemia by combining metformin and repaglinide.

However, both drug have short half-life of the drugs (MH/ 0.9–2.6 h, RG/1.3 h) (Corti et al., 2008; Soegondo et al., 2004), patients have to take the ordinary compound tablets 2 to 3 times every day; thus, causing inconvenience to patient and fluctuations. MH is poorly permeable in the gastrointestinal tract. Its oral bioavailability is in the range of 40 to 60% decreasing with increasing dosage, which suggests some kind of saturable absorption process. It also has very high water solubility leading problems in controlling the initial burst of drug (Kumar and McGuffy, 2003). RG is having bioavailability 56% and metabolize completely by oxidative bio-transformation; its metabolites do not contribute to antidiabetic action. RG is rapidly eliminated from the blood stream with a $t_{1/2} \leq 1$ thus, frequent administration required that make patients compromised for the treatment (Venkataramudu et al., 2012). Providing both drug in a separate layer in sustain release formulation for 12 h, can reduce dosing frequency, improve bioavailability and combination of different glucose lowering mechanism will assure patient therapy and compliance especially for 2nd line treatment of T2DM.

An ideal oral medication is that which after ingestion quickly achieve therapeutic concentration and maintain steady state plasma concentration for longer period of time so less dosage amount required, less frequency of administration and
increase patient compliance with higher bioavailability of active constituent can be achieved (Robinson and Lee, 2003; Lee and Robinson, 2001).

8.2 METHODOLOGY

8.2.1 ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

Metformin hydrochloride (MH) and Repaglinide (RG) were analyzed by UV spectrophotometric–simultaneous estimation method using Shimadzu-1800 double beam UV-Visible spectrophotometer in addition with UV-probe_2.33 software and the analytical method was developed and validated. The estimation was carried out by absorbance correction method (Younis and Algobahi, 2012) utilizing concept of standard addition in two dissolution medium i.e. 0.1 N HCl-gastric fluid and pH 6.8 phosphate buffer as a solvent. The proposed methods were validated accordance to ICHQ2 (R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification (ICH Guidelines Q2(R1), 1996)

8.2.2 PREFORMULATION STUDIES

Preformulation studies are the foundation for formulation of robust and effective dosage form. The study gives understanding for the physicochemical properties of the drug molecule. Efforts given on preformulation studies can provide cost cutting, by lowering challenges in formulation development. Physical state, color, and clarity, solubility, melting point, FTIR, solid state reaction study was carried out for both MH and RG. Based on the observation the solubility enhancement of RG was carried out by Complexation with β-cyclodextrin. The micromeritic study viz. angle of repose, carr’s index and Hausner’s Ratio was calculated for MH and RG that may help to decide method of formulations.

8.2.3 DRY GRANULATION

The dry granulation was done using Guar gum (GG); HPMC K100 M; Eudragit RSPO (E-RSPO) in 10-30% for MH and 20%, 30% and 40% polymer and 10% dry binder on trial and error bases with Gum Acacia (AG), Carbopol-971 and HPMCK 100M concentration under high pressure. The compression achieved by two processes: producing a huge tablet like mass known as slug in a heavy-duty tableting
press; the process is known as slugging or squeezing powder between two rollers to produce a sheet of material known as roller compaction. In both cases slug/pressed sheet of materials are broken using a suitable milling technique and sieved to produce granular material (El-say et al., 2010, Kondeti et al., 2014).

8.2.4 WET GRANULATION

Wet granulation involved mixing of dry drug, polymer 20% Polymer (Xanthan gum, Guar gum, HPMC K100 M, Eudragit RSPO) for MH and 40% Gum Acacia (GA), Carbopol-971 and HPMC K 100 M for RG and many times binder mixture using a granulating agent PVP k30 5%. The fluid in granulating agent contains a solvent which should be volatile so that it can be evaporate on drying. Thus prepared mass was passed through a sieve that produces granules and agglomerates. The solvent was allowed to evaporate and agglomerates again passed through sieve to produce likely uniform size particle (Kondeti et al., 2014).

8.2.5 MELT GRANULATION

Melt granulation techniques required use of meltable binders, Hydrogenated castor oil (HCO), stearic acid (SA) and bees wax (BW) for MH and Polyethylene glycol 6000 and poloxamer 188 for RG. In this technique binder alone or in combination with control release polymers required to achieve desire drug release profile and their usage depends on nature of polymer, nature of drug and amount of dose of a drug to be controlled for release (Wadher et al., 2010; Sharma et al., 2014). Further to choose optimum variables that give desire drug release, $3^2$ full factorial design was employed. The data were analysed by regression analysis and optimised batch was selected.

Preparation of Bilayer Tablets

The bilayer tablets were prepared manually by double compression method in all case after satisfactory pre-compression evaluations results for both drug granules. In this double compression method, pre weighted quantity of MH was compressed with low pressure and then along with it pre weight repaglinide compressed. Necessary cavity adjustment was carried out. The final pressure was kept same for all formulation. (Sharma R et al., 2014).
8.2.6 EVALUATIONS

All prepared formulations were evaluated for Pre-compression evaluation viz.

I. Angle of repose

II. Carr’s index

III. Hausner’s Ratio

Post-compression evaluation parameter viz.

I. Uniformity of unit dosage,

II. Crushing strength,

III. Friability test,

IV. Dimensions

V. Assay and

VI. *In-vitro* dissolution test.

VII. Drug release kinetics & release mechanism

VIII. Stability study

The prepared formulations were compared to each other based on above evaluation parameter and based on its results the final formulations were selected. $3^2$ full factorial designs were used when above evaluation results were insufficient to decide final formulation. In Factorial design the results were analyzed by multiple regression analysis and hypothesis.

Optimized formulations were further evaluated for *in-vivo* drug release study. Plasma-drug concentration curve were plotted and different kinetic parameter were determined.
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8.3 RESULTS AND DISCUSSION

8.3.1 ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

In simultaneous estimation of MH and RG Absorption correction method with standard addition was successfully developed. Following formula obtained to determined drug concentration \([C_x \text{ and } C_y = \text{Concentration of MH and RG respectively (gm/100 ml)}]\)

For Estimation of drug in 0.1N HCL

\[ C_y = \frac{\text{absorbance at } \lambda_2(299)}{71.1944} \]

\[ C_x = \frac{\text{absorbance at } \lambda_1(208) - (C_y \times 678.417)}{91.1833} \]

For Estimation of drug in Phosphate buffer pH 6.8

\[ C_y = \frac{\text{absorbance at } \lambda_2(283)}{78.77} \]

\[ C_x = \frac{\text{absorbance at } \lambda_1(232.4) - (C_y \times 678.417)}{113.93} \]

The developed method was validated for Linearity & Range, Accuracy, Precision, Limit of detection (LOD) and Limit of quantification (LOQ). The developed method also compared with available only two but different method for simultaneous estimation. After results it was concluded that the current developed method is more accurate and economic and the method complies with detection of drugs as per their label claim. Further no additional derivetization or modification in spectra is required so the proposed method can be said as simple accurate and economic as compared to other published method.

8.3.2 PREFORMULATION STUDY

The organoleptic properties and melting point showed that both drugs were in control/limit compared to the limits described in pharmacopoeia. The results of solubility study revealed that RG had low solubility and in order to achieve comparative drug release to that of MH, solubility enhancement of RG is necessary.

Complexion technique was employed for solubility enhancement of RG. RG’s complexation was done using β-CD. A phase solubility curve was prepared and with
the help of saturation solubility with β-CD, best ratio was selected that enhance solubility of RG. 1:1 molecular ration of drug to β-CD was selected that give highest but different solubility in 0.1n HCl and pH6.8 phosphate buffer. The prepared complexation was confirmed by DSC study. The evaluation of physical properties of both drug showed that RG- β-CD complex had very poor flow property and in spite of good flow properties of MH, direct compression technique was not employed as it required higher amount of polymer compared to granulation technique. It also required higher compression force for matrix tablet formulation and sometimes friability problem observed when polymer / excipients not selected properly.

8.3.3 FORMULATION BY DRY GRANULATION

The results for formulation F1-F11 (for MH layer) showed that all pre-compression parameter were in control but in post formulation compression the friability was not observed well in limit. All other parameters were found in limit. In comparison to all formulation F11 showed good in-vitro drug release and was used for RG layer selection. The formulation F12-23 showed good flow properties, hardness 7.2-8 kg/cm². The friability was quite bellow the limit ranging from 0.80-0.96% and assay were in prescribed limit. From the invitro drug release study F20 showed good drug release and was selected.

The drug release mechanism of selected bilayer tablet based on best curve fit method showed, MH layer released drug by first order drug release kinetic that is concentration dependent drug release and RG showed Zero order drug release rate indicating concentration independent or erosion controlled drug delivery from the layer. The value of coefficient (n) for MH layer was 0.41, (< 0.5) conforms fickian diffusion based mechanism of drug release and for RG, it is 0.88, indicating non fickian drug release and conforms zero order drug release mechanism (>0.85). The results of stability study are most important parameter. The stability study showed that formulation after 6 month in accelerated stability showed almost unacceptable friability. Marginal reduction in hardness was observed and invitro drug release study based on similarity index showed 92% similar drug release.
8.3.4 FORMULATION BY WET GRANULATION

In this technique preliminary study was carried out to select binder for successful wet granulation. Preliminary study P1-P6 showed comparison between starch, pregelatinized starch and PVP K30 as binders. The results showed during granulation drying time taken by different batches was starch > pregelatinized starch > PVP. It was may be due to high viscosity of starch paste > pregelatinized starch > PVP. PVP showed more hardness than starch and pregelatinized starch. The friability results showed that PVP showed good friability control (<1). Thus PVP k 30 was used in further studies.

Wet granulation using PVP K30 (5% in IPA) was carried out for different batches (F15-F24) for MH layer. Based on results combination of polymer was used to achieve better control of drug release. Among batch F28-F30 (combination of Eudragit RSPO and Guargum), batch F29 and among batch F31-F33 (combination of Eudragit RSPO and HPMCK100M), batch F33 showed good initial drug release and sustain release till 12 hour. Both formulations were tested for mechanism of drug release and drug release rate. the value of Korsmeyer and Peppas’ coefficient (n) was 0.61 & 0.47 for F29 & F33, indicates that F29 gives anomalous drug release i.e. mixture of diffusion and dissolution and F33 give purely diffusion based drug release (higuchi $R^2=0.9984$). Further n=0.61 is more closure to 0.8 than 0.47, meaning F29 is closer to zero order drug release than F33 (>0.85 indicate zero order drug release) and thus F29 (containing mixture of 15% guargum and 5% Eudragit-RSPO) was selected for RG layer study and stability study.

In wet granulation formulation study with F34 to F38 showed all formulation were in control limit for pre and post compression evaluation parameter. Based on RG release study, F36 and F38 were tested for various kinetic models using M. S. Excels. The value of Korsmeyer and Peppas’ coefficient (n) was 0.96 & 0.98 for F36 & F38, indicates that both gave dissolution controlled zero order drug release and F38 was more robust than F36. Batch F38 was intended to check that whether reducing amount of polymer can produce same result as F36 or not and the results shows that batch F38 succeeded in intention.
The stability study was carried out for F38 that containing both selected MH and RG layer. The dissimilarity index ($f_1$) for *in vitro* drug release before and after 6 month obtained was 1.67 and 1.59 for MH and RG. The similarity index of MH layer was 94.27% and for RG layer was 93.53% thus both dissolution profile were considered similar.

### 8.3.5 FORMULATION BY MELT GRANULATION

Melt granulation technique employed 10, 15 and 20% stearic acid (SA), hydrogenated castor oil (HCO) and bees wax (F39-F49). The prepared tablets were evaluated for pre and post compression evaluation parameter. The results showed that hardness and friability both were improved than other two techniques. All other parameters were in control limit. The comparison of *in vitro* drug release study showed that 15 % HCO and SA showed comparatively good drug release. The change in diluent from MCC to DCP improved the % drug release for both formulations. F48 and F49 both showed similar drug release kinetics i.e. first order drug release with anomalous drug release; both showed similar flow properties, compression properties and other evaluation parameter. It was difficult to choose any one of them based on their evaluation parameter.

RG layer was prepared with melt binders PEG 6000 and Poxamer 188 (F50-F53) the results showed that both binders were acting as just a binder and role in release retardation was present but it was limited. RG is low soluble drug and use of hydrophilic binders were insufficient to control the drug release thus hydrophilic release retarding polymer-HPMC K 100 M was used on trial and error bases. The results of % drug release showed positive effect of polymer. The obtained results from $3^2$ full factorial design were tested by multiple regression analysis with hypothesis ($H_0$) that the results obtained are by chance or model has no predictive capability. The hypothesis was tested by F test and with 5% probability. The results showed that the developed model was significant and $H_0$ was rejected. For the developed polynomial model equation the values were predicted using contour plot and response surface plot. For the predicted check points, dependent variables were predicted theoretically and they were compared with actual value obtained after preparation of that formulation also with that optimized batch was also selected.
The stability study was carried out for two different formulations. In both formulation similar RG layer and in MH layer one contains 15% HCO (F67) and another 15% SA (F66). The results of accelerated stability study showed that both formulations were stable based on comparison made by similarity index. Formulation F67 had lower similarity index than F66 thus it was considered that F66 is more robust formulation than F67 and was selected as optimized batch.

8.3.6 IN VIVO PHARMACOKINETICS STUDY

In this study rabbits of either sex weighing (2.9-3.4 kg) were divided into 3 groups, each consisting of 2 animals for the pharmacokinetics study. First group received the immediate release tablets formulation available in market, the second group received the tablets optimized from wet-granulation approach and third and fourth group received the tablets prepared from the Melt-granulation approach. For the obtained blood plasma was separate and plasma-drug concentration was determined. On the bases of plasma-drug concentration curve numbers of pharmacokinetic terms were calculated and compare with marketed immediate release formulation.

The comparison of parameters showed that with similar AUC, the T_max obtained was increased in optimised formulations (MH 9h, RG 4h) compared to conventional formulation (1.5h); conforms drug release was sustain. The value of relative bioavailability, $F_r$ (%) for F38, 98.11 and 102.05 and for F66 it is 101.97 and 102.96 respectively for MH and RG, indicating bioequivalence and both drug release simultaneously for 12 h time period. Although MH and RG having different solubility properties and elimination $t_{1/2}$, interestingly both formulations showed almost similar pharmacokinetic behavior and for both formulation mean retention time for MH and RG was close to each other thus both drug will be available together for almost same time period to provide combine effect for treatment of diabetes.
8.3.7 INFLUENCE OF VARIOUS FACTORS ON DRUG RELEASE

In current study various factors viz. granulation technique, nature polymer, effect of binder, effect of diluents, effect of crushing strength, binder addition rate, cooling of melt granules, effect of dissolution media, paddle rotation intensity were observed as they may affect formulation and its performance.

**Granulation Methods:** In current study three different granulation technologies were used. (1) Dry granulations (2) Wet granulations and (3) Melt granulation. All three techniques involved their different technique and that affect the drug release. In dry granulation the compaction, either by roller-compaction or slug preparation for granulation of powder blend was very important it was observed that higher the force involved for compaction of mass, harder the granules were obtained and hardness of tablets was increased. In wet granulation granulating agent its properties were important that gave the tablet excellent strength, uniform weight and controlled friability Ex. Starch, Pre-gelatinised starch and PVP (formulations P1-P6). Although addition of additional ingredient (binder), with or without costly solvent, its drying at desire temperature and time period are critical point that may increase cost and delay in formulation. In melt granulation use of melting binders creates difference in all other techniques as they act as binders as well as release retarding agent. It require melting of binders at desirable temperature that affect the cost but in contrast to that elimination and reduction of release retarding agent as observed in case of MH layer and RG layer respectively; without use of toxic solvent and no delay in formulation related to drying of granules, reduces the overall cost and time. In all three methods melt granulation technique showed least % friability and highest hardness.

**Nature of Polymer:** Nature of polymer is an important factor that affected mainly drug release. Nature of polymer viz. Natural polymer (Guar gum, Xanthan gum, gum acacia, Bees wax, carnauba wax), synthetic polymer (HPMC, polxamer, PEG), semisynthetic polymer (hydrogenated castor oil, steatic acid) acrylic polymer (Eudragit S, Eudragit RSPO), hydrophilic polymer (HPMC, Guar gum, Xanthan gum, gum acacia, PEG ), lipophilic polymer (Bees wax, carnauba wax, hydrogenated castor oil, steatic acid), swellable polymer (carbomer) were the different polymer with different nature were used in current study. It was observed that usage of these
polymers depends on granulation technique used and solubility nature of drug. In current study highly water soluble MH and low soluble RG were used. MH required hydrophobic and acrylic polymer to retard drug release and RG required hydrophilic and/or swellable polymer.

**Effect of Binder:** Different types of binders were required as per the formulation technique. It was observed that binder play important role in SR tablet formulation. In dry granulation dry binders were required that were mixed with drug and polymer; in wet granulation binder were used to prepare wet granules that required role of granulating fluid and in melt granulation melting point of binders were unique properties. In gry granulation binder provided physical strength to tablets and for that higher amount of binder required which unnecessarily increased volume of tablet. In wet granulation, the amount of binder reduced as the granulating fluid was used in addition. In melt granulation requirement of amount of binder was quite higher side than other binder but it also gave retardant effect and eliminate release retarding agents (in case of MH layer by melt granulation)

**Effect of diluents:** in current study diluents played essential and various roles in different formulations. Diluents usually used as diluting agents but here they acted as binders in dry granulation, as filler, as release controlling or release modifying agent. These roles were different depending upon different diluents. In current study, MCC, Lactose and DCP were used as diluent. With MCC it was observed that in dry granulation it provided mechanical strength to the tablets for MH layer. In same technique, for RG layer, it was observed that MCC played important role in controlling drug release and in compared to that when lactose was tried higher & unwanted drug release was achieved. MCC is cellulosic hydrophobic polymer that swell up to some extent and that was responsible for controlled release; Lactose is water insoluble but hydrophilic material that released drug by erosion. In melt granulation for MH layer when MCC replaced by DCP, it improved dissolution characteristics. DCP is also having similar properties like lactose that erode from lipophilic binder and improvise dissolution.

**Effect of Hardness:** Hardness or crushing strength of the tablets is very important and distinctive property of SR formulation that may or may not affect drug release and friability. In current study all formulation were given similar compaction
force but different formulations achieved different hardness. It was observed hardness obtained for different was lower to higher in formulation batches prepared with dry-granulation <wet-granulation<melt-granulation techniques and with that friability obtained was higher to lower and no correlation observed for drug release. The drug release in different formulations was based on usage of different polymer and binder rather than hardness.

**Binder Addition Rate:** The addition of binder in case of wet-granulation found important for dissolution profile. It was observed that slower addition rate gave uniform and reproducible drug release rather than addition at once. The slow addition of binder makes uniform mixture of drug, polymer and binder and by that reproducible result can be achieved. Thus addition of binder at slower rate is very important.

**Cooling Rate of Binder:** It was observed that cooling of melted binder after addition of drug affected drug release. When rapid cooling at freezing temperature was done unexpected drug release was observed. The reason for this may be uneven distribution of binder when rapid cooling was done. Cooling of binder at room temperature produced better and reproducible drug release. The formulations were evaluated by Raman spectroscopy for that test formulation prepared by rapid cooling of melted binder was done and it was compared with optimized formulation. The microscopy clearly indicated that rapid cooling made clumps of binder and uneven distribution occurs, compare to cooling at room temperature for optimized formulation.
8.4 CONCLUSION

Three formulations dry granulation, wet granulation and melt granulation were prepared in this study. In comparison to all, wet granulation and melt granulation provided better *in vitro* dissolution profile and optimized formulation characteristics. The melt granulation technique provided formulations with minimum % friability and ideal hardness. The optimised formulations from melt and wet granulation technique were compared to marketed formulation with *in vivo* pharmacokinetic study. The results showed that formulation prepared with melt granulation technique gave better relative bioavailability.

Finally it was concluded that SR bilayer formulation of MH and RG by melt granulation technique,

1. Will provide drug release for 12 h,
2. Drug release kinetics will be almost zero order,
3. Will provide better bioavailability,
4. Reduction in overall cost by,

- Does not use organic solvent that is costly and not safe to human and environments
- It does not required time for drying of granules
- Fewer processing steps
- It eliminates and/or reduces use of release retarding polymer

Thus an optimised SR Bilayer tablet by melt granulation technique is a promising formulation technique.