Original Research Article

Preformulation Study of Glimepiride: An Insight for Formulation and Development of Parenteral Formulation

ABSTRACT

Aim: The objective of preformulation study is to develop the elegant, stable, effective and safe dosage form by establishing kinetic profile, compatibility with other formulation excipients and physico-chemical parameters of new drug substance. This could provide important information for formulation design or support the need for molecular modification. So, in the present study preformulation studies were performed on Glimepiride (GMP) to assess its suitability for parenteral formulation. Glimepiride is the first IIIrd generation sulphonyl urea used to treat type –II diabetes mellitus.

Methods: The authenticity of GMP was established by DSC and FTIR spectra. A UV spectrophotometric method and HPLC method were employed for determination of GMP in bulk active pharmaceutical ingredient (API).

Results: The UV method was linear in the range of 3-10 μg/ml. The low % CV values of intra-day and inter-day variations revealed that the proposed method is robust. The retention time of GMP in HPLC method was found to be 1.9 min. The method was proven robust by obtaining very high regression coefficient value (0.999).

Conclusions: The results of the physicochemical study of drug revealed suitability of GMP for parenteral route. Moreover, the drug was found stable in both solid as well as liquid state at different conditions.

Keywords: *Preformulation, Glimepiride, Parenteral formulation, Stability.*

1. INTRODUCTION

Preformulation is a group of studies that focus on the physicochemical properties of a drug candidate that could affect the drug performance and the development of dosage form [1]. Also, it could provide important information for formulation design or support the need for molecular

modification. Every drug has intrinsic chemical and physical properties which have been considered before development of pharmaceutical formulation. This property provides the framework for drugs combination with pharmaceutical ingredients in the fabrication of dosage form [2].

The objective of preformulation study is to develop the elegant, stable, effective and safe dosage form by establishing kinetic profile, compatibility with other formulation excipients and establish physic-chemical parameter of new drug substance. The classic preformulation study requires drug characterization in solid as well as liquid phase. Preformulation can help in cost cutting for effective therapeutic development of the product.

Diabetes mellitus is a condition in which a person has a high blood sugar level, either because the body doesn't produce enough insulin, or because body cells don't properly respond to the insulin that is produced [3,4]. In 2021, Approximately 537 million adults (20-79 years) are living with diabetes. The total number of people living with diabetes is projected to rise to 643 million by 2030 and 783 million by 2045 [5].

Glimepiride is the first III generation sulphonyl urea. Glimepiride is a sulfonyl urea used to treat type –II diabetes mellitus. Molecular formula of glimepiride is C24H34N4O5S with a molecular mass of about 490.617g/mol [6]. It belongs to class-II of Biopharmaceutical classification system. It is completely insoluble in water, acidic media and slightly soluble in various buffers and organic solvents [7]. The mechanism of action of Glimepiride in lowering blood glucose appears to be dependent on stimulation the release of insulin from functioning pancreatic beta cells, and increasing sensitivity of peripheral tissues to insulin [8].

Polymeric microspheres as a parenteral drug delivery system are primarily developed for sustained release of drugs for prolonged systemic therapeutic effects after subcutaneous (SC) or intramuscular (IM) administration. Polymers used for formulation of microspheres are biodegradable and biocompatible. In the present research PLGA is used as a polymer [9,10]. This polymer is usually used for biodegradable controlled release microparticles. In the present research solvent evaporation method is applied to formulate microparticles for injectable controlled release drug delivery system. Microspheres in the finished product are in a dry powder form. Prior to administration, a microsphere product is reconstituted in a liquid diluent which can be supplied in a separate container or in the liquid compartment of a dual-chamber prefilled syringe [11,12].

So, in the present study focus was given on preformulation studies of GMP. The main objective of the study was to assess GMP for its suitability to be formulated as SR microspheres for parenteral delivery.

2. MATERIAL AND METHODS

Material

Biodegradable polymer, poly(lactide-co-glycolide), RESOMER® RG 502 H (lactide:glycolide = 50:50, Mw: 15000), RESOMER® RG 503 H (lactide:glycolide = 50:50, Mw: 35000), RESOMER® RG 504 H

(lactide:glycolide = 50:50, Mw: 50000), RESOMER® RG 750 S (lactide:glycolide = 75:25, Mw: 1.25K dalton) were obtained as a gift sample from Evonik, and all of them were stored at -25℃ to 8℃ prior to use. Glimepiride API was obtained as a gift sample from Acron Pharmaceuticals, India. Methylene chloride (DCM) was obtained as a gift sample from Final Limited, India. N-Methyl-2-Pyrrolidone was obtained as a gift sample from Avantor Performance Materials India Limited, India. Chemicals and solvents used were of high-performance liquid chromatography (HPLC) grade. Freshly prepared distilled water was used throughout the study.

2.1 Drug Identification

The drug identification was performed by organoleptic properties, melting point, UV, HPLC, FTIR and DSC.

2.2 Determination of thermodynamic solubility

Solubility study of drug was performed using different solvents such as methanol, ethanol, dimethyl sulfoxide, ethyl acetate, Dichloromethane (DCM) and N-Methyl-2-Pyrrolidone. Samples were shaken on a rotary shaker at 37ºC for 24 hours. The two phases are then separated by filtration [6]. Amount of solute in supernatant is then determined using UV spectrophotometric analysis at the corresponding λmax of each solvent.

2.3 Analytical Preformulation

2.3.1 Analysis of GMP by UV spectrophotometry method[13]

Standard stock solutions of GMP was prepared in acetonitrile and scanned spectrophotometrically over the range of 200–400nm with double beam spectrophotometer (Shimadzu UV spectrophotometer, 240 j/PC, Japan), against the respective blank, to determine wave length of maximum absorbance (λmax).

A stock solution containing 1000 µg/ml GMP was prepared by dissolving 25 mg GMP in 5 ml of acetonitrile in a 25 ml of volumetric flask and volume was made upto 25 ml with the acetonitrile. From these stock solutions, suitable aliquots were taken and diluted using appropriate solvent to get dilutions of 3-10 µg/ml. The determinations were conducted in triplicate and studied for three days to check intra and inter day variations.

Calibration curve was constructed at concentrations range 3-10 µg/ml. Absorbance of each solution was measured at the wavelength of 228 nm. Calibration curve was constructed for GMP by plotting absorbance versus concentration at 228 nm wavelength. The determination was conducted in triplicate.

2.3.2 Analysis of GMP by HPLC method[13]

Glimepiride was quantified using a Shimadzu prominence-iLC2010 high performance liquid chromatography (HPLC) system equipped with isocratic pump, auto sampler, and photodiode array detector. The mobile phase was water: acetonitrile, 50:50 (v/v). The system was equipped with an all-time C18 column (30 x 4.6 mm, 5µ), temperature of column was ambient and the flow rate was set to 1

mL/min. The injection volume for drug loading samples was 10 µl. The chromatographs were analyzed with empower software at 228 nm. Linearity of the method was proved for concentration range of 0.4ppm to 150ppm.

2.4 Drug-Polymer Compatibility Study

The physical stability of glimepiride with polymer was evaluated at 25℃ and 60% relative humidity (RH). Additionally, the samples were also closed in vials and stored in refrigerator (2–8℃). The samples were removed after 30 days.

2.4.1 Fourier transform-infrared (FTIR) study[14]

The FTIR analysis was used for qualitative estimation and identification of functional group present in the compound. GMP was mixed with each of the components at an appropriate ratio; equivalent to that used in formulation process. Each mixture was stored in USP type-1 glass vial at 25°C±5°C, 60±5% RH (relative humidity) for one month. FTIR spectroscopy, Shimadzu, Model 8400, Japan, was used to study the compatibility of pure drug and other preparation composites, by KBr pellet method and scanned from 4000 to 400 cm^{-1}.

2.4.2 Differential Scanning Calorimetry (DSC)[15]

DSC is the thermal analysis method by which we can measure the interaction of drug with polymer. The thermal analysis of Drug, PLGA, physical mixture of Drug and PLGA was performed by using 3-5 mg of samples in a standard thermal aluminum pan with a comparable lid and heated from 0 to 300°C at a 10°C/min heating rate in METTLER TOLEDO DSC (METTLER TOLEDO, Switzerland).

3. RESULTS AND DISCUSSION

3.1 Drug Identification

3.1.1 Organoleptic properties and Melting Point

Glimepiride is odourless and almost white powder which is sticky in nature. The melting point of drug was in the range 207–209°C.

3.1.2. Drug identification by UV

The maximum absorbance of GMP in acetonitrile was found at 228 nm as depicted in figure 1.

Figure 1: UV spectra of GMP in acetonitrile at 228 λ-max

3.1.3. Drug identification by HPLC

The peak retention time of GMP was found to be 1.9 min as observed from figure 2.

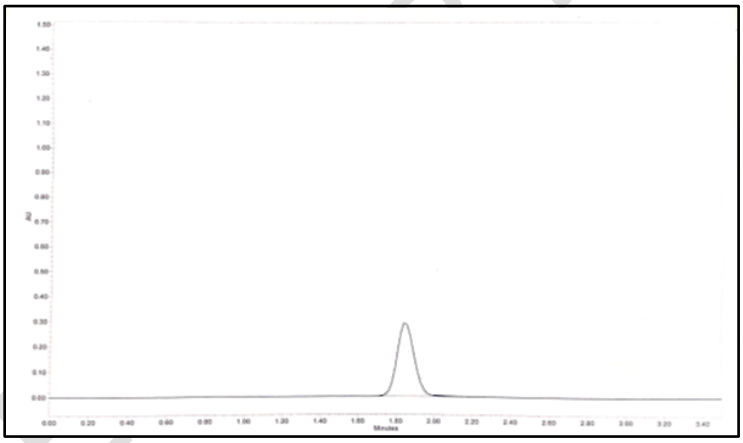

Figure 2: Peak of Glimepiride by HPLC

3.1.4 Drug identification by FTIR

The characteristic absorption peaks of GMP in FT-IR spectra as shown in Figure 3 and the functional groups responsible for characteristic peaks of GMP are mentioned in Table 1.

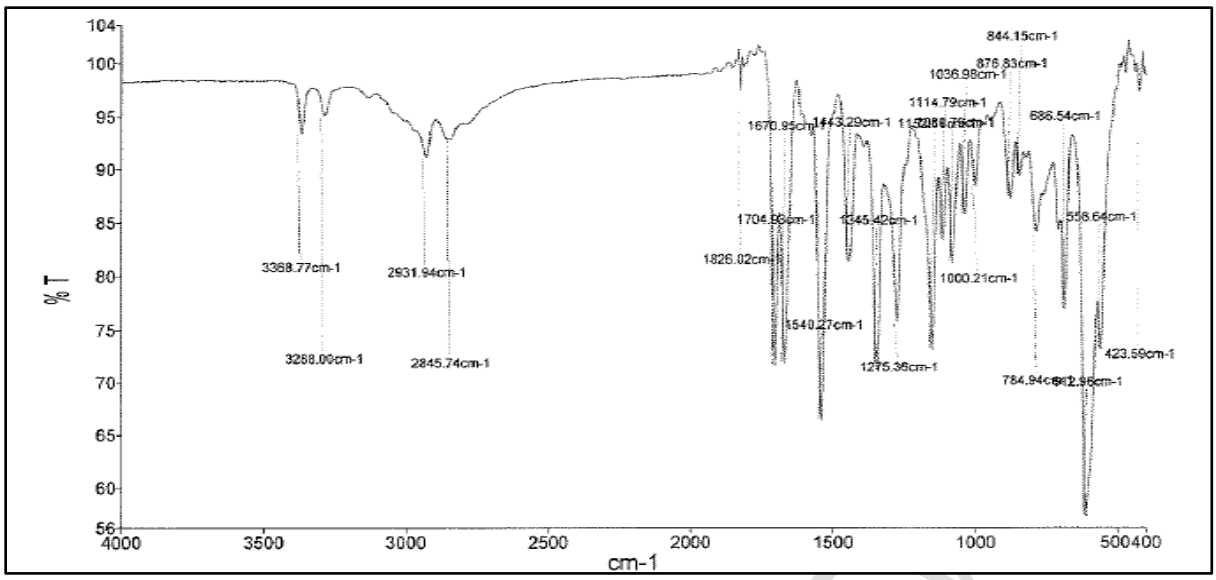

Figure 3: Fourier transform-infrared spectrum of Glimepiride

Table 1. Stretching bending of Glimepiride

Peak at wave number (cm-1)	Interpretation
3368.77, 3288	N-H stretch (Secondary amine)
2931.94	C-H stretch (aliphatic)
1704.93	C=O stretch
1670.95	N-C=O stretch
1345.42	O=S=O

3.1.5 Drug identification by DSC

DSC thermogram of GMP is shown in Figure 4. Reported melting point value was found 207℃ and practically melting point value were found 207-209°C and 218.48°C by capillary method and DSC, respectively.

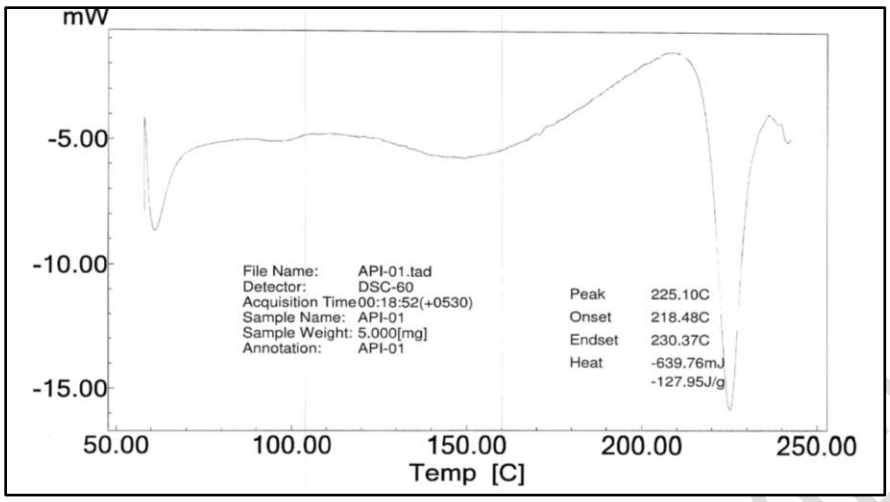

Figure 4: Differential Scanning Calorimetry (DSC) of Glimepiride

3.2 Determination of thermodynamic solubility

Glimepiride is a practically insoluble in water, soluble in dimethyl formamide and N-Methyl-2-Pyrrolidone, sparingly soluble in dichloromethane, very slightly soluble in methanol, ethanol and ethyl acetate. The solubility of GMP in various solvents is shown in Table 2.

Table 2. Solubility parameters of different solvents

Solvents	Solubility (mg/mL)
Methanol	3.0±0.15
Ethanol	3.0±0.15
Dimethylformamide (DMF)	57.0±2.85
Ethyl Acetate	1.0±0.05
Dichloromethane (DCM)	5.0±0.25
N-Methyl-2-Pyrrolidone	50.0±2.5

3.3 Analytical Preformulation

3.3.1 Analysis of GMP by UV spectrophotometry method

The development of spectrophotometry methods for the determination of drugs has been increased considerably in recent years because of their importance in pharmaceutical analysis. Based on the experimental data the standard calibration curves were plotted. The regression analysis showed very good correlation (r^2=0.9999) in acetonitrile. These solutions obeyed Beer-Lambert's law and the linearity was found in concentration range of 3-10 µg/ml in acetonitrile. The standard curve of GMP is shown in Figure 5.

Table 3. Standard curve of Glimepiride in Acetonitrile by UV

Conc. (ppm)	Absorbance at 228nm			Average	Std. Deviation	% RSD
0	0	0	0	0.000	0.000	0.000
3	0.307	0.311	0.3	0.306	0.006	1.820
4	0.405	0.408	0.402	0.405	0.003	0.741
5	0.515	0.511	0.507	0.511	0.004	0.783
6	0.617	0.615	0.609	0.614	0.004	0.678
7.5	0.771	0.768	0.765	0.768	0.003	0.391
10	1.01	1.03	0.999	1.013	0.016	1.551

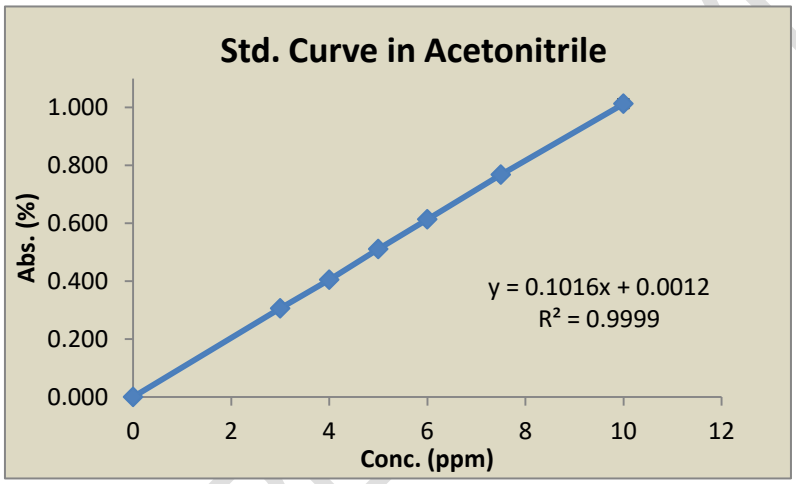

Figure 5: Standard curve of Glimepiride in Acetonitrile

3.3.2 Analysis of GMP by HPLC method

The method was developed to quantify Glimepiride for assay and *in-vitro* release. The chromatography was checked for its linearity and was proved to be linear from 0.40 ppm to 149.87 ppm which covers the linearity requirements for assay and *in-vitro* release, the chromatograph of the same is depicted in figure 6. The quantification of % Assay and/or % *in-vitro* release was done by estimating area of sample peak with area of standard peak of known concentration using formula Au/As*Cs/Cu*100 after conforming above mentioned system suitability requirements and the results are mentioned in Table 4. Method was found to be suitable for its intended uses.

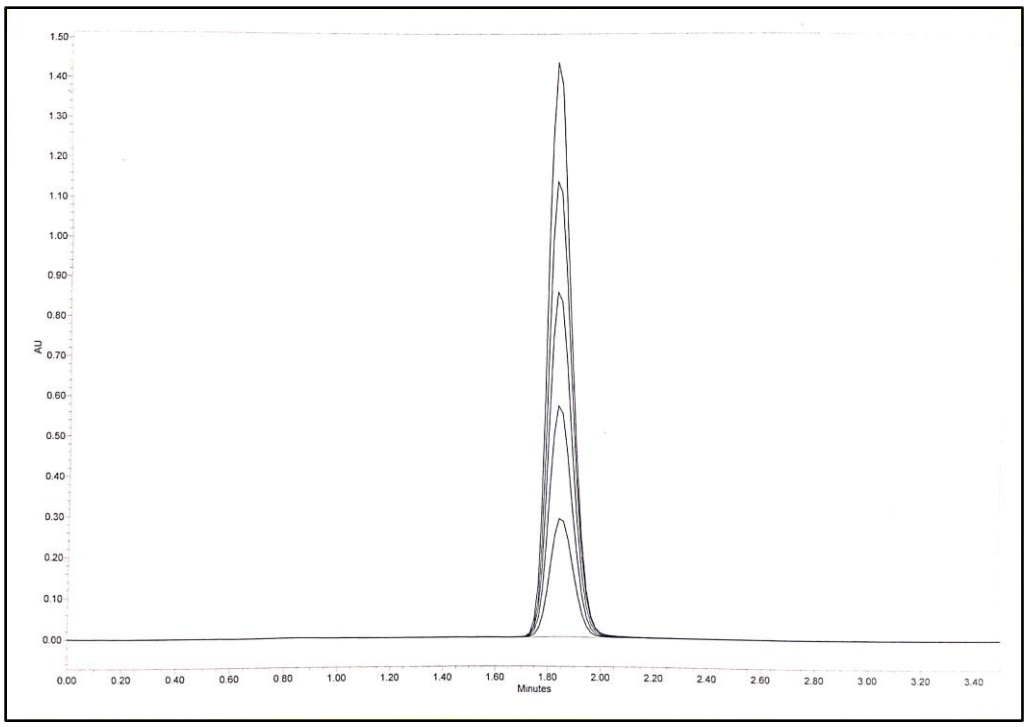

Figure 6: Linearity of Glimepiride by HPLC

Table 4. Linearity curve of Glimepiride

PPM with Potency	Set-1 Area	Set-2 Area	Set-3 Area	Average Area
0.4	8782	8798	8787	8789
2.5	55096	55079	55125	55100
9.99	210979	210959	211029	210989
24.98	550483	550495	550522	550500
49.96	1110121	1110087	1110095	1110101
99.92	2200138	2200145	2200162	2200148
149.87	3300576	3300589	3300538	3300568
		Slope		22040.042
		R^2		0.9999
		Intercept		-709.1137
		Y Bias		-0.0322

3.4 Drug-Polymer Compatibility Study

The characteristic absorption peaks of GMP in FT-IR spectra as shown in Figure 3 proves stable and pure drug profile. Further, stability of GMP has been also assessed at various temperatures, moisture,

light and oxidation condition. The results obtained from stability study under preformulation exhibited stable characteristics of drug at different storage conditions which are shown in Table 5.

Table 5. Drug stability under preformulation study at different conditions

No.	Influencing factor	Test Sample	Packing material	Storage condition	Storage time(weeks)	Physical degradation	Drug content
1	Moisture	Pure drug	Open container	25℃/75% RH	0	No	99.65
					1	No	99.48
2	Temperature Pure drug	Pure drug	50 ml glass container with twist-off closure	70℃	0	No	99.73
					2	No	100.05
					4	No	99.95
3	Oxidation	1%aqueous solution in 0.35 H_2O_2 Solution	25 mL glass flask with glass stopper	50℃	0	No	99.59
					1	No	99.99
					3	No	99.37
4	Light	Pure drug substance	Open petridish	Xenon lamp	24 hrs	No	99.45
					48 hrs	No	100.02
			Amber colour petridish	Xenon lamp	24 hrs	No	99.79
					48 hrs	No	99.83

3.4.1 Fourier transform-infrared (FTIR) study

The FTIR spectral analysis showed that there is no appearance or disappearance of any characteristic peaks of pure drug glimepiride and in the physical mixture which confirms the absence of chemical interaction between drug and polymers. The FT-IR spectra of physical mixture in initial condition and after 1 month study are shown in figure 7 and 8 respectively and the functional groups responsible for characteristic peaks are mentioned in Table 6.

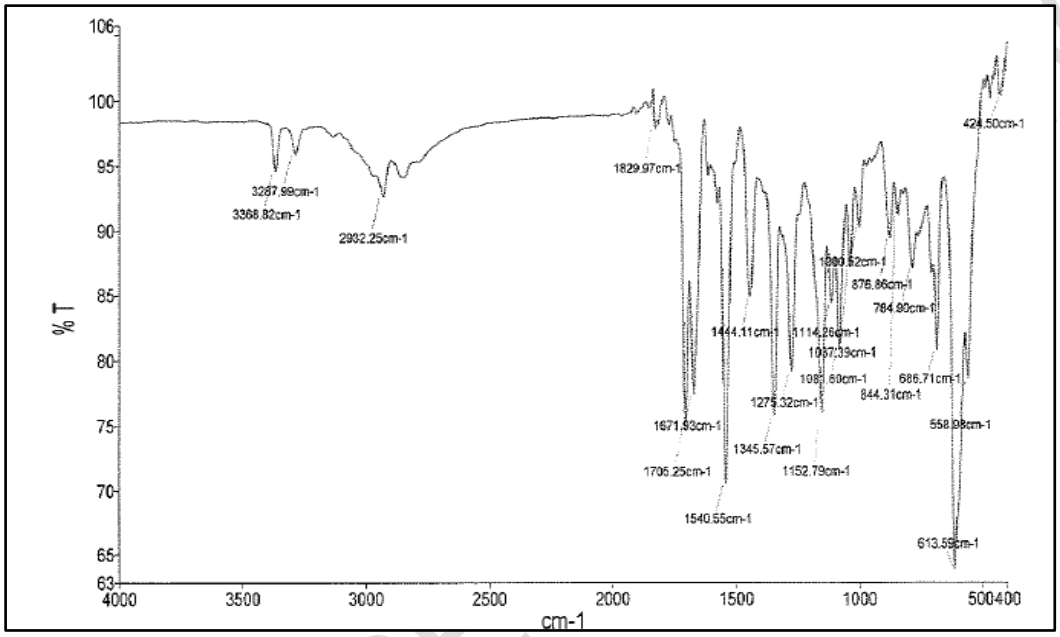

Figure 7: Fourier transform-infrared spectrum of Glimepiride-Polymer mixture (Initial)

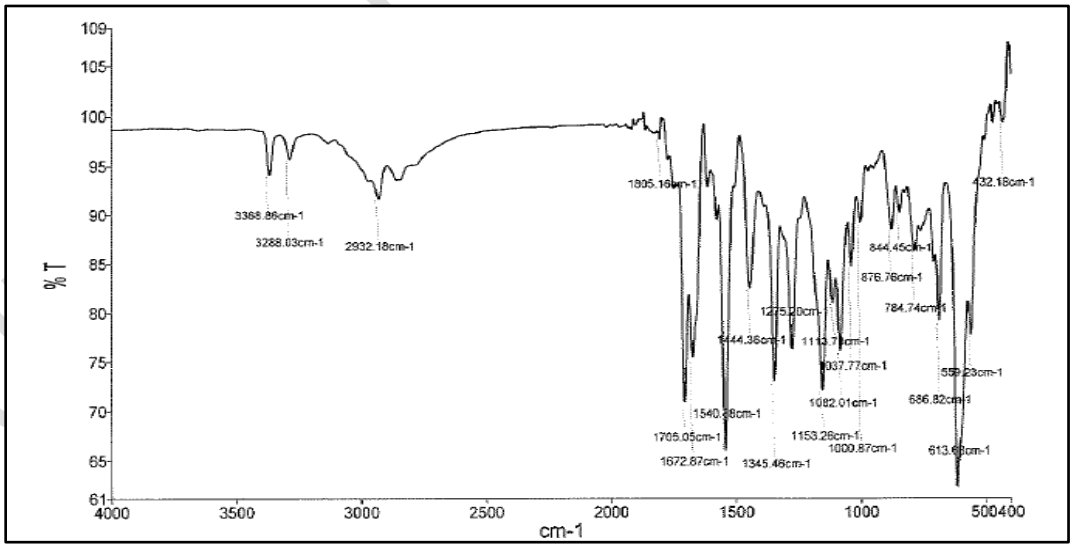

Figure 8: Fourier transform-infrared spectrum of Glimepiride-Polymer mixture

(1 Month, 25℃/60%RH)

Table 6. Compatibility of Glimepiride-Polymer mixture by FTIR.

Glimepiride (API) X (cm^{-1})	Glimepiride + Polymer mixture (Initial) X (cm^{-1})	Glimepiride + Polymer mixture (1 M 25°C/60% RH) X (cm^{-1})	Interpretation
3368.77	3368.82	3368.86	N-H stretch (Secondary amine)
3288	3287.99	3288.03	N-H stretch (Secondary amine)
2931.94	2932.25	2932.18	C-H stretch (aliphatic)
1704.93	1705.25	1705.05	C=O stretch
1670.95	1671.93	1672.87	N-C=O stretch
1345.42	1345.57	1345.46	O=S=O

3.4.2 Differential Scanning Calorimetry (DSC)

DSC thermogram of GMP and polymer mixture showed that there is no change observed in the endothermic peak of drug and polymer in physical mixture at initial condition and after 1 month, which confirms the absence of chemical interaction between drug and polymers as shown in figure 9 and 10 respectively.

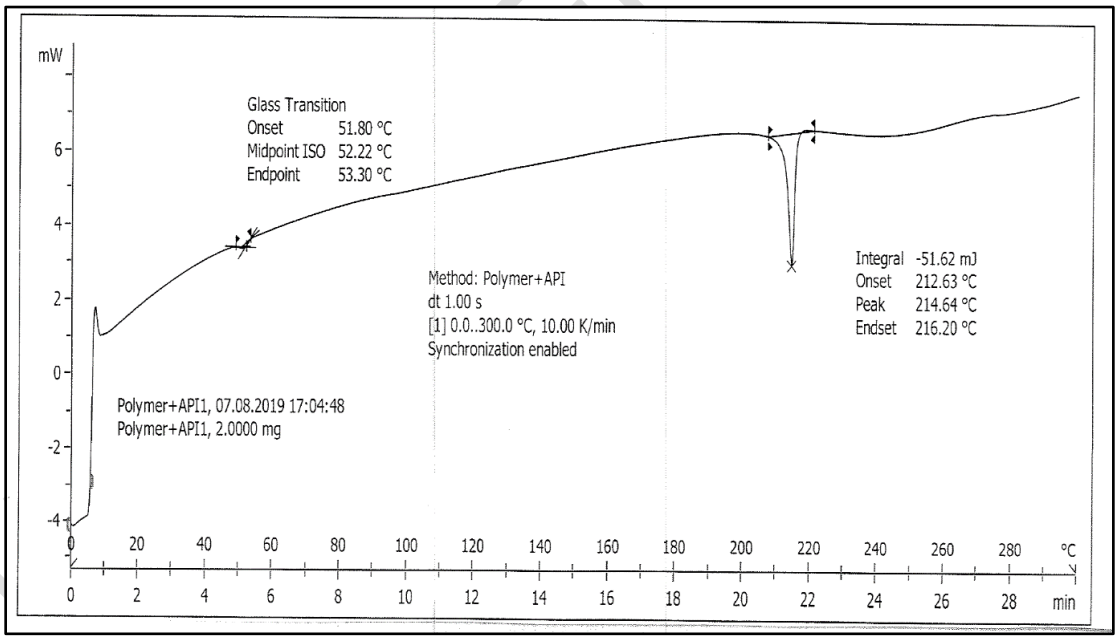

Figure 9: Differential Scanning Calorimetry (DSC) of Glimepiride-Polymer mixture (Initial)

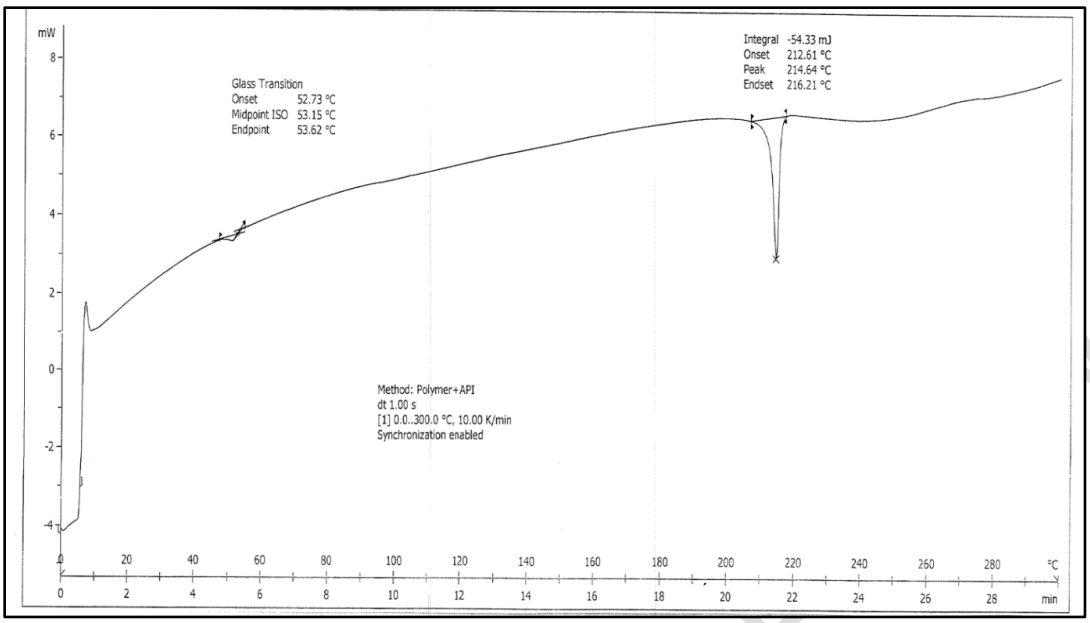

Figure 10: Differential Scanning Calorimetry (DSC) of Glimepiride-Polymer mixture (1 Month, 25℃/60%RH)

4. CONCLUSION

From the results of the different preformulation studies, it can be concluded that GMP is suitable for sustained release microsphere parenteral formulation. The results of UV, HPLC, FT-IR and DSC suggested the drug is authentic. The UV method and HPLC method showed good correlation indicating they can be used for quantification of drug in bulk and *in vitro* studies. The solubility study of drug suggested that it is soluble in organic media suggesting its suitability for sustained release formulation. Stability study under preformulation studies revealed stable characteristics of drug confirming final stability of formulation.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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