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Formulation, optimization and evaluation of pellets loaded glipizide co amorphous mixture using central composite design to enhance solubility

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ABSTRACT: The objective of this study was to prepare a co-amorphous blend of BCS class II drug, glipizide (GPZ), using arginine (ARG) as co former. The co-amorphous GPZ-ARG mixtures (molar ratios 1:1, 1:2 and 2:1) were prepared by mechanical activation (ball milling) with different milling times. The obtained mixtures were characterized with respect to their thermal properties, possible molecular interactions, dissolution properties and physical stability and compared to the behaviour of pure crystalline form. Solid-state characterization of mixture revealed amorphization of GPZ after 90 min. of ball milling. GPZ-ARG co amorphous mixture (1:2) upon ball milling for 90 min. exhibited maximum solubility (3876.8 µg/ml) compared to pure drug (300.89 µg/ml). The resulting best co amorphous blend was incorporated into pellets which were prepared by extrusion spheronisation technique. The parameters related to process of pelletisation and product related parameters were optimized by using 4-factor, 5-level Central Composite Design. The pellets containing co amorphous blend, prepared by using optimal parameter settings, showed 95.67% Drug Release in 120 min. (F23). The co-amorphous mixture and the optimized formulation were found to be stable when subjected to stability study as per ICH guideline. It was concluded that co amorphous is a promising approach to overcome the solubility constraint of Glipizide which would be helpful in better management of the disease.

KEYWORDS: Glipizide; Co-amorphous; Central Composite Design; ball milling; pellets.

1. INTRODUCTION

One approach that has been explored to overcome the problem of poor aqueous solubility is to convert crystalline drugs into their amorphous counterparts, thus increasing dissolution rate and apparent solubility of the compounds [1,2]. The main drawback of this approach is that amorphous systems are thermodynamically unstable and tend to recrystallize during manufacturing or storage [3]. Thus, producing amorphous formulations that are sufficiently stable for practical applications is a significant challenge. Many studies reported the improvement in the dissolution rate of a co-amorphous system of different drugs such as indomethacin and naproxen, simvastatin [4–6], carbamazepine, lurasidone [7], quercetin [8]. Co-amorphous drug-amino acid systems have increasingly become well-studied systems to improve the dissolution rate of poorly water-soluble drugs [9]. GPZ is an oral hypoglycemic agent used in treatment of diabetes mellitus. With the drug being BCS class II, it is poorly soluble but sufficiently permeable, so formation of a better dissolving and stable co-amorphous mixture could provide a promising candidate for better therapeutic action. In this study, the co-amorphous GPZ-ARG mixtures were prepared by ball milling at various milling times i.e. 30, 60 and 90 min. and characterized with respect to their thermal and structural properties, possible molecular interactions, physical stability and dissolution properties.

In present research co-amorphous mixtures of GPZ and ARG of different molar ratios were prepared using the ball milling method. Further, a mixture with the highest solubility was formulated in pellets dosage form by extrusion spheronization method using MCC PH 101 as pelletising aid, PVP K 30 as binder and SSG as

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superdisintegrant for faster release. Response surface methodology (RSM) is a rapid technique used to empirically derive a functional relationship between an experimental response and a set of input variables in the development and optimization of drug delivery systems [10]. In this work, central composite design (CCD) was employed to simultaneously study the effect of speed and time of spheronisation, amount of MCC PH101 and PVP K30, against response variables i.e. %Drug Release, Angle of Repose, Aspect Ratio and Disintegration time of pellets. CCD is a response surface design which provides information on direct effects, pair-wise interaction effects, curvilinear variable effects, and widely used for formulation and process optimization in the field of pharmaceutics [9,11].

2. RESULTS

2.1 Solubility study

The prepared co-amorphous mixtures of (GPZ: ARG) with different molar ratios (1:1, 1:2 and 2:1) showed markedly enhanced solubility over the pure drug in different pH (Figure 1). The maximum solubility was observed in phosphate buffer pH 7.4 (3876.8±21.56 µg/ml) of GPZ:ARG molar ratio (1:2) compared with pure drug (300.89±10.21) and other ratios. When milling time was increased from 30 min to 90 min the solubility was also increased, the maximum solubility was found in molar ratio (1:2) at 90 min compared to 1:1 and 1:2 ratios.

2.2 Solid-State Characterization

2.2.1 Fourier Transform Infrared Spectroscopy (FTIR) study

The spectra of samples are shown in Figure 2. The IR spectra of GPZ showed characteristic peak at 3248.13 cm^{-1} which is assigned to N–H stretching, 1330.88 cm^{-1} because of C=C stretching of aromatic ring, 1687.71 cm^{-1} due to C=O stretch of amide, 1647.21 cm^{-1} attributed to C=N stretching,1157.29 cm^{-1} assigned to C–N stretching vibration of amine, 1440.83 cm^{-1} due to C=C stretching of aromatic ring, band at 1031.92 cm^{-1} is attributed to S=O and a strong band at 1525.69 cm^{-1} is assigned to N-H bending vibrations [12].

2.2.2 Differential Scanning Calorimetry (DSC) study

In DSC thermograms of the Glipizide, Arginine and Co amorphous blend of GPZ: ARG is shown in Figure 3. The thermogram of Glipizide showed sharp endothermic peak at around 212.40°C corresponding to its melting point which revealed highly crystalline in nature and the value obtained was corresponding to its melting point reported in literature [13].

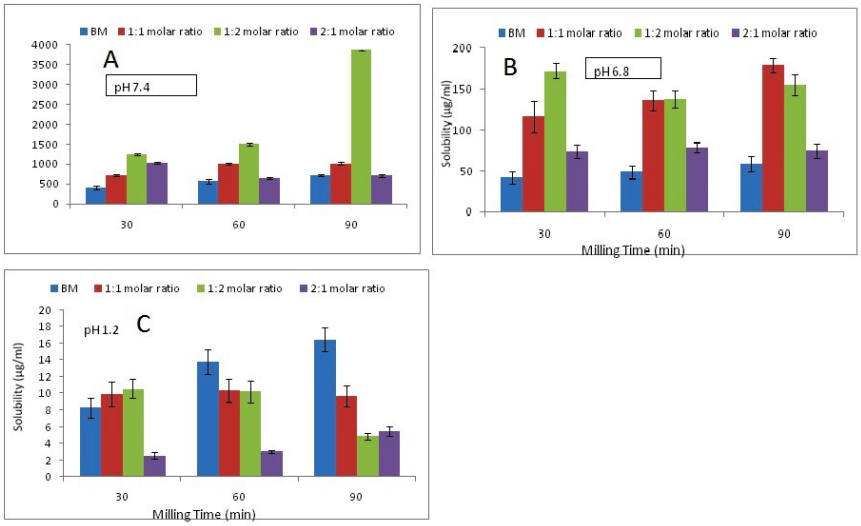

Figure 1. A) Solubility study in pH 7.4, B) Solubility study in pH 6.8 and C) Solubility study in pH 1.2

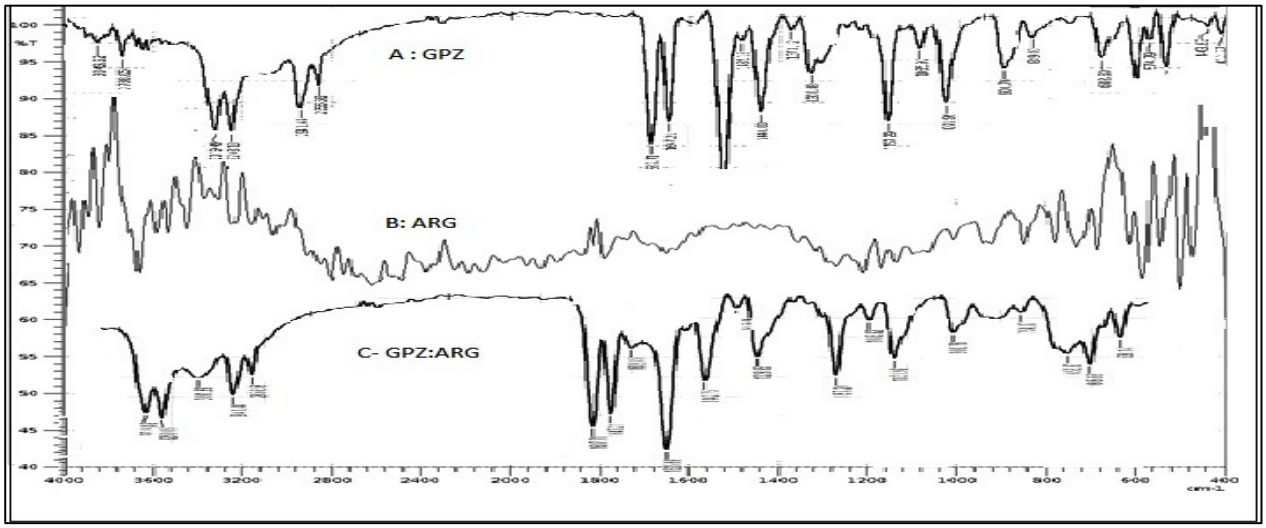

Figure 2. FTIR of A) Glipizide, B) Arginine and C) GPZ: ARG Coamorphous mixture

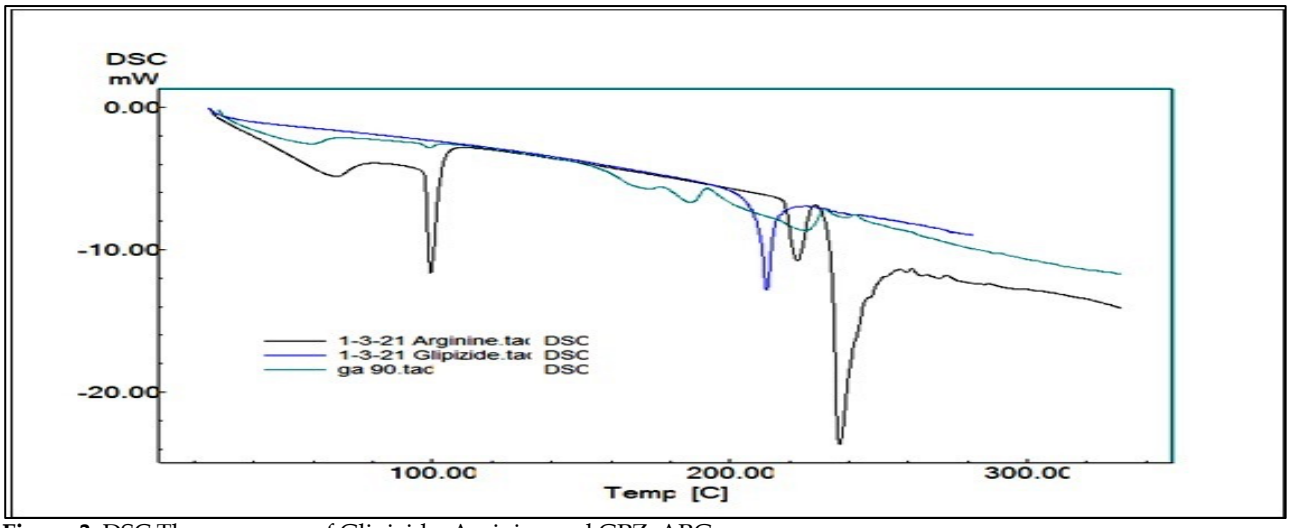

Figure 3. DSC Thermograms of Glipizide, Arginine and GPZ: ARG

2.2.3 Powder X-ray diffraction (PXRD)

In PXRD pattern (Figure 4) indicate GPZ in crystalline form, observable by several sharp peaks values at 77.29°, 10.89° ,15.53°, 16.82°, 17.91°, 18.39°, 19.14°, 21.69°, 24.14°, 24.16°, 26.61° at 2θ as reported earlier (6,13). The XRPD pattern of mixtures at 18.53°, 18.82°, 21.83°, 22.19°, 23.41°, 25.73 °, 26.45°, 28.41°, 29.23°, 32.99°, 40.81° at 2θ confirms the co amorphisation of GPZ by absence of sharp peaks of the drug (Figure 4).

2.3 Experimental design for pellets

In study independent variables (amount of MCC, PVP, Spheronisation time and Speed of Spheronisation) showed a significant effect on the Percent Drug Release, Disintegration Time, Aspect Ratio and Angle of Repose (⁰). The results for the dependent variables are shown in Table 1.

2.3.1 Data analysis for Percent Drug Release

The response obtained was subjected to multiple regression analysis and Final Equation in Terms of Actual Factors is as below

% DR = +89.43000-0.542917 Spheronisation time -0.400417 Spheronisation speed -0.650417 MCC PH 101 1.68208 PVP K30

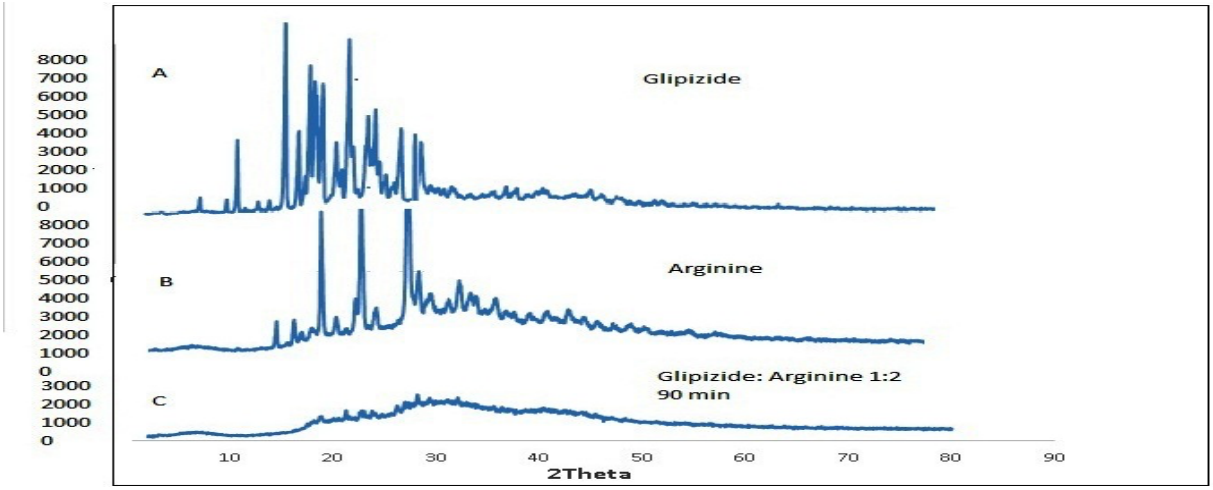

Figure 4. Powder X Ray Diffractograms of A) GPZ, B) ARG, C) GPZ: ARG 1:2 90 min.

Table 1. Coded value and responses of Central Composite Design

Std	Run	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3	Response 4
		A:Spheronisation time	B:Spheronisation speed	C:MCC PH 101	D:PVP K 30	% DR	Angle of Repose	Aspect Ratio	Disintegration time
		(min)	(rpm)	(g)	(g)	(%)	(degrees)	-	(min)
3	1	-1	1	-1	-1	92.6	28	1.289	8
1	2	-1	-1	-1	-1	93.24	29	1.473	9
4	3	1	1	-1	-1	92.34	26	1.169	7
9	4	-1	-1	-1	1	91.53	25	1.094	10
10	5	1	-1	-1	1	90.97	25	1.137	9
11	6	-1	1	-1	1	91.08	26	1.183	8
15	7	-1	1	1	1	85.73	28	1.632	10
5	8	-1	-1	1	-1	90.47	30	1.519	8
16	9	1	1	1	1	84.67	27	1.157	7
23	10	0	0	0	-2	95.67	30	1.622	6
21	11	0	0	-2	0	88.34	29	1.341	7
19	12	0	-2	0	0	86.91	28	1.296	10
18	13	2	0	0	0	87.48	26	1.178	9
25	14	0	0	0	0	85.74	29	1.213	8
7	15	-1	1	1	-1	91.08	28	1.369	7
6	16	1	-1	1	-1	89.93	27	1.253	6
13	17	-1	-1	1	1	88.39	27	1.172	8
8	18	1	1	1	-1	89.61	26	1.099	7
17	19	-2	0	0	0	89.36	29	1.317	8
12	20	1	1	-1	1	85.89	25	0.998	9
20	21	0	2	0	0	88.59	29	1.384	7
22	22	0	0	2	0	89.06	27	1.167	10
2	23	1	-1	-1	-1	90.36	26	1.054	7
14	24	1	-1	1	1	91.08	28	1.264	8
24	25	0	0	0	2	85.63	26	1.019	10

2.3.2 Data analysis for Disintegration time

The response obtained was subjected to multiple regression analysis and Final Equation in Terms of Actual Factors is as below

Disintegration Time = +8.12000-0.250000 Spheronisation time -0.333333 Spheronisation speed +5.15697 MCC PH 101 +0.750000 PVP K30

2.3.3 Data analysis for Aspect ratio

The response obtained was subjected to multiple regression analysis and Final Equation in Terms of Actual Factors is as below

Aspect Ratio = +1.25596 -0.078250 Spheronisation time +0.004417 Spheronisation speed +0.030000 MCC PH 101 -0.074750 PVP K30

2.3.4 Data analysis for Angle of Repose

The response obtained was subjected to multiple regression analysis and Final Equation in Terms of Actual Factors is as below

Angle of Repose = +27.36000-0.708333 Spheronisation time -0.041667 Spheronisation speed + 0.291667 MCC PH 101 -0.708333 PVP K30

2.3.5 Response surface plot

The response surface plots obtained during optimization through central composite design for Drug Release, Disintegration Time, Aspect Ratio and Angle of Repose are shown in figure 5.

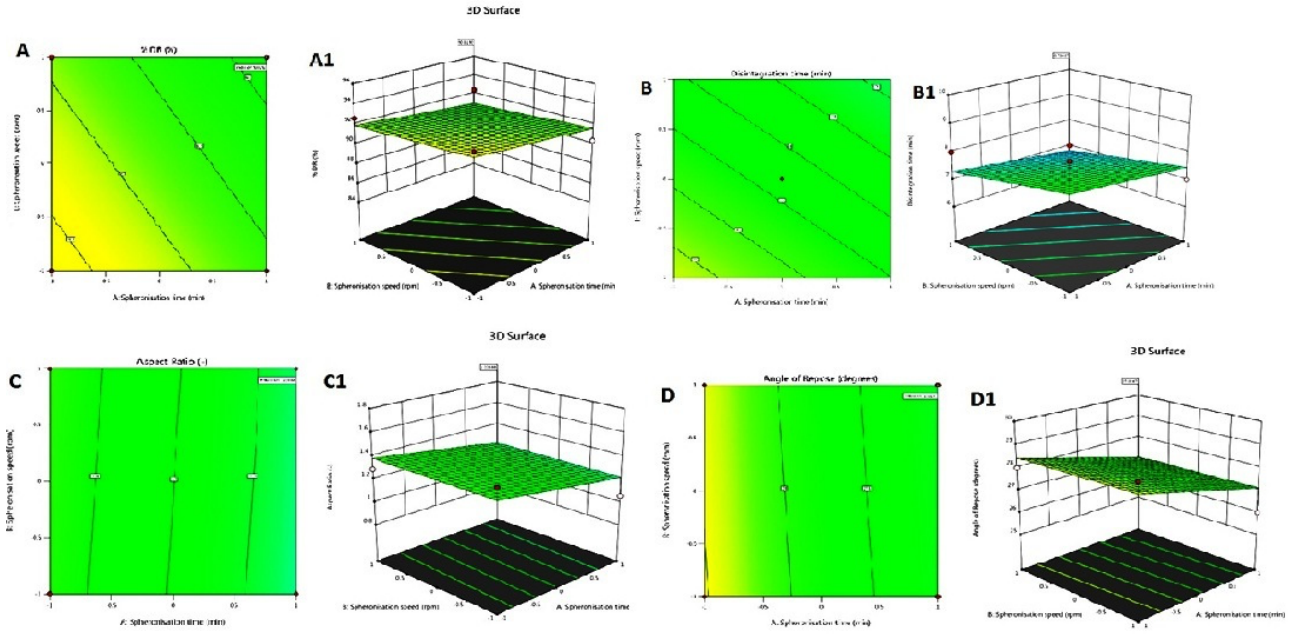

Figure 5. Contour plot and 3D surface response plot for A, A1) Drug Release, B, B1) Disintegration Time, C,C1) Aspect Ratio and D,D1) Angle of Repose.

2.4 Characterization of pellets

The results of characterization study of the co amorphous (GPZ: ARG) mixture and the pellet formulations are given in Table 2. The pellets showed good flow properties as evidenced by the micromeritic study. When CI value is less than 10 or HR value less than 1.11 is treated 'excellent' flow, whereas CI greater than 38 or HR greater than 1.60 is treated as 'very very poor' flow. In study we found CI and HR values for co amorphous (GPZ: ARG) mixture were 9.73 ± 0.61 and 1.08 ± 0.42 %, respectively. For pellet formulations the values were ranged from 3.02± 0.52 to 8.56 ± 0.36 and 1.00± 0.28 to 1.08± 0.62, demonstrating good compression properties of pellets.

2.5 In-vitro dissolution studies

In this study it was observed that the percent drug release (95.67 ± 3.59 %) of F23 (Run 10) showed significant difference (P<0.005) compared to other formulations as shown in Figure 6.

Table 2. The results of bulk density, tap density, hausner ratio, carr's index, and friability parameters

Parameters	Co amorphous (GPZ: ARG)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Bulk density	1.053 ± 0.08	0.925 ± 0.010	0.869 ± 0.08	0.826 ± 0.021	0.858 ± 0.081	0.883 ± 0.045	0.880 ± 0.026	0.885 ± 0.040	0.816 ± 0.074	0.826 ± 0.068	0.825 ± 0.010	0.829 ± 0.08	0.826 ± 0.021
Tap density	1.074 ± 0.06	0.820 ± 0.028	0.793 ± 0.025	0.796 ± 0.060	0.806 ± 0.090	0.789 ± 0.080	0.756 ± 0.083	0.740 ± 0.056	0.720 ± 0.063	0.721 ± 0.044	0.720 ± 0.028	0.782 ± 0.025	0.786 ± 0.060
Hausner Ratio	1.08 ± 0.42	1.10± 0.28	1.13± 0.34	1.07± 0.16	1.04± 0.25	1.02± 0.28	1.13± 0.25	1.02± 0.26	1.06± 0.18	1.08± 0.62	1.00± 0.28	1.02± 0.34	1.06± 0.16
Carr's index	9.73 ± 0.61	3.12± 0.52	8.42± 0.60	8.76± 0.36	5.20± 0.56	8.28± 0.42	3.40± 0.60	5.32± 0.32	3.70± 0.78	3.80± 0.42	3.02± 0.52	8.22± 0.60	8.56± 0.36
Friability test	—	0.7± 0.1	0.6± 0.1	0.5± 0.2	0.4± 0.2	0.3± 0.2	0.2± 0.1	0.4± 0.1	0.3± 0.2	0.2± 0.2	0.6± 0.1	0.5± 0.1	0.4± 0.2

Parameters	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24	F25
Bulk density	0.848 ± 0.081	0.883 ± 0.045	0.880 ± 0.026	0.885 ± 0.040	0.825 ± 0.010	0.829 ± 0.08	0.826 ± 0.021	0.848 ± 0.081	0.883 ± 0.045	0.880 ± 0.026	0.885 ± 0.040	0.816 ± 0.074	0.826 ± 0.068
Tap density	0.796 ± 0.090	0.789 ± 0.080	0.756 ± 0.083	0.740 ± 0.056	0.720 ± 0.028	0.782 ± 0.025	0.786 ± 0.060	0.796 ± 0.090	0.789 ± 0.080	0.756 ± 0.083	0.740 ± 0.056	0.720 ± 0.063	0.721 ± 0.044
Hausner Ratio	1.04± 0.25	1.02± 0.28	1.13± 0.25	1.02± 0.26	1.00± 0.28	1.02± 0.34	1.06± 0.16	1.04± 0.25	1.02± 0.28	1.13± 0.25	1.02± 0.26	1.06± 0.18	1.08± 0.62
Carr's index	5.20± 0.56	8.28± 0.42	3.40± 0.60	5.32± 0.32	3.02± 0.52	8.22± 0.60	8.56± 0.36	5.20± 0.56	8.28± 0.42	3.40± 0.60	5.32± 0.32	3.70± 0.78	3.80± 0.42
Friability test	0.4± 0.2	0.3± 0.2	0.2± 0.1	0.4± 0.1	0.6± 0.1	0.5± 0.1	0.4± 0.2	0.4± 0.2	0.3± 0.2	0.2± 0.1	0.4± 0.1	0.3± 0.2	0.2± 0.2

2.6 Morphological analysis

Scanning Electron Micrographs (SEM) of pellets are given in Figure 7. Examination of the surface morphology showed good roundness and a smooth surface and also showed that the pellets were discrete and free from cracks.

2.7 Stability studies

In stability study, there were no physical changes observed in co amorphous (GPZ: ARG) mixture of ratio (1:2) and F23 pellets formulation during the three months study period. As shown in Figure 8 A, the PXRD pattern showed no occurrence of any peak which suggests no recrystallization of (GPZ: ARG) mixture was seen during storage condition. The PXRD results further supported by no significant (P>0.05) difference was seen dissolution profile (Figure 8 B) of pellets. This indicates that the co-amorphous (GPZ: ARG) blend of (1:2) ratio formulated pellets are more stable at the ICH guidelines storage conditions.

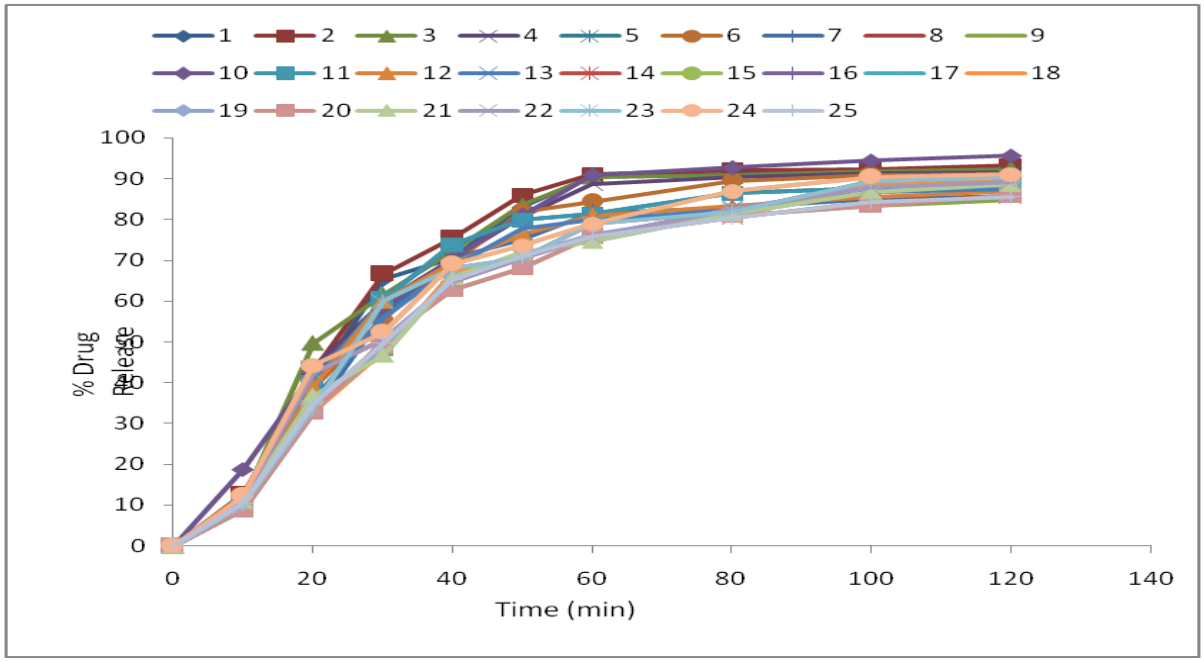

Figure 6. *In- vitro* dissolution profile of F1-F25 formulations.

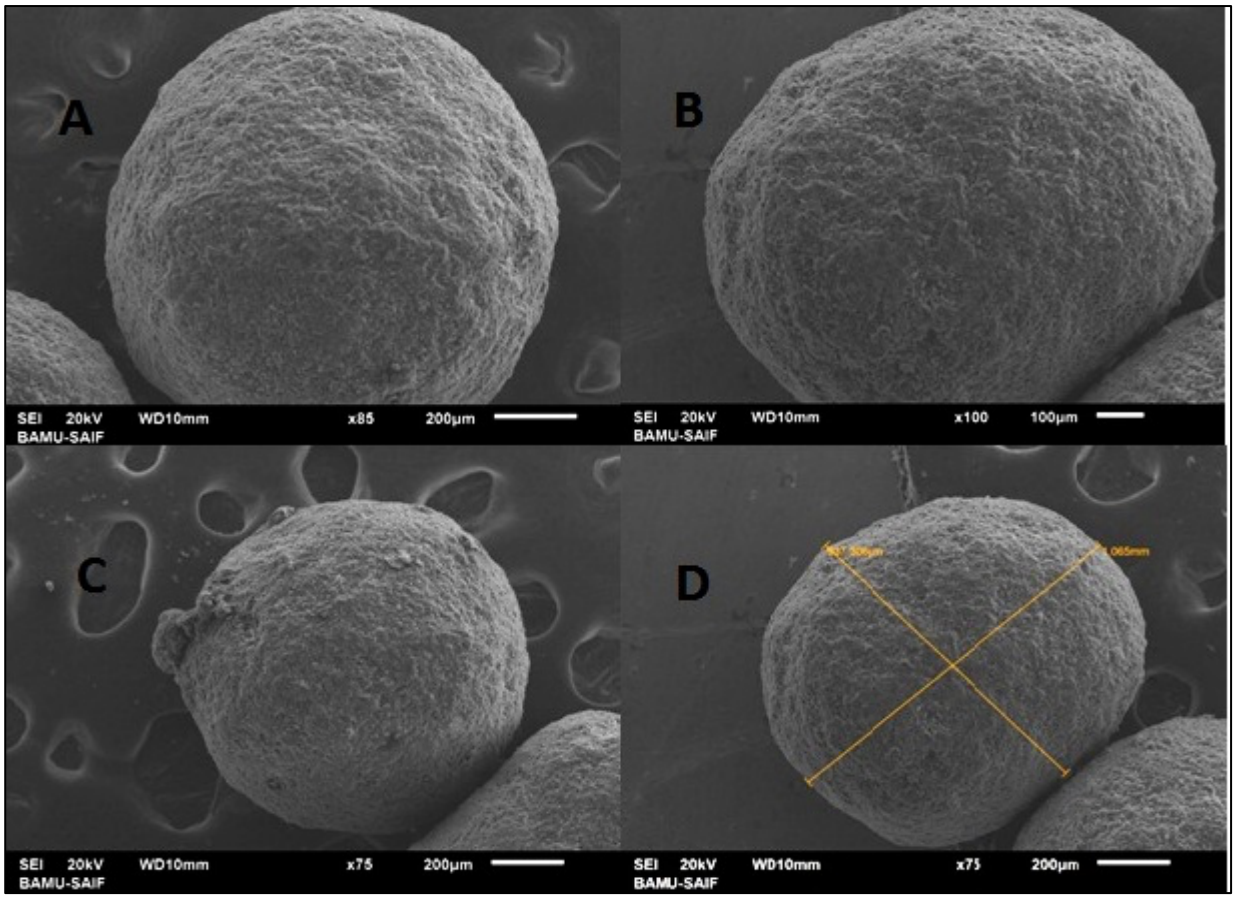

Figure 7. Scanning Electron Micrographs of optimized batch of pellets

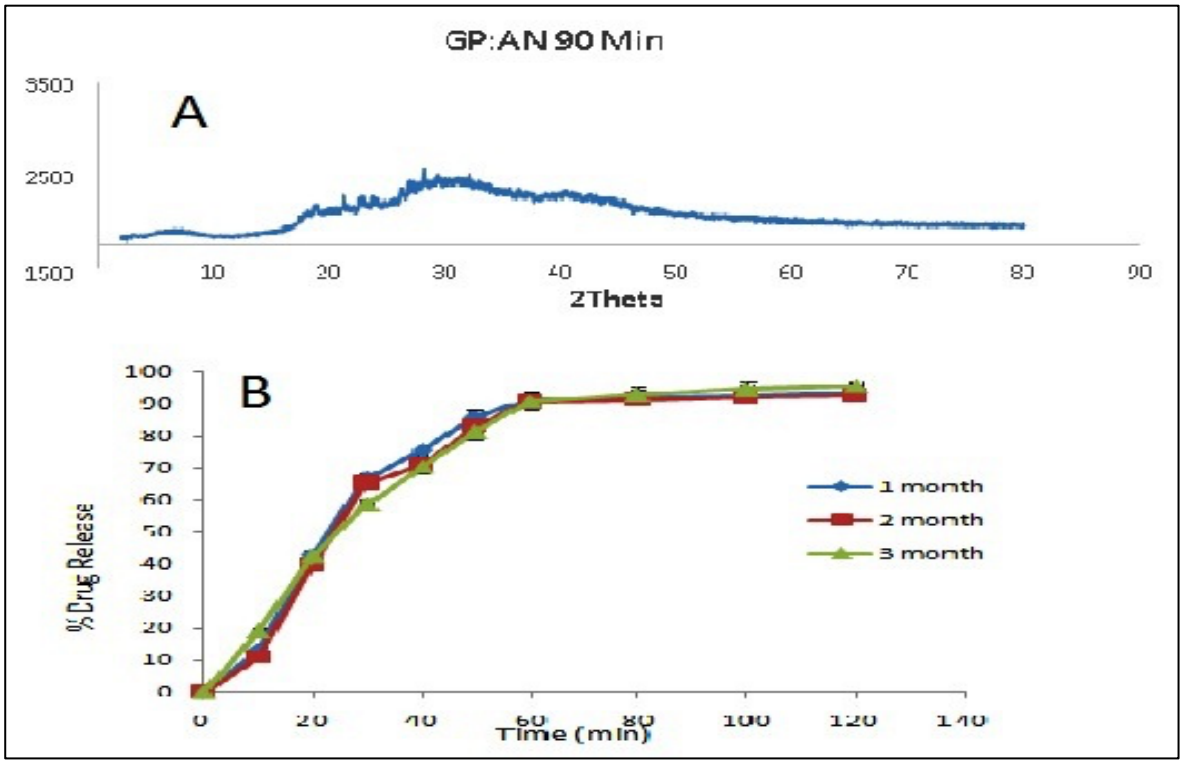

Figure 8. Stability results of optimized F23 batch A) PXRD of co amorphous mixture after three months B) *In vitro* dissolution profile during stability study

3. DISCUSSION

3.1 Solubility study

Owing to molecular interactions and possible salt formation there is increase in solubility, improved dissolution rate and better physical stability of co-amorphous drug-amino acid systems compared to the amorphous drug [14,15]. The "arginine assisted solubalisation system" approach also contributed to increased solubility [16]. Additionally, the particle size reduction due to milling might have contributed to solubility enhancement. Milled particles have higher surface free energies and this, coupled with their thinner diffusional boundary layers which leads to the increase in dissolution rate. To gain a better insight of this possibility, the pure amorphous GPZ was prepared by ball milling crystalline GPZ under the same conditions as the co amorphous mixtures. It showed only 2.4-fold increase in solubility as compared to 13-fold increase observed in co-amorphous mixture. Hence, in mixtures, the highest solubility of GPZ was seen in ratio GPZ: ARG (1:2) at 90 min ball milling and chosen for further solid- state evaluation study.

3.2 Solid-State Characterization

3.2.1 Fourier Transform Infrared Spectroscopy (FTIR) study

In co-amorphous mixture with ARG, slight or no changes were observed in GPZ vibrations and bands which appeared unchanged compared to those in the pure drug spectra(Figure4).Further, peaks were less intense and slightly broader, and sometimes accompanied by shoulders resulting from conformer vibrations. In general, conversion of a crystalline compound into its amorphous counterpart results in broadening and shifting of peak due to the change molecular arrangement and short-range order in compound [16]. Therefore, slight changes in the vibrational modes of drug are due to amorphization of the mixture.

3.2.2 Differential Scanning Calorimetry (DSC) study

The mixture showed a decrease in the magnitude of peak gradually with heating and also broad endotherm without a sharp melting point which confirmed the transition of the glipizide to the amorphous phase during ball milling (Figure 17). These results indicate that glipizide and the arginine mixture is converted to amorphization in co-amorphous solid. From the observation of broad DSC thermogram with no sharp melting point and the amorphous form in DSC pattern, it becomes obvious that an amorphous

mixture rather than the crystalline structure was formed. In addition, no separate Tg was observed for the single components present in the mixture, indicating the formation of a single-phase co-amorphous mixture [17].

3.2.3 Powder X-ray diffraction (PXRD)

No crystalline peaks of GPZ was seen in the blend (GPZ: ARG) of different ratios which indicates transformation of crystalline drug to amorphous state. Further, the 2 theta values of GPZ and ARG in mixtures of different ratios suggested that there is no change in the location of Bragg peaks, but extremely reduce the peak intensity with the milling time, indicates formation of co-amorphous form.

3.3 Experimental design for pellets

The design indicated that increasing the amount of MCC PH 101, PVP K 30 and increasing the time and speed of spheronisation decreases the drug release. MCC PH 101 is used as pelletising aid, but increasing its amount leads to pellets which becomes hard and delays disintegration of pellets [18] This might be the possible reason of decrease in drug release.

The effect of MCC PH 101 showed prominent positive effect on the angle of repose, which was expected as reported in previous study, MCC (Avicel PH-101), is a very good excipient for extrusion spheronization aid since it can reduce friability, improve pellet sphericity. Whereas, PVP K30 showed negative effect on angle of repose. Increasing the spheronisation time leads to formation of good spherical particles which enhances the flow property of pellets.

The plot suggests that the percent drug release is decreases in proportion to the amount of MCC and PVP (Figure 7), whereas spheronisation time and speed was found to be inversely proportional to drug release. The negative effect was observed on drug release when amount of MCC increases from 50 to 70 %. The disintegration time increases in proportion to the amount of MCC and PVP (Figure 8) whereas spheronisation time and speed was found to be in direct proportion to disintegration time. Increasing the time and speed of spheronisation make the pellets harder which delays disintegration.

3.4 Characterization of pellets

Based on angle of repose value, selected pellets as a dosage form showed good flow property when compared to co amorphous (GPZ: ARG) mixture as a powder form. Flow property of material is done on the basis of compressibility index and hausner ratio [19]. Therefore, F1-F25 formulations showed excellent carr's index and hausner ratio values. Flowability improvement benefits in easy filling of pellets in hard gelatin capsule.

3.5 In-vitro dissolution studies

There was an increase observed in drug release of co amorphous GPZ as compared to pure crystalline GPZ. The most likely reason could be the amorphization of GPZ. The increase in dissolution may be contributed to a phenomenon termed as AASS ("arginine-assisted solubilization system"[20]. In addition, during the dissolution, no energy need to split the crystal lattice in co-amorphous systems [21] which results in increase in the solubility of co-amorphous mixture compared to free drug. Immediate drug release was detected in the dissolution medium, pH 6.8 phosphate buffer. Immediate release was observed owing to presence of SSG which quickly absorbs water, which causes swelling, which leads to rapid disintegration of pellets [22].

3.6 Morphological analysis

More hydrophilic polymer produced a wet mass with the lowest mean torque value when mixed with MCC, due to which wet mass is easily extruded, resulting pellets formed with less rough surfaces [23] as well favored more spherical and smooth pellets [24].

3.7 Stability studies

As shown in Figure 13 A, the PXRD pattern showed no occurrence of any peak which suggests no recrystallization of (GPZ: ARG) mixture was seen during storage condition. The PXRD results further supported by no significant (P>0.05) difference was seen dissolution profile (Figure 13 B) of pellets. This

indicates that the co-amorphous (GPZ: ARG) blend of (1:2) ratio formulated pellets are stable at the ICH guidelines storage conditions.

4. CONCLUSION

The Co amorphization of Glipizide with Arginine was successfully achieved after 90 mins. of ball milling. The prepared blends were analyzed with respect to their thermal properties, possible molecular interactions, dissolution properties and physical stability and compared to the behaviour of pure crystalline form. GPZ-ARG co amorphous mixture (1:2) exhibited maximum solubility compared to pure drug. This co amorphous mixture was incorporated into pellets prepared by extrusion spheronisation. The parameters viz. spheronisation time, speed of spheronisation, amount of MCC PH 101 and amount of PVP K30, were successfully optimized using a Central Composite Design. Further, stability studies were conducted on optimized batch and it was found to be stable during storage.

5. MATERIALS AND METHODS

Glipizide (M 445.5 g/mol) was gifted by Wockhardt Ltd. Arginine (M= 174.2 g/mol), Microcrystalline cellulose PH 101, Poly Vinyl Pyrrolidone K 30, Sodium Starch Glycolate and Lactose were generously provided by Wockhardt Ltd., Aurangabad, India. All other chemicals and solvent used in the study were of analytical reagent grade.

5.1 Preparation of various molar ratios

Accurately weighed total mass of 3 g containing GPZ and ARG of different molar ratios (Table. 3) was mixed uniformly with the help of mortar and pestle for 60s prior to the ball milling (Bionics Scientific Technologies Ltd, India).

Table 3. Composition of molar ratios mixture of GPZ and ARG

Sample name	Molar ratio	Weight ratio (g)
GPZ:ARG	(1:1)	2.157:0.843
GPZ:ARG	(1:2)	1.683: 1.317
GPZ:ARG	(2:1)	2.509: 0.491

5.2 Preparation of Co amorphous mixtures

The co amorphous mixtures of GPZ and ARG were prepared by ball milling (BM). A total mass of 3 g of the GPZ and ARG at a molar ratio of 1:1, 1:2 and 2:1 was placed in 25 ml milling jars with three stainless steel balls having 18mm, 15mm and 12mm of the diameter. All the mixtures were ball milled 60, 90 and 120 min. The ball milling process was performed in a cold room (5°C) to avoid an increase in sample temperature during milling. The pure crystalline GPZ was milled under same conditions. After BM, the jars were placed in vacuum desiccators to reach room temperature before opening to avoid moisture absorption [4].

5.3 Solubility study

Solubility of crystalline GPZ, ball milled GPZ and different co-amorphous mixtures of different molar ratios of GPZ and ARG was measured by adding a known excess quantity of every mixture in different pH (1.2, 6.8 and 7.4) and kept in a shaking water bath at 37 ± 0.5 °C for 24 hr. The samples were removed from the bath and after equilibration the filtration was done using a membrane filter. The filtrate was analyzed at λ_{max} 274 nm using UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan).

5.4 Fourier Transform Infrared Spectroscopy (FTIR) study

FTIR analysis of pure GPZ and co-amorphous mixtures was carried out using the FTIR spectrophotometer (JASCO FTIR-410 Japan) by KBr pellet method. The scanning range was 400 to 4000 cm^{-1} and the resolution was 4 cm^{-1}

5.5 Differential Scanning Calorimetry (DSC) study

DSC analysis of pure GPZ and co-amorphous mixture were subjected using differential scanning calorimeter (Mettler Toledo 823e, Switzerland). The measurements were carried out under nitrogen flow (40 ml/min) at a scanning rate of 10°C/min from 30°C to 300°C [4].

5.6 Powder X-ray diffraction (PXRD)

The sample of GPZ and co-amorphous mixture was subjected to X-ray diffraction analysis using X-ray powder diffractometer (Bruker D8 AXS Advance, Germany). The studies were performed by exposing samples to Cu sealed X-ray tube with the voltage of 40 kV and 30 mA of current on a flat plate with θ/2θ geometry, where 2θ ranged from 5 to 50°, with a step width of 0.020.

5.7 Preparation of pellets containing Co amorphous mixture

5.7.1 Experimental Design (Central Composite Design)

CCD has been widely used for fitting a second-order model and to require a minimum number of experiments to be performed. The independent process variables including spheronisation time (X1) , speed of the spheronizer (X2), amount of MCC PH101(X3) and PVP K30(X4) were the factors, percent Drug Release(Y1), Angle of Repose (Y2), Aspect Ratio(Y3) and Disintegration Time of pellets (Y4) were used separately as the responses in the CCD. The data obtained for the four responses in each trial were fitted to the classical second-order polynomial model. The mathematical models were expressed as follows.

Second-order polynomial model:
$y = b0 + b1x1 + b2x2 + b3x12 + b4x22 + b5x1x2$
where x1, x2 represents the main effect, x12, x22 the quadratic effect and x1x2 is the interaction effect, y is the measured response, b0 is an intercept, b1–b5 are the regression coefficients. Data were analyzed by nonlinear estimation using Design Expert® software 13.0. Against the four factors, graphs of surface responses were plotted with the response variation.
The effects were evaluated statistically at 0.1 level (α = 1.141). The process variables with their relative experimental values are shown in Table 4. Pellets were produced according to different levels of the factors as reported in Table 4. Each experiment was executed in triplicate.

Table 4. Experimental Conditions for the study.

Symbols	Factors	Experimental Values	
		Low Level	High Level
X1	Spheronisation Time	5 min	25 min
X2	Spheronisation Speed	800 rpm	1200 rpm
X3	Amount of MCC PH 101	40%	60%
X4	Amount of PVP K30	1%	3%

5.8 Formulation of Immediate Release Pellets

The pellets were prepared by pelletization method (Table 5) using extrusion/ spheronization technique. All the ingredients used for preparing pellets were passed through sieve no. 40 before pelletization and mixed uniformly in a mortar and pestle. Water: IPA (1:1) was added drop wise to the mixture and mixed for 30 min. The obtained dough mass was extruded using a screw extruder (1 mm orifice, Kalweka, India). The prepared extrudates were transferred directly in spheronizer and rotated for different time and speed of spheronisation as suggested by CCD, keeping air velocity of 1 kg/cm2 until spherical pellets were obtained. The spheronizer disc having groove pattern of "waffle iron" type was used with 2.0 mm pitch plate. The pellets were dried at room temperature and kept in a desiccator till further use [25].

Table 5. Factors and Levels for Central Composite Design

Std	Run	Factor 1	Factor 2	Factor 3	Factor 4
		Sphr time	Speed of sphr	PH 101	PVP K30
3	1	-1	1	-1	-1
1	2	-1	-1	-1	-1
4	3	1	1	-1	-1
9	4	-1	-1	-1	1
10	5	1	-1	-1	1
11	6	-1	1	-1	1
15	7	-1	1	1	1
5	8	-1	-1	1	-1
16	9	1	1	1	1
23	10	0	0	0	-2
21	11	0	0	-2	0
19	12	0	-2	0	0
18	13	2	0	0	0
25	14	0	0	0	0
7	15	-1	1	1	-1
6	16	1	-1	1	-1
13	17	-1	-1	1	1
8	18	1	1	1	-1
17	19	-2	0	0	0
12	20	1	1	-1	1
20	21	0	2	0	0
22	22	0	0	2	0
2	23	1	-1	-1	-1
14	24	1	-1	1	1
24	25	0	0	0	2

5.9 Characterization of pellets

5.9.1 Angle of repose

To study the flowability of pellets the angle of repose (θ) was determined, by a fixed funnel method [26].

5.9.2 Tap and bulk density

The Carr's index and Hausner ratio (HR) of the pellets were calculated on the basis of tapped and bulk density. Tap and bulk density of the pellets was evaluated using tap density tester (Electrolab, India).

5.9.3 Disintegration time

The disintegration time of pellet in water was evaluated by Tablet disintegration test apparatus IP (ElectroLab India).

5.9.4 Scanning electron microscopy

The morphological characteristics of pellets were observed by scanning electron microscopy (SEM). Pellets were coated with platinum by means of sputter coater (JEOL, JSM-6510, Tokyo, Japan) to assure conductivity.

5.9.5 Content Uniformity

Accurately weighed samples of pellets (100 mg) from prepared formulations were dissolved in methanol, filtered after appropriate dilution with 0.1 N HCl at λ_{max} 274 nm using a UV-Vis spectrophotometer (UV-1700, Shimadzu, Japan). Experiments were performed in triplicate.

5.9.6 Friability test

In friability test the percent friability was determined by placing the known mass of pellets in a Roche Friability tester (Electro lab Friability tester, EF -2, India) and rotated at 25 rpm for 4 min. and percent friability was calculated by using following formula:

$$\% \text{ Friability} = \frac{W1 - W2}{W1} * 100$$

Where, W1= Initial weight and W2= Final weight

5.10 *In vitro* dissolution study

In vitro dissolution study was performed using USP dissolution apparatus I (Electro lab TDT- 06PL Dissolution tester) containing 900 ml of dissolution medium. The accurately weighed amount (equivalent to 5mg of GPZ) of the pellets were weighed and filled in hard gelatin capsule was placed in the basket and immersed into the dissolution medium (Phosphate Buffer pH 6.8, containing 0.75%SLS, at 37 ± 0.5 ^{o}C) [27]. Sample volume used for analysis was replaced by fresh dissolution medium to maintain the sink conditions analyzed at $\lambda_{max.}$ 274 nm using UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan) and each batch percentage drug release was analyzed in triplicate.

5.11 Stability study

In accordance with ICH Q1 (R2) guideline, the stability study was performed on optimized formulations, the formulations were packaged in a sealed container and store at 40°C ±2 °C/75% ±5% RH in a stability chamber (HmG, India) for three months [28,29]. The co amorphous (GPZ:ARG) mixtures were investigated by PXRD study and the pellets was determined for *in-vitro* dissolution study.

5.12 Statistical analysis

The results were analyzed by one-way ANOVA using Data Analysis ToolPak in Excel.

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