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CHARACTERIZATION OF GLICLAZIDE RELEASE FROM ISABGOL HUSK HYDROGEL BEADS BY VALIDATED HPLC METHOD

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Abstract: *Isabgol* husk, a medicinally important natural polysaccharide was applied for fabrication of hydrogel beads by ionic gelation method to incorporate gliclazide. Different strengths of *Isabgol* husk and sodium alginate were utilized for assessing the process variables on formulation performance. Aqueous solution of calcium chloride in 2, 5 and 8% w/v strength was used as cross-linker for polymeric blends of *Isabgol* husk and sodium alginate. The formulations were characterized for various parameters such as particle size, swelling index, entrapment efficiency, *in vitro* release, and release kinetics. The quantification of gliclazide throughout the study was performed by HPLC method which was validated according to ICH guidelines for system suitability, linearity, accuracy, sensitivity, precession, robustness, and ruggedness. The surface morphology of beads was observed by scanning electron microscopy. The formed beads were brown, free flowing, spherical, and irregular in structure. The size in different formulations varied from 752.83 ± 0.630 to 838.62 ± 0.741 µm. The beads remained for 2–3 h in alkaline phosphate buffer (pH 7.4), after that they showed disintegration. The formulations released up to 95% of loaded gliclazide in phosphate buffer (pH 7.4) within 8 h. No significant difference was observed in parameters studied such as particle size, entrapment efficiency and swelling index for hydrogel beads during accelerated stability study ($p > 0.05$). The regression equation developed by HPLC method was linear ($r^2 > 0.9990$) over the range 2.5 to 10 µg/mL. The limit of detection (LOD) and limit of quantification (LOQ) were 0.037619 µg/mL and 0.113997 µg/mL, respectively. The observed values for number of theoretical plates ($N \geq 2000$), tailing factor ($T \leq 2$), asymmetry factor ($AF \leq 1$), and relative standard deviation ($RSD \leq 1\%$) of applied method showed the reliability for gliclazide estimation in *Isabgol* husk hydrogel beads.

Keywords: validation, *Isabgol* husk, cross-linking, *in vitro* release

The pharmaceutical formulations such as conventional and controlled release are prepared by using different excipients with drug(s) and are used to solve the main aim of making the incorporated drug(s) available at desired site of action in required quantity. In conventional dosage forms, the fluctuations of drug concentration in blood occur that sometimes create the available dose level of drug below and upper to the therapeutic window. Amongst different categories of drug delivery devices, solid formulations are administered through oral route and follow a certain rate to achieve therapeutic drug concentration. Before absorption, these formulations undergo disintegration and dissolution in gastrointestinal fluid. This step of absorption from gastrointestinal tract (GIT) affects the bioavailability of the formulations. The fluctuations in bioavailability are considered mainly due to insufficient dissolution and subsequent absorption of the drug from the GIT. In case of water insoluble drugs, these factors have more impact on desired therapeutic level of the drug. In sustained release dosage forms, the release of drug is slow and compatible with the rate of absorption that results minimal loss of drug in GIT by presystemic metabolism. Hence, it becomes necessary to a drug delivery device to release the incorporated drug(s) in required manner to attain a desired concentration for a desired time in systemic circulation (means bioavailability) so that it may achieve the therapeutic goal, e.g., therapeutic efficacy. In sustained release formulations, special attention is given to microparticulate drug delivery devices due to their efficient volume to surface area ratio, small spherical size and retention to desired sites in the body in case of technically modified surface.

The applicability of different fabrication techniques depends upon the active pharmaceutical

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ingredient (API) and polymeric carrier. The performance of the formulation to achieve the desired goal of therapeutics is also governed by polymeric network. The drug carrier of natural origin such as gums, mucilage, resins, latex etc., have been applied in development of conventional and modified drug delivery devices and draw a marked consideration due to their eco-friendly nature, low cost, safety, biocompatibility, and availability for development of novel drug delivery systems (1).

Isabgol husk is obtained from epidermal and collapsed adjacent layers removed from the seeds of *Plantago spp.* and is well known for enormous water holding capacity in contact with water for forming mucilage. It is widely used for its different therapeutic effects, e.g., ulcerative colitis, hemorrhoids, constipation, hypercholesterolemia, diabetes mellitus, colorectal cancer etc. (2). *Isabgol* husk has been used for the development of hydrophilic matrix and microparticulate system for different drugs (3). Grafted *Isabgol* structure with polyacrylamide and polyacrylonitrile have been reported for the use in flocculation study (4). Alginates are also naturally occurring polysaccharides obtained from marine brown-algae consisting of two monomeric units; β-D-mannuronic acid (M) and α-L-guluronic acid (G). These residues are arranged in homopolymeric blocks (GG, MM) and in heteropolymeric blocks (MG). Sodium alginate shows gelling properties in the presence of multivalent cations such as Ca^{2+}, Ba^{2+}, Al^{3+} etc. The cross-linked structure has marked swelling in water and acidic environment but it is sensitive towards the alkaline phosphate condition as the egg-box like structure of cross-linked alginates ruptures.

Gliclazide is an oral hypoglycemic second generation sulfonyl urea, which is useful for long term treatment of non-insulin dependent diabetes mellitus (NIDDM) (5). Previous studies showed that gliclazide possesses good general tolerability, low incidence of hypoglycemia, and low rate of secondary failure (5). For a hypoglycemic drug to be effective, rapid absorption from the gastrointestinal tract is required. However, the absorption rate of gliclazide from the gastrointestinal tract is slow and varied amongst subjects. Several studies on healthy volunteers and diabetic patients revealed that the time to reach plasma concentration (t_{max}) ranged from 2 to 8 h following a single oral administration of 80 mg of gliclazide tablet (5). The slow absorption has been suggested to be due to either hydrophobic nature or poor permeability of the drug across the GI membrane. Due to this, controlled release formulations of the said drug are available in the market as incorporation of gliclazide in such preparations may control its absorption from gastrointestinal tract and overcome the variability problems.

The present study was undertaken to assess the participation of *Isabgol* husk with sodium alginate in controlling gliclazide release from hydrogel beads and the impact of process variables on entrapment efficiency, *in vitro* release and other formulation related factors. Due to insoluble nature of gliclazide in water, aqueous ionic gelation-cross-linking technique may be an appropriate method for sustained release formulation development of gliclazide.

Besides the release of incorporated drug from drug delivery devices, the quantification of drug by suitable and routine analytical technique becomes necessary. The selective and sensitive analytical methods for quantitative determination of drugs and their metabolites are essential for successful evaluation of clinical pharmacology, pharmacokinetics (PK), bioavailability (BA) and bioequivalence (BE) studies. In this study, HPLC method was used for gliclazide quantification. The proposed method was validated for the parameters like system suitability, linearity, accuracy, sensitivity, precision, robustness and ruggedness according to ICH guidelines (6). The linearity of an analytical method is its ability to elicit that test results are proportional to the concentration of drug in samples within a given range. It is generally determined by constructing calibration curve. The accuracy is the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions. It is determined by calculating % RSD of various measurements at different time, equipment or analyst.

EXPERIMENTAL

Materials

Isabgol husk, as readymade herbal remedy (Sidhpur, Gujarat), was procured from local market. Gliclazide was obtained as gift sample from Comed Pharmaceuticals Ltd., Baroda. HPLC grade methanol, water and acetonitrile were procured from Rankem, New Delhi. All other regents and chemicals were of analytical grade and used without further purification and modification.

Methods

Formation of hydrogel beads of *Isabgol* husk by aqueous ionic gelation cross-linking technique (7)

Hydrogel beads of gliclazide composing of *Isabgol* husk and sodium alginate were fabricated by

using *Isabgol* husk in different strength for facilitating the spherical beads formation. Sodium alginate was added for its hardening effect during cross-linking with calcium chloride. About 3% w/v dispersion of *Isabgol* husk and sodium alginate was prepared in distilled water and gliclazide was added in dispersion, which was homogenized at 500 rpm at room temperature. The dispersion containing gliclazide was added *via* 23-gauge needle-syringe into a gently agitated 5% w/v calcium chloride solution. The droplets instantaneously gelled into discrete, free flowing, brown colored beads upon contact with calcium chloride solution. These whitish and spherical beads were left for 30 min in cross-linker solution for curing and hardening. After curing, calcium chloride solution was decanted and each batch was washed three times successively with 500 mL of distilled water for removing unreacted calcium chloride from the surface and the beads were dried at 60°C for 10 h. Different variables such as calcium chloride concentration and sodium alginate to *Isabgol* husk ratio were studied to analyze the effect of these factors on particle size, swelling behavior, drug entrapment efficiency, *in vitro* release and release kinetics.

Particle size determination

The particle size of beads was measured microscopically by observing about 250–300 particles on a glass slide under optical microscope (Olympus, Japan) at 5× magnification. In microscopic observation, the divisions in eye piece covered by particles were counted and by multiplication with least count of ocular micrometer, the size range of particles was determined.

Swelling index

Swelling of formulations was measured gravimetrically by taking initial weight and weighing after complete swelling in swelling media on single pan electronic balance with least count of 0.1 mg. The swelling media used for swelling were distilled water, 0.1 M HCl and phosphate buffer (pH 7.4). The swelling index was calculated by following expression:

$$\text{Swelling index}(\%) = \frac{\text{Weight after swelling} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

HPLC method development and optimization

The HPLC system used in the study was of CECIL (UK) CE 4200 with UV detector CE 4201; HPLC pump; injector loop Rheodyne (Model No. 2767, 20 µL volume loop). Data acquisition was performed by the POWERSYSTEM software. Chromatographic analysis was performed on a C-18 column (250 × 4 × 60 mm, 5 µm, Luna 5u, Phenomenex). The mobile phase consisted of phosphate buffer (pH 3.4) : acetonitrile (20 : 80 v/v) and the flow rate was set at 1 mL/min. The mobile phase was filtered through 0.45 µm filter under vacuum and untrasonicated before pumping into HPLC system. The column was maintained at ambient temperature and equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The absorbance of the effluent was monitored by UV detection at 227 nm.

Drug entrapment efficiency

About 50 mg formulation was taken into 50 mL media composed of 30 mL of phosphate buffer (pH 7.4) and 20 mL methanol taken in 100 mL volumetric flask for 48 h with occasional shaking. After it, the beads with media were disintegrated for 30 min in ultrasonicator, crushed in pestle mortar and then filtered through Whatman filter paper. The filtrate following suitable dilution was analyzed by HPLC at 227 nm. The drug entrapment efficiency was determined by the following relation (7):

$$\text{Drug entrapment efficiency}\ (\%) = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

Surface morphological study

It was performed by scanning electron microscope (ZIESS EVO 40EP, Carl Zeiss, Cambridge, UK) for formulations prepared by sodium alginate, and *Isabgol* husk-sodium alginate combination. The surface study was also performed for the formulation remained after dissolution to assess the impact of dissolution on beads.

Dissolution test

The dissolution test was performed in basket type dissolution test apparatus (Model: DR-08, Campbell Electronics, Mumbai) in six replicates for each formulation at 50 rpm. The dissolution media used in the study were: distilled water, 0.1 M HCl and phosphate buffer (pH 7.4). The dissolution media were maintained at 37.5 ± 0.5°C. The sampling was performed by withdrawing 5 mL of the sample at preset time interval such as 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300 and 480 min and the dissolution medium was replenished by pre-warmed dissolution medium to maintain the volume constant. The samples after suitable dilution were analyzed by determining absorbance at 227 nm by HPLC method. The respective concentration of gliclazide in dissolute samples was calculated from the calibration curve obtained from

pure sample of gliclazide. The dilution factor was applied as follows to determine the accurate concentration of gliclazide in each sample withdrawn:

$$C_n = O_n\left[\frac{V_1}{V_t - Vs}\right] \times \left[\frac{C_{n-1}}{O_{n-1}}\right]$$

where C_n is the corrected concentration of n^{th} sample, O_n is the original concentration of the n^{th} sample, V_t is the volume of the dissolution medium, Vs is the volume of the sample withdrawn, C_{n-1} is the corrected concentration of the $(n-1)^{th}$ sample and O_{n-1} is the original concentration of the $(n-1)^{th}$ sample.

Release kinetics analysis

The release behavior of gliclazide in different media was analyzed by zero order, first order, Korsmeyer-Peppas, Higuchi and Hixon-Crowell models by using the following expressions (8):

Zero order : $M_t = K_o t$

First order : $\log M_t = \log M_o - K_1 t/2.303$

Korsmeyer-Peppas : $\ln Mt = n \ln t + \ln K$

Higuchi : $M_1/M_\alpha = K_h \cdot t^{1/2}$

Hixon-Crowell : $M_t^{1/3} = M_o^{1/3} - kt$

In above equations, M_t is the amount of drug released at time t and K_0, K_1, and K_H are the coefficient of respective equations.

Stability study

The accelerated stability testing of incorporated gliclazide in hydrogel was performed at 40°C and 75% RH in stability chamber (NSW-175, New Delhi) up to six months. About 100 mg of each formulation was kept in open mouth HPTE bottles at preset experimental conditions for 6 months. After 1, 3, and 6 months, the formulations were analyzed for particle size, drug content, swelling behavior and scanning of gliclazide by validated HPLC method.

Statistical analysis

In all cases, the experimentation was performed in triplicate (n = 3) where the number of studies are not mentioned. Significance of results was tested by ANOVA using Sigma State software (Sigma State 2.03, SPSS, Chicago, USA). Significance of differences was defined at $p < 0.05$.

RESULTS AND DISCUSSION

The beads formed after hydrogelation of *Isabgol* husk and sodium alginate were spherical in shape with irregular surface that may be due to drying effect of hydrogel at surface. The drug crystals observed on the surface were probably formed as a result of their migration along with water to the surface during drying. Similar results were also observed by Fathy et al. (9) for alginate beads composed of tiaramide. The SEM photographs of the preparations formed by *Isabgol* husk-sodium alginate and sodium alginate are shown in Figure 1. The formulations prepared with their characteristics are summarized in Table 1. The particle size of *Isabgol* husk-sodium alginate (1 : 2) beads prepared in 2% w/v calcium chloride solution was found to be 825.47 ± 1.149 µm, which decreased in 8% w/v strength of calcium chloride to 790.41 ± 1.201 µm. The effect of calcium ions on particle size was also observed in higher ratio of *Isabgol* husk to sodium alginate (3 : 4). In 2% w/v strength, the particle size was measured as 872.03 ± 0.2933 µm which was decreased to 810.33 ± 0.5533 µm in 8% w/v strength of calcium chloride. The decrement in particle size emphasized the role of calcium in tight junction formation in cross-linked polymeric network resulting smaller in size. The smaller bead size e.g., 872.03 ± 0.2933 µm on higher concentration of *Isabgol* husk on applying similar calcium ion strength for low *Isabgol* husk ratio revealed the participation of *Isabgol* husk in cross-linking. Similarly, on increasing the strength of calcium chloride from 2 to 8% w/v, the particle size was also decreased from 804.63 ± 1.4278 µm to 790.21 ± 1.201 µm and from 813.41 ± 0.9233 µm to 790.12 ± 0.241 µm in sodium alginate beads, respectively. The particle size of different formulations is shown in Table 1.

The swelling index (%) of beads in distilled water has also been summarized in Table 1. In formulations of 1 : 2 *Isabgol* husk-sodium alginate prepared in 2 to 8% w/v calcium chloride strength, the swelling index (%) decreased from 354 ± 4.71 to 296 ± 3.42, while in 3 : 4 *Isabgol* husk-sodium alginate formulations, it decreased from 426 ± 3.54 to 176 ± 1.23. Similarly, in sodium alginate formulations such as AA, AA/2 and AA/4, it decreased from 406 ± 2.43 to 352 ± 1.12. Here, it was considered due to more involvement of calcium in cross-linking resulting in tight network of polymer that may create hindrance in water diffusivity to cross-linked *Isabgol* husk-sodium alginate beads. Secondly; on increasing the concentration of cross-linking agent, reduction in the mobility of the polymer chains and consequently, a decrease in porosity may occur resulting in slow swelling. The results also showed that the swelling was related to the polymer concentration because more swelling was observed on increased polymer content. The loading efficiency of the majority of the systems prepared was high

Table.1 Formulations of *Isabgol* husk-sodium alginate beads fabricated by aqueous ionic gelation-crosslinking technique.

	Formulation code	Conc. of sodium alginate (g)	Conc. of *Isabgol* husk (g)	Conc. of $CaCl_2$ (% w/v)	Particle size (µm ± S.D.)	Entrapment efficiency (%)	Swelling index (%)
1.	A/2	1	0.5	2	825.47 ± 1.149	89.54 ± 1.26	354 ± 4.71
2.	A	1	0.5	5	813.78 ± 0.7485	93.45 ± 0.134	326 ± 2.34
3.	A/4	1	0.5	8	794.41 ± 0.7485	94.16 ± 1.23	296 ± 3.42
4.	AA/2	1	Ż	2	804.63 ± 1.4278	88.41 ± 0.07	406 ± 2.43
5.	AA	1	Ż	5	781.57 ± 0.4872	87.65 ± 0.118	342 + 1.21
6.	AA/4	1	Ż	8	790.21 ± 1.201	90.06 ± 2.34	352 ± 1.12
7.	BB/2	0.75	Ż	2	813.41 ± 0.9233	83.14 ± 0.12	376 ± 1.78
8.	BB	0.75	Ż	5	752.83 ± 0.6300	83.46 ± 0.031	330 ± 3.56
9.	BB/4	0.75	Ż	8	790.12 ± 0.241	95.78 ± 1.23	291 ± 2.23
10.	B/2	1	0.75	2	872.03 ± 0.2933	91.66 ± 0.89	426 ± 3.54
11.	B	1	0.75	5	838.62 ± 0.7412	95.12 ± 1.112	338 ± 1.23
12.	B/4	1	0.75	8	810.33 ± 0.5533	96.10 ± 1.16	176 ± 1.23

*Values shown in parentheses are the S.D. of three successive results

Table 2. Results of the system suitability studies.

Property	Mean ± S.D.*	% RSD	Required limits
Retention time (t_R) (s)	161.2 ± 1.13	0.7045	RSD ≤ 1%
Theoretical plates (N)	7281.058 ± 7.67	0.1053	N ≥ 2000
Tailing factor (F)	1.66 ± 0.01	0.6024	T ≤ 2
Asymmetry factor (AF)	0.766 ± 0.005	0.7530	AF ≤ 1

*Average of six determinations

Table 3. Calibration data of gliclazide by HPLC method.

No.	Concentration (µg/ mL)	Peak area (mV.S) (Mean ± S.D.)*	%RSD
1.	2.5	1303.533 ± 7.902742	0.606255
2.	5.0	2624.233 ± 5.772844	0.219982
3.	7.5	3975.203 ± 4.87455	0.122624
4.	10.0	5393.233 ± 5.997077	0.111196

* The mean of six successive readings

Table 4. Accuracy of method in terms of recovery results.

Sample %	Area (mV.s) (Mean* ± SD)	Initial amount (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL)	Recovery %	% RSD
80	5337.908 ± 2.265582	2	8	9.98	99.99672	0.042443
90	5863.704 ± 6.813041	2	9	10.95	100.061	0.127635
100	6364.534 ± 1.4851	2	10	11.89	99.97656	0.023334
110	6951.695 ± 0.956106	2	11	12.98	99.98622	0.013754
120	7562.257 ± 3.567373	2	12	14.12	99.94802	0.047173

* Average of six successive determinations

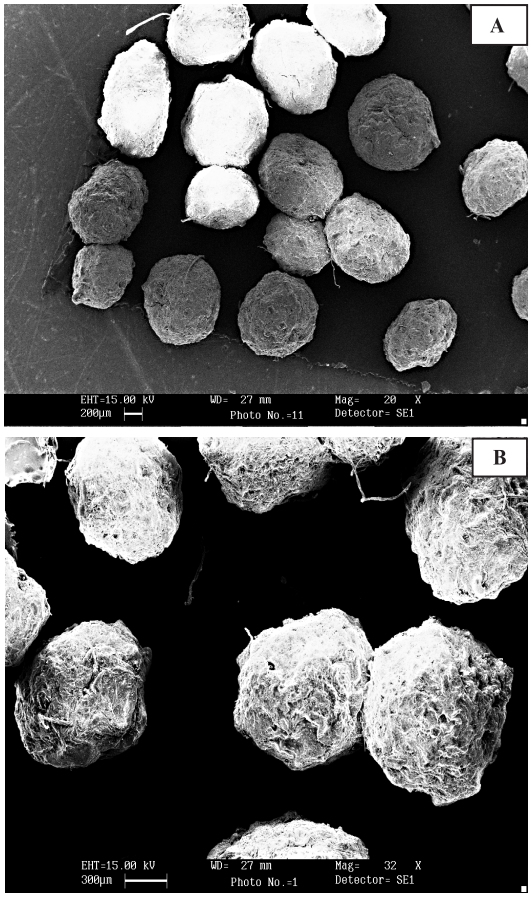

Figure 1. Photomicrographs of gliclazide loaded hydrogel beads of (**A**) sodium alginate and (**B**) *Isabgol* husk-sodium alginate

owing to the low water solubility of gliclazide in water and the minimum loss of the drug during preparation of the beads as well as in washings with water prior to drying. A relation with increment of entrapment efficiency with decrement in particle size was observed that may be due to more cross-linking resulting in less loss of drug in dispersed media during fabrication

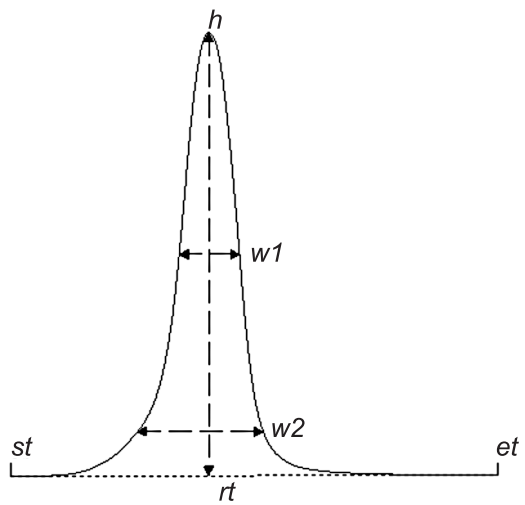

Figure 2. Chromatogram of peak width at 50% height (w_1) and 10% height (w_2), retention time (rt), and peak height (h) of gliclazide

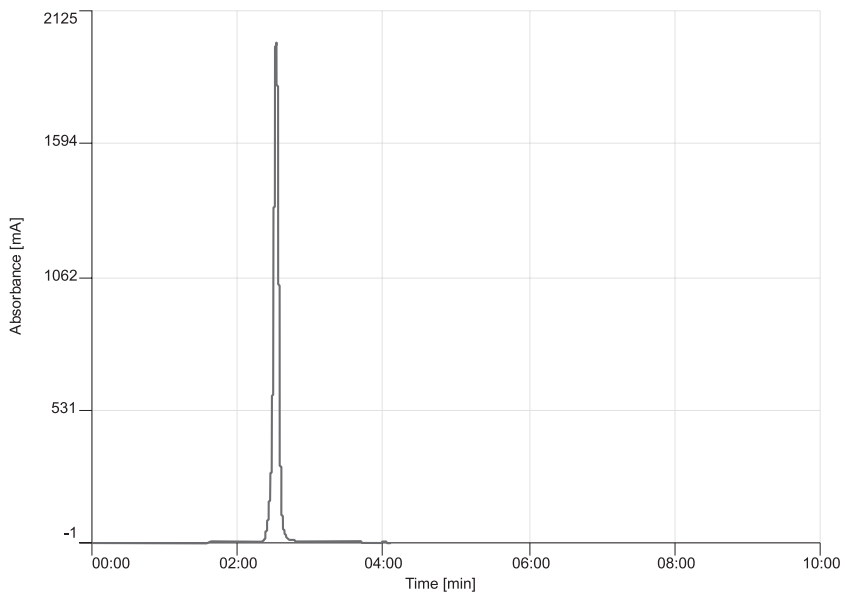

Figure 3. Unimodel, positively skewed chromatographic peak of gliclazide in mobile phase of phosphate buffer (pH 3.4) and acetonitrile (20 : 80 v/v)

Table 5. Linear regression characteristics parameters of gliclazide by HPLC method.

Parameters	HPLC method
Calibration range (µg/mL)	2.5–10
Detection wavelength	227 nm
Mobile phase, phosphate buffer (pH 3.4) : acetonitrile) v/v	20 : 80
Regression equation (Y)	$y = 538.19x - 33.053$, $R^2 = 0.9997$
Retention time (s)	160.4
Slope (m)	538.19
Intercept (c)	33.053
Correlation coefficient (r^2)	0.9997
Limit of detection (LOD) (µg/mL)	0.037619
Limit of quantification (LOQ) (µg/mL)	0.113997

Table 6. Intra-day precision results of gliclazide.

No.	Concentration (µg/mL)	Retention time (s)	Area (mV.s)
1.	15	160.3	8041.95
2.	15	160.8	8038.721
3.	15	161.2	8036.03
4.	15	161.3	8043.564
5.	15	160.4	8037.106
6.	15	160.7	8036.03
7.	15	160.9	8041.95
8.	15	161.1	8044.103
9.	15	161.8	8043.564

Table 7. Inter-day precession results of gliclazide determination by HPLC method.

No.	Concentration (µg/µL)	Retention time (s)	Area (mV.s)
1.	15	161.2	8040.335
2.	15	161.5	8039.259
3.	15	161.8	8038.721
4.	15	160.9	8038.182
5.	15	160.8	8036.03
6.	15	161.3	8037.106
7.	15	161.7	8042.488
8.	15	160.4	8044.641
9.	15	160.2	8038.721

Table 8. Robustness study of HPLC method for gliclazide estimation.

No.	Flow rate (mL/min)	Peak area (Mean ± S.D.)	Retention time (s) (Mean ± SD)*	Mean % RSD (for retention time)	Mean % RSD (for area)
1.	0.5	4287.711 ± 26.92009	160.125 ± 0.330404	0.206341	0.627843
2.	1	4286.635 ± 27.8305	160.25 ± 0.896289	0.559306	0.649239
3.	1.5	4307.899 ± 29.60815	161.175 ± 0.670199	0.415821	0.687299

*Average of six successive determinations

Table 9. Ruggedness studies of HPLC method for gliclazide determination.

Injection number	User-1			User-2		
	Area (mV.s)	Retention time (s)	Theoretical plates (N)	Area (mV.s)	Retention time (s)	Theoretical plates (N)
5 µg/mL	2627.66	161.2	7115.519	2622.78	160.8	6775.759

Table 10. Release behavior of hydrogel beads of sodium alginate and *Isabgol* husk-sodium alginate in distilled water, 0.1 M HCl, and phosphate buffer (pH 7.4)

No.	Formulation code	Time (min) required for 50% release of loaded gliclazide ($t_{50\%}$)		
		In distilled water	In 0.1 M HCl	In phosphate buffer (pH 7.4)
1.	A/2	741.0642	466.6285	256.4819
2.	A	922.0937	478.5284	286.9242
3.	A/4	988.0937	478.5284	315.9242
4.	AA/2	620.7927	375.3471	249.835
5.	AA	688.8649	397.5341	283.3517
6.	AA/4	728.8649	417.5341	323.3517
7.	BB/2	598.9712	342.1793	213.3317
8.	BB	694.9605	357.4922	243.0078
9.	BB/4	724.9605	371.4922	253.0078
10.	B/2	912.112	524.1456	248.8384
11.	B	963.931	596.5950	283.1824
12.	B/4	1058.612	648.2974	323.1824

Validation of HPLC method

In the study, the determination of gliclazide in all samples was performed by HPLC method that was carried out according to the recommendations for analytical method validation.

System suitability

System suitability tests are an integral part of method development and are to ensure adequate performance of the chromatographic system. Retention time (t_r), number of theoretical plates (N), asymmetry factor (AF), and tailing factor (F) were evaluated by applying following expressions for six replicate injections of the drug at a concentration of 5 µg/mL. The presentation of peak width at 50% and 10% height is shown in Figure 2. The numbers of theoretical plates (N) are also countable for assessing the column efficiency. The mathematical concept of theoretical plates was applied by using half peak height method as following:

$$N = 5.545 \left(\frac{t_R}{W_h}\right)^2$$

Table 11. Release kinetics parameters of sodium alginate and *Isabgol* husk-sodium alginate beads crosslinked by calcium chloride.

Formulation code	Name of kinetic models									
	Zero order		Korsmeyer-Peppas		First order		Higuchi		Hixon-Crowell	
	k	r^2	n	r^2	k	r^2	k	r^2	k	r^2
In distilled water										
B	0.0508	0.9111	0.5453	0.9787	-0.0003	0.9264	1.2317	0.986	-0.004	0.5797
AA/2	0.0411	0.9168	0.5087	0.9861	-0.0002	0.9331	0.9991	0.9899	-0.0036	0.555
In 0.1 M HCl										
B	0.1142	0.956	0.6664	0.9788	-0.0007	0.9682	2.6497	0.9814	-0.0057	0.6954
AA/2	0.1135	0.7847	0.6497	0.9641	-0.0008	0.8431	2.9038	0.9792	-0.0048	0.4742
In phosphate buffer (pH 7.4)										
B	0.1425	0.8205	0.6735	0.951	-0.0012	0.9325	3.626	0.9717	-0.0051	0.463
AA/2	0.1456	0.7095	0.6797	0.909	-0.0015	0.8727	3.8806	0.9726	-0.0051	0.463

where N is a number of theoretical plates; t_R = retention time, and 'W_h' = peak width at half height (in units of time). Columns with high plate numbers are considered to be more efficient, that is, have higher column efficiency, than columns with a lower plate count. A column with a high number of theoretical plates will have a narrower peak at a given retention time than a column with a lower theoretical plates number. High column efficiency is beneficial in case of less peak separation (meaning lower α value) and on the other hand, more efficient columns are needed. Column efficiency is a function of the column dimensions (diameter, length and film thickness), the type of mobile phase and its flow rate, nature of compound to be separated, and its retention.

The tailing factor was determined by using the following formula:

$$T = \left(\frac{a + b}{2a}\right)$$

where T is the tailing factor (measured at 10% of peak height), b = distance from the point at peak midpoint to the trailing edge and a = distance from the leading edge of the peak to the midpoint. The peak asymmetry was calculated using the following equation:

$$Asymmetry = \frac{N_{10}}{N_{50}}$$

where N_{10} is the plate efficiency at 10% of the peak height and N_{50} is the plate efficiency at 50% of the peak height. Here, the plate efficiency at 10 and 50% of the peak height was determined by the following formulae:

$$\text{Plate efficiency @50\%} = 05.54 \left(\frac{\text{Retention time}}{w_1}\right)^2$$

$$\text{Plate efficiency @10\%} = 18.55 \left(\frac{\text{Retention time}}{w_2}\right)^2$$

The results are shown in Table 2. The number of theoretical plates (N) was greater than 2000 ($N \geq 2000$) and the tailing factor was less than 2 ($T \leq 2$). Similarly, the asymmetry factor was less than 1 ($AF \leq 1$). In all six replicates of the experimentation according to ICH guidelines, the % RSD was also less than one ($RSD \leq 1\%$). All these results indicated the suitability of the applied HPLC method for gliclazide estimation. It was found that the regression coefficient for linear curve fitting for calibration was 0.9997 ($r^2 > 0.9990$). The higher value of regression coefficient 'r' indicated the linear kinetics as best curve fitting model for developed HPLC method. The unimodel, sharp, and pointed chromatographic peak of gliclazide in mobile phase of

Table 12. Stability study analysis of *Isabgo*l husk hydrogel beads.

Formulation code	Time (months)	Particle size (µm)	Entrapment efficiency (%)	Swelling index (%)
A	1	814.26 ± 0.26	92.66 ± 0.631	331 ± 0.42
	3	816.14 ± 1.45	93.04 ± 0.12	335 ± 1.43
	6	815.12 ± 0.89	92.98 ± 1.78	334 ± 1..98
A/2	1	820.05 ± 2.02	89.88 ± 0.87	359 ± 2.23
	3	823.18 ± 1.45	90.42 ± 2.56	358 ± 3.12
	6	826.23 ± 0.59	90.12 ± 1.72	361 ± 0.59
A/4	1	797.45 ± 1.22	95.53 ± 0.45	299 ± 1.38
	3	799.89 ± 2.55	94.89 ± 1.23	301 ± 0.58
	6	803.23 ± 0.87	95.67 ± 2.14	298 ± 1.22

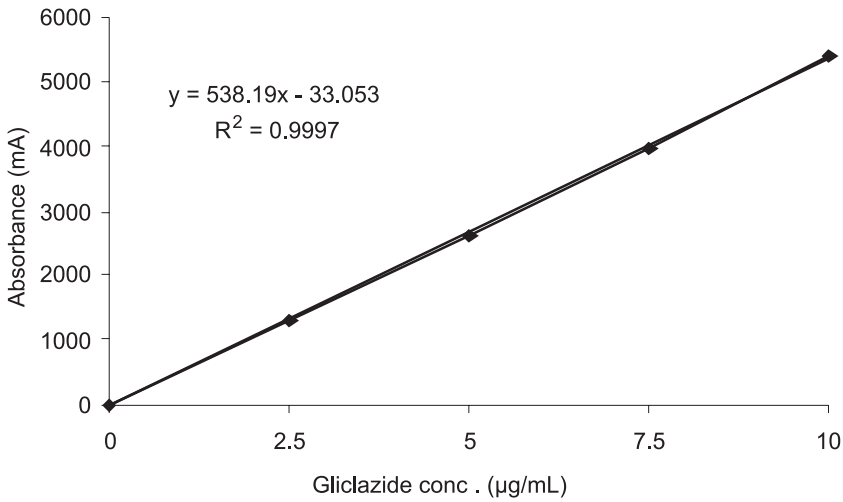

Figure 4. Calibration curve of gliclazide through HPLC method in mobile phase

phosphate buffer (pH 3.4) and acetonitrile (20 : 80 v/v) is shown in Figure 3.

Linearity

Several aliquots from the standard stock solution of gliclazide (10 mg/mL) in different strengths such as 2.5, 5, 7.5 and 10 µg/mL were prepared in the mobile phase. The detection of gliclazide was performed by UV at 227 nm. The peak was recorded for all samples and calibration graph was obtained by plotting peak area *versus* concentration of gliclazide. The plot of peak area against concentration of gliclazide was found to be linear in the range of 2.5 to 10 µg/mL with the correlation coefficient of 0.9997. The calibration data of gliclazide are given in Table 3 and the calibration curve of gliclazide is shown in Figure 4.

Accuracy

The accuracy was performed in triplicate after spiking pure drug equivalent to 80, 90, 100, 110 and 120% of the standard concentration of gliclazide (10 µg/mL). The results obtained after drug analysis are shown in Table 4. The results indicated the excellent recovery of gliclazide. In all the samples, the recovery of the drug was not less than 99.94%. The recovery results indicated that the method is highly accurate for determination of gliclazide.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were determined from standard deviation and slope method according to ICH guidelines. The LOD was found to be 0.037619 µg/mL and LOQ was found to be 0.113997 µg/mL.

The results of linear regression with LOD and LOQ are presented in Table 5.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies.

Intra-day precision

In the intra-day studies, six injections of the standard solution (15 µg/mL) were injected into the chromatographic system in different time interval within a day. The results of gliclazide potency in the samples are shown in Table 6 with % RSD.

Inter-day precision

In the inter-day variation studies, six injections of standard solution (15 µg/µL) were injected at different days. The results of gliclazide potency in samples are shown in Table 7 with % RSD.

Robustness

Robustness of the method was determined by making slight changes in chromatographic condi-

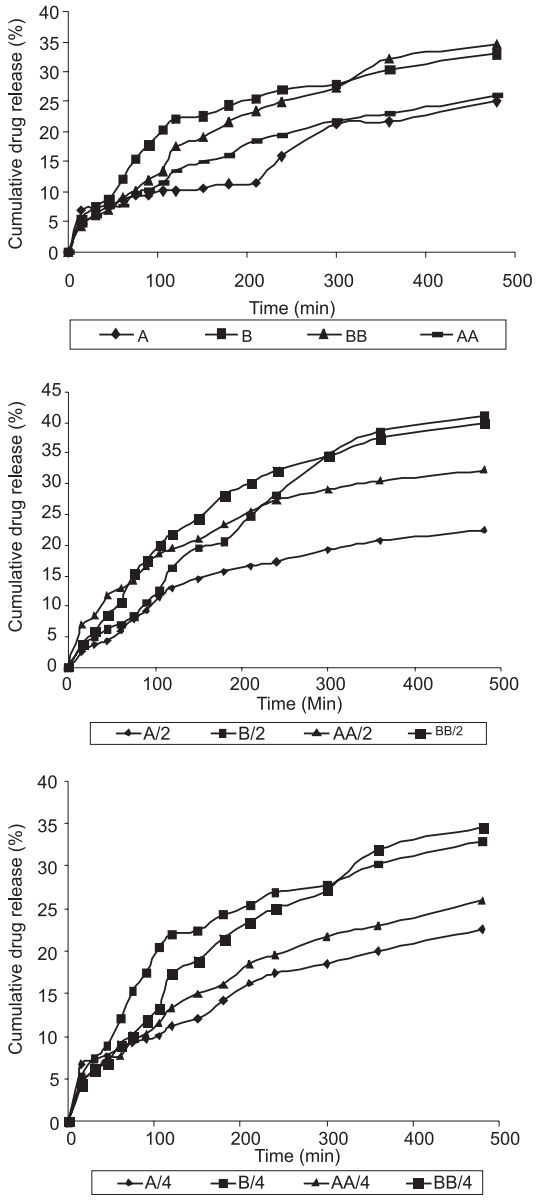

Figure 5. (i). Dissolution pattern of different formulations in distilled water

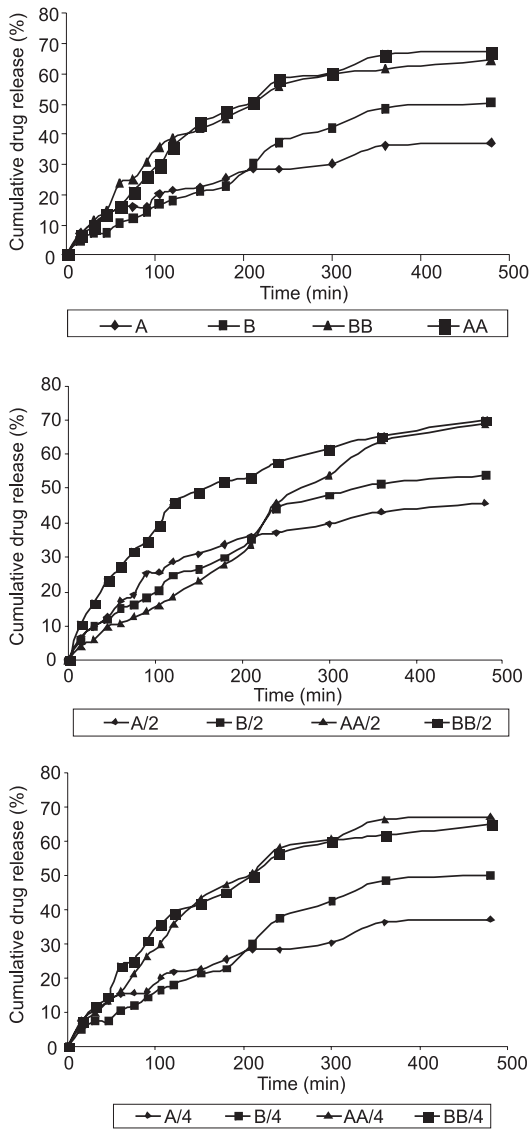

Figure 5. (ii). Dissolution pattern of different formulations in 0.1 M HCl

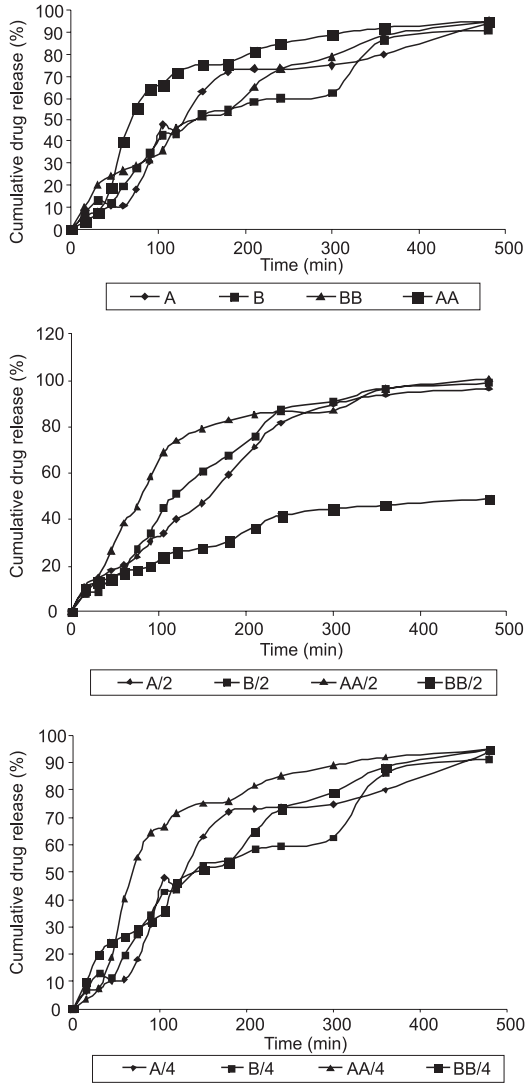

Figure5. (iii). Dissolution pattern of formulations in phosphate buffer (pH 7.4)

tions such as change in flow rate from 0.5 to 1.5 mL/min. It was observed that there were no marked changes in the chromatograms, which demonstrated that the method applied for determination of gliclazide is robust. The results of robustness of the method are represented in Table 8.

Ruggedness

It was analyzed by determining precession on the same instrument but by the different user. Results of the reproducibility of the method are shown in Table 9.

***In vitro* drug release**

The release pattern of formulations in distilled water, 0.1 M HCl and phosphate buffer (pH 7.4) has been shown Figure 5 (i, ii, and iii). Due to sensitivity of *Isabgol* husk-sodium alginate cross-linked beads towards ionic media, the release pattern of beads was different in distilled water, 0.1 M HCl and phosphate buffer ($p < 0.05$). The time required for 50% release of loaded gliclazide from formulations has been summarized in Table 10. It was found that $t_{50\%}$ value was lower in phosphate buffer (pH 7.4) than in distilled water and 0.1 M HCl for all formulations. It indicated the faster drug release in phosphate buffer (pH 7.4) than in 0.1 M HCl and distilled water. The slow release of gliclazide at pH 1.2 has been observed due to the stability of alginate at low pHs and the conversion of calcium alginate to the insoluble but swelling alginic acid (10, 11). In phosphate buffer (pH 7.4), the rapid swelling and erosion of the beads has also been observed in swelling study that may greatly contribute in facilitating the fast release. The value of $t_{50\%}$ was comparatively larger for formulations containing more *Isabgol* husk (B/2, B, and B/4) than those containing less amount of *Isabgol* husk (A/2, A, and A/4). Here, it was inferred that more amount of *Isabgol* husk may provide the thicker hydrogel coating on gliclazide dispersed in beads and hence resulting in slow release.

Release kinetics of gliclazide

The release kinetics of sodium alginate and *Isabgol* husk-sodium alginate beads has been summarized in Table 11. In B and AA/2 formulations, the value of 'n' was greater than 0.5 ($n > 0.5$) that indicated anomalous drug diffusion mechanism. In case of formulation B, it was found that 'n' was 0.6664 and 0.6735 in 0.1 M HCl and phosphate buffer (pH 7.4) dissolution media, respectively. Similarly, for the formulation AA/2, the value of 'n' was found to be 0.6497 and 0.6797 in 0.1 M HCl and phosphate buffer (pH 7.4) dissolution media, respectively. The increment in 'n' value in 0.1 M HCl and phosphate buffer (pH 7.4) was considered due to swelling controlled diffusion of gliclazide from sodium alginate and *Isabgol* husk-sodium alginate polymeric network cross-linked by calcium chloride. It was observed that the value of regression coefficient 'r' was closer towards '1' in case of Higuchi model that indicated the diffusion of drug followed by relaxation of polymeric network of the beads. As the penetration of swelling media causes the swelling, the channels of cross-linked matrix start to open and cause the drug release.

The diffusion of drug from beads has also been shown in photomicrographs taken by SEM in Figure 6. The surface was found porous and hard. Some

of the drug particles were also seen on the surface as well as nearby to the pores boundary. This porous structure on the surface may develop due to diffusion of drug from the matrix. The surface of *Isabgol* husk-sodium alginate beads was more rigid as these were formed by possible involvement of polymeric structure of *Isabgol* in cross-linking that resulted in hard surface structure

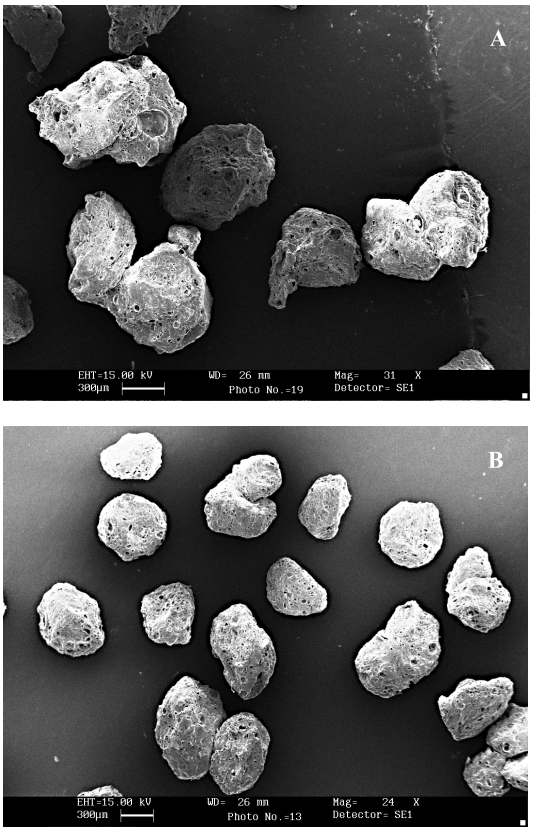

Figure 6. Photomicrographs of gliclazide loaded hydrogel beads (A) after dissolution in distilled water (B) after dissolution in 0.1 M HCl

The hydrogel beads of gellan gum cross-linked by calcium chloride and zinc sulfate also showed the non-fickian release of cephalexin due to relaxation of polymeric chains in dissolution media (12). Similar mechanism of gliclazide release from gliclazide/metformin tablets fabricated by using Eudragit NE30D in wet granulation method has been observed by Arno et al. (13).

During stability testing study, it was observed that swelling index (%) of beads varied from 331 ± 0.42 to 335 ± 1.43, 358 ± 3.12 to 361 ± 0.59, and 298 ± 1.22 to 301 ± 0.58, for A, A/2 and A/4, respectively. The variation in particle size was also observed i.e., 814.26 ± 0.26 to 816.14 ± 1.45 µm, 820.05 ± 2.02 to 826.23 ± 0.59 µm and 797.45 ± 1.22 to 803.23 ± 0.87 µm for A, A/2 and A/4, respectively. For the formulations A, A/2 and A/4 undergone stability study, the entrapment efficiency (%) varied from 92.66 ± 0.631 to 93.04 ± 0.12, 89.88 ± 0.87 to 90.42 ± 2.56, and 94.89 ± 1.23 to 95.67 ± 2.14, respectively. However, variation in results of entrapment efficiency, swelling index and particle size was not statistically significant from the initial values for these parameters ($p > 0.05$). When the entrapped gliclazide in spinking media containing mobile phase was scanned at 227 nm during stability study of A, A/2 and A/4 formulations, a unimodel sharp peak was obtained for each formulation

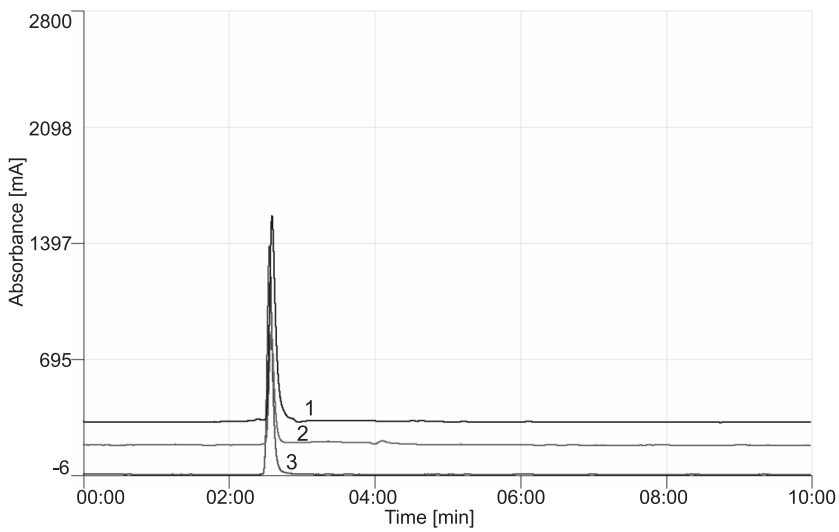

Figure 7. Chromatograms of gliclazide through stability study of A (1), A/2 (2), and A/4 (3)

at every time of the study and the peak can be overlaid to one another as shown in Figure 7.

CONCLUSION

The formulations prepared with high concentration of calcium chloride strength were more spherical and smaller size and showed marked effect on gliclazide entrapment and its release in dissolution media. Due to aldobiouronic content, *Isabgol* may participate in complex formation with Ca^{2+} ions. Due to chelation with phosphate in alkaline phosphate buffer (pH 7.4), the beads were unstable. The stability of formulations in terms of swelling index, particle size and entrapment efficiency revealed the reliability and suitability of the fabrication technique for sustained release gliclazide beads development of *Isabgol* husk. The study also revealed that the polymers have their significant impact on drug release from drug delivery devices and it is more effective in sustained release formulations. The release behavior followed by gliclazide in all dissolution media was diffusion followed by relaxation of the polymer chains. The release mechanism of gliclazide from beads may be fruitful as the initial fast release and then slow release, as shown in Higuchi model, will increase the absorption content and hence bioavailability of the drug. The quantification of gliclazide in formulations by HPLC method indicated high degree of regression coefficient ($r < 0.9990$) and relative standard deviation (%RSD < 1). The results of limit of detection (LOD) and limit of quantification (LOQ) were also of significant importance. All other results of applied HPLC method such as selectivity, sensitivity, precession, accuracy, ruggedness, and robustness indicated the applicability of this method in gliclazide determination in pure form and in formulations.

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