RESEARCH ARTICLE

Development and validation of discriminatory dissolution procedure for poorly soluble glyburide

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In the present study, parameters such as solubility, medium pH, surfactant type and dissolution behavior of formulations, influence of sink conditions, stability and discriminatory effect of dissolution testing were studied for the selection of a proper dissolution medium for glyburide (BCS Class II drug). Results of solubility data revealed that solubility increased with an increase in pH. Sink conditions were exhibited in the 0.05 M borate buffer pH 9.6, 0.05 M phosphate buffer pH 6.5 containing 0.1-2% (w/v) cetyl trimethyl ammonium bromide (CTAB) and 0.05 M phosphate buffer pH 7.4 containing 0.1-2% CTAB (w/v), respectively. The 0.05 M phosphate buffer at pH 6.5 containing 0.1% CTAB (w/v) with an agitation speed of 50 rpm (USP II) showed a more discriminating drug release profile when compared with 0.05 M phosphate buffer at pH 7.4 containing 0.1% CTAB (w/v) with an agitation speed of 75 rpm. The spiked samples have shown better recovery at 50, 100 and 150% levels. There was no degradation, as observed in the mass spectrum of recovered dissolution samples when compared with the mass spectrum of standard drug solution, which promised that the method was specific and can be used for routine Quality Control analysis as well as for assessment of formulation variables in future dissolution studies of glyburide.

Key words: *CTAB, dissolution, glyburide, mass spectrum, spiked samples*

INTRODUCTION

Dissolution testing has emerged in the pharmaceutical field as a very important tool to characterize drug product performance. The development of a meaningful dissolution procedure for drug products with limited water solubility has been a challenge to both the pharmaceutical industry and the agencies that regulate them. *In vivo*, the dissolution process depends on physicochemical parameters, which may be affected by the intraluminal conditions in the body. Naturally occurring surfactants solubilize sparingly soluble drugs in the body and help in the absorption process. A dissolution medium containing surfactant can better simulate the environment of the gastrointestinal tract than a medium containing organic solvents or other nonphysiological substances, making the dissolution test conditions more useful in evaluating drug quality.[1,2] Specific information about the drug substance solubility, drug substance stability as a function of pH, and BCS Classification will direct the expedient selection of a proper dissolution medium. A sensitive, reliable *in vitro* dissolution procedure is used to determine the quality of a product and to advance the evolution of dissolution technology. A clear trend has emerged where the dissolution test has moved from a traditional quality control test to a surrogate *in vitro* bioequivalence (BE) study.[3,4] Dissolution has become an important and widely utilized test receiving more emphasis worldwide from regulatory authorities during the last 15 years. The significance of a dissolution test is based on the fact that for a drug to be absorbed and available to the systemic circulation, it must previously be dissolved.[5] Therefore, dissolution tests are used not

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only for quality control of finished products, but also to assess several stages of formulation development, for screening and proper assessment of different formulations.[6] Basically, the dissolution test makes it possible to assess the dissolution properties of the drug itself and thereby to select the most appropriate excipients and to optimize proportions among them to obtain the desired drug release behavior.

Glyburide (BCS Class II drug), is a sulfonyl urea derivative and is widely used as an oral antihyperglycemic agent. Its solubility in water as per the literature is 4 mg/L.[7] The official medium prescribed in FDA's CDER guidelines[8] for its non-micronized form is, 0.05 M borate buffer pH 9.6 (500 ml) using USP II, paddle apparatus at 75 rpm for 60 mins at 37±0.5°C. Moreover, for its micronized form the prescribed media is 0.05 M phosphate buffer pH 7.5 (900 ml) using USP II, paddle apparatus at 50 rpm for 60 mins at 37±0.5°C (CDER, 2004). pH above 8.0 is not relevant to the human gastrointestinal physiology.[9] Basic media, mostly above 8.0 causes column degradation and thus challenges the developed dissolution method.[10] Micronization of the drug may increase the time and cost of production during the scale-up and manufacturing process, as the drug is having better permeability (BCS Class II). Moreover, can the use of media having pH 9.6, be a better approach for an *in vivo – in vitro* correlation of glyburide?

The objective of the present study was to develop and validate a discriminating dissolution method for glyburide at a pH, which must satisfy the aforementioned issues related with the existing dissolution procedure for glyburide.

MATERIALS AND METHODS

Materials

Glyburide working standard was a gift sample from Dr. Reddys Laboratory, Hyderabad, India. Glyburide active pharmaceutical ingredient (API) was procured from Cadila Pharmaceuticals, India. Sodium lauryl sulfate (SLS), Tween 40, cetyl trimethyl ammonium bromide (CTAB), potassium dihydrogen orthophosphate, sodium dihydrogen orthophosphate (Qualigens, Mumbai), sodium hydroxide (S.D. Fine Chemicals, Mumbai), methanol (AR grade) and hydrochloric acid (Merck, Darmstadt, Germany) were used as received. Double distilled water was used throughout the study. Dissolution apparatus (Lab India DS 8000, India), UV/Vis spectrophotometer (Cyberlab UV-100, USA), LC/MS (Shimadzu 2010A LC- MS, Japan) were used. Instat software was used for statistical analysis.

Methods

Preparation of stock standard solution and calibration curve samples

One hundred milligrams of glyburide working standard was weighed accurately and transferred to 100 ml standard volumetric flask; the volume was adjusted to 100 ml using 0.2 M sodium hydroxide solution at a concentration of 1mg/ml. The glyburide stock standard solution was diluted to obtain the known standard concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 μg/ml. The 0.2 M sodium hydroxide solution was prepared by weighing 0.8 g of sodium hydroxide pellets and transferring it to 1000 ml volumetric flask; the volume was adjusted to 1000 ml using double distilled water. UV absorbance of each standard solution was measured spectrophotometrically (UV/Vis spectrophotometer, Cyberlab UV-100) at 230 nm with the mean data (n=6) used for calibration curve.

Saturation solubility study

The saturation solubility study of glyburide was determined in the following media: double distilled water; 0.1N HCl pH 1.2, acetate buffer pH 4.8 and 5.0, phosphate buffer pH 6.2, 6.5, 6.8, 7.2, 7.4, 7.8 and 8.0, alkaline borate buffer pH 9.6, 0.5, 1.0, 1.5 and 2% (w/v) SLS in water and in pH 7.4 phosphate buffer, 0.25, 0.5, 0.75, 1 and 2% Tween 40 in water and in pH 7.4 phosphate buffer, 0.1, 0.25, 0.5, 1 and 2% (w/v) CTAB in water, pH 7.4 phosphate buffer, 6.5 phosphate buffer, pH 5.0 acetate buffer and pH 1.2 hydrochloric acid buffer, respectively, at room temperature. As per BCS guidelines for solubility studies,[11] highest single-dose strength (5 mg) of glyburide was added to 250 ml of the aforementioned media for saturation solubility study in 500 ml conical flasks and agitated continuously at room temperature for 24 hrs on a mechanical shaker. The solutions were then filtered (Cutoff 0.2μm, Ministart SRP 25, Sartorius) and analyzed spectrophotometrically at 230nm (UV/Vis spectrophotometer, Cyberlab UV-100). The studies were repeated three times and mean data was recorded.

In vitro drug release studies

Glyburide tablets from three different brands namely Glinil (Cipla), Euglucon (Piramal Healthcare) and Gluconil (Bal Pharma) each containing 5 mg of glyburide (glibenclamide) (A, B and C) were procured for comparative dissolution studies. The dissolution study was performed using USP Apparatus 2 at 37±0.5°C with paddle speeds 50±5rpm and 75±5 rpm in 900-ml dissolution medium [0.05 M phosphate buffer at pH 6.5 containing 0.1% CTAB (w/v) and 0.05 M phosphate buffer at pH 7.4 containing 0.1% CTAB (w/v)]. A 10-ml sample was withdrawn at different time interval and filtered (Cutoff 0.2 μm, Ministart SRP 25, Sartorius) and analyzed spectrophotometrically at 230 nm. Withdrawn samples were replaced with 10 ml of fresh medium. The percentage cumulative drug release [% cumulative drug release (CDR)] of the three brands was calculated.

Comparison of dissolution profiles by a model-independent method

This study utilized a model-independent approach in which the dissolution profiles of two drug products are compared using the fit factor. This fit factor directly compares the difference between percent drug dissolved per unit time for a test and a reference product. The fit factor, *f2*, is defined by the following:

$$\text{Similarity factor } f_2 = 50 \times \log \left[(1 + 1/n \sum_{t=1}^{n} (R_t - T_t)^2)^{-0.5} \times 100\right]$$

Where, *n* is the number of dissolution sampling times, and *Rt* and *Tt* are the individual or mean percent dissolved at each time point for the reference and test dissolution profiles, respectively. f_2 values greater than 50 (50–100) would indicate sameness or equivalence of the two curves.[12] The drug release profile of three formulations A, B and C containing same dose of glyburide were compared for the selected two different media at different stirring speeds using f_2 values.

Recovery studies

The recovery of the dissolution method is done to prove the method accuracy. The standard glyburide was spiked with the placebo at three different recovery levels (50, 100 and 150% recovery level).[13] 2.5 mg (50%), 5 mg (100%) and 7.5 mg (150%) of glyburide working standard was spiked with 95 mg of the placebo individually in 900 ml of 0.1% (w/v) CTAB in pH 6.5 phosphate buffer. Ten milliliter samples were withdrawn, filtered (Cutoff 0.2 μm, Ministart SRP 25, Sartorius) and analyzed spectrophotometrically at 230 nm. The studies were repeated six times and the mean data was recorded.

Stability studies

Solutions of pure glyburide and the recovery samples of three different levels were stored in the dark at ambient temperature and 2-8°C for upto 7 days. Sample aliquots of 5 ml were withdrawn and analyzed spectrophotometrically after every 24-hr period. Each day the concentration of the drug found in the standard and recovery samples were compared with concentrations of drug found in the same samples stored at 2-8°C. The absolute difference between the results at time zero and the time indicated for stability were determined by analyzing the content using UV-visible spectrophotometer. The mean (n=6) recovery of the different level's recovery samples with compared to the amount of drug added (initial) during spiking after 3 hrs, 6 hrs and 1 week was recorded.

Method specificity

The dissolution analysis method must be specific for the bulk drug substance in the presence of a placebo.[13] As a UV spectrum of the solutions is not sufficient in determining degradation since many degradation products will have the same UV spectrum as the parent compound. Therefore, specificity testing was confirmed by analyzing accuracy samples using LC-MS. Specificity testing was done by injecting working standard of drug and accuracy samples (dissolution media samples i.e., mixture of drug, dissolution media and excipients) in the mass spectrophotometer and the spectra were recorded. An analytical column, Phenomenex C_{18} (150×4.6 mm i.d., 5 μ) was used for chromatographic separation. The mobile phase consisted of acetonitrile–ammonium acetate buffer, 5 mM (45:55, v/v) without any pH adjustment and was operated isocratically at a flow-rate of 1 ml/min. Atmospheric pressure ionspray was used as an interface.

RESULTS AND DISCUSSIONS

The mean (n=3) solubility profile of glyburide in pH 1.2 to 9.6 is shown in Table 1 and Figure 1. The solubility of glyburide in double-distilled water was found to be 3.98 μg/ml (3.98 mg/L) as shown in Table 1. The results of the mean solubility data (n=3) and the influence of sink conditions with the use of various surfactants are summarized in Table 2, which showed that there was a significant increase in solubility with increasing pH. The addition of different concentrations of SLS in double distilled water provided the maximum solubility of 4.15 μg/ml. The SLS failed to prove its use to enhance the dissolution of glyburide, as the sink condition value was 0.75 [i.e., the ratio of saturation solubility to the dose in 900 ml dissolution medium (C_S/C_D) must be ≥ 3.0]. Moreover, the turbidity was seen with the use of SLS in phosphate buffer at pH 7.4 which challenged the stability of SLS in pH 7.4 phosphate buffer. The 2% concentration of Tween 40 in phosphate buffer has provided a solubility of 11.52 μg/ml (11.52 mg/L), but was unable to provide the sink condition. The sink condition was well achieved with all concentrations of CTAB in phosphate buffer pH 6.5 and pH 7.4. As the objective was to use the lowest percentage of CTAB in the medium; phosphate buffer pH 6.5 and 7.4 containing 0.1% CTAB (w/v) were used as a dissolution medium.

Table 1: pH Solubility profile of glyburide from 1.2 to 9.6 (Number of replicates of studies conducted (n) = 3)*

Dissolution medium (250ml)	Solubility* (mean ± SD)	Sink condition µg/ml (Cs/CD)
pH 1.2 HCl buffer	2.13±0.050	0.38
pH 4.5 Acetate buffer	2.48±0.055	0.50
pH 5.8 Acetate buffer	2.59±0.005	0.50
pH 6.8 Phosphate buffer	3.18±0.009	0.57
pH 7.0 Phosphate buffer	3.38±0.003	0.61
pH 7.4 Phosphate buffer	3.67±0.012	0.66
pH 7.8 Phosphate buffer	3.95±0.153	0.71
pH 8.0 Phosphate buffer	4.57±0.023	0.82
pH 9.6 Borate buffer	17.51±0.015	3.15
Double distilled water	3.98±0.020	0.72

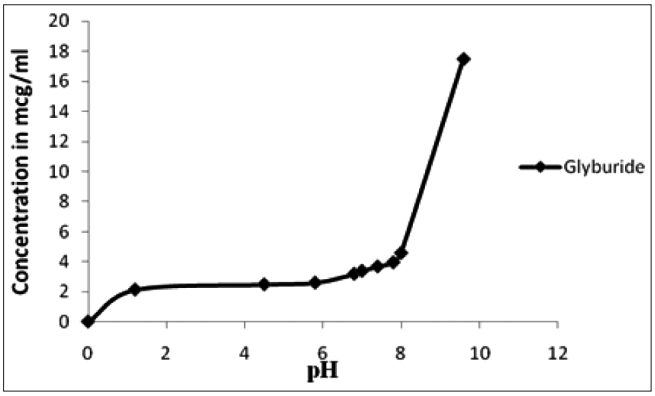

Figure 1: pH Solubility profile of glyburide from 1.2 to 9.6 (Number of replicates of studies conducted = 3)

Greater than 80% drug release was found within 15 mins in 0.1% (w/v) CTAB in pH 6.5 (900 ml), in pH 7.4 phosphate buffer (900 ml) and 0.05 M borate buffer pH 9.6 (500 ml) medium, respectively. The results of dissolution studies are shown in Table 3.

The percentage cumulative drug release profiles (%CDR) of marketed formulations A, B and C in 0.1% CTAB (w/v) in pH 6.5 [Figures 2 and 3] and 0.1% CTAB (w/v) in pH 7.4 [Figures 4 and 5] phosphate buffer were compared at 50±5 rpm and 75±5 rpm.

Table 2: Saturation solubility of glyburide and relative sink conditions in different dissolution Media (n=3)* [Where, n = number of replicates of studies conducted]

Solubility medium (250ml)	Solubility* (mean ± SD) µg/ml	Sink Condition (C_S/C_D)
1.0% SLS (w/v) in DD Water	3.24±0.010	0.58
2.0% SLS (w/v) in DD Water	4.15±0.060	0.75
1.0% SLS (w/v) in phosphate buffer pH 7.4	2.79±0.010	0.50
2.0% SLS (w/v) phosphate buffer pH 7.4	3.51±0.010	0.63
1.0% Tween 40 in DD Water	5.22±0.020	0.94
2.0% Tween 40 in DD Water	9.85±0.130	1.77
1.0% Tween 40 in phosphate buffer pH 7.4	8.90±0.020	1.60
2.0% Tween 40 in phosphate buffer pH 7.4	11.52±0.030	2.07
0.25% CTAB (w/v) in DD water	8.10±0.010	1.46
0.1% CTAB (w/v) in DD water	5.77±0.586	1.04
0.5% CTAB (w/v) in DD water	9.75±0.010	1.75
1.0% CTAB (w/v) in DD water	14.96±0.040	2.69
2.0% CTAB (w/v) in DD water	15.10±0.090	2.72
0.1% CTAB (w/v) in phosphate buffer pH 7.4	19.94±0.060	3.59
0.25% CTAB (w/v) in phosphate buffer pH 7.4	19.96±0.080	3.59
0.5% CTAB (w/v) in phosphate buffer pH 7.4	19.98±0.050	3.60
1.0% CTAB (w/v) in phosphate buffer pH 7.4	19.98±0.020	3.60
2.0% CTAB (w/v) in phosphate buffer pH 7.4	20.12±0.100	3.63
0.1% CTAB (w/v) in phosphate buffer pH 6.5	19.46±0.050	3.51
0.25% CTAB (w/v) in phosphate buffer pH 6.5	19.88±0.013	3.58
0.5% CTAB (w/v) in phosphate buffer pH 6.5	19.91±0.016	3.59
1.0% CTAB (w/v) in phosphate buffer pH 6.5	19.91±0.060	3.59
2.0% CTAB (w/v) in phosphate buffer pH 6.5	19.98±0.034	3.60
0.1% CTAB (w/v) in acetate buffer pH 5.0	3.31±0.012	0.60
0.25% CTAB (w/v) in acetate buffer pH 5.0	5.63±0.027	1.01
0.5% CTAB (w/v) in acetate buffer pH 5.0	12.27±0.022	2.21
1.0% CTAB (w/v) in acetate buffer pH 5.0	12.37±0.023	2.23
2.0% CTAB (w/v) in acetate buffer pH 5.0	19.50±0.016	3.51
0.1% CTAB (w/v) in pH 1.2 HCL buffer	2.75±0.012	0.50
0.25% CTAB (w/v) in pH 1.2 HCL buffer	5.42±0.015	0.98
0.5% CTAB (w/v) in pH 1.2 HCL buffer	5.92±0.015	1.10
1.0% CTAB (w/v) in pH 1.2 HCL buffer	6.70±0.016	1.21
2.0% CTAB (w/v) in pH 1.2 HCL buffer	7.17±0.019	1.30

Table 3: Mean % Cumulative dissolution data of glyburide standard tablet and its marketed formulations at pH 6.5 and 7.4 at 50 and 75 rpm, respectively (n=6)*

Time in minutes	*Mean % Cumulative Dissolution Data of Glyburide Tablets											
	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_4	N_1	N_2	N_3	N_4
0	0	0	0	0	0	0	0	0	0	0	0	0
5	70.0	76.3	77.9	83.1	81.5	82.5	82.3	84.4	81.7	89.4	84.6	82.2
10	78.6	84.0	83.8	89.9	85.9	89.4	88.2	89.8	86.7	89.4	89.3	88.4
15	88.7	93.2	90.1	93.3	88.9	95.0	94.5	95.1	89.5	94.9	93.4	93.1
30	92.9	96.3	95.0	96.2	93.0	97.2	96.5	97.4	93.2	96.9	96.2	95.8
45	96.0	98.4	98.2	98.4	97.0	99.2	98.3	98.8	96.5	98.8	98.6	98.3
60	99.0	100.3	100.2	100.3	100.1	100.6	100.5	100.6	100.2	100.5	100.6	100.4

S_1- Formulation A in 0.1% CTAB (w/v) in pH 6.5 at 50 rpm; S_2 - Formulation A in 0.1% CTAB (w/v) in 6.5 at 75 rpm; S_3 - Formulation A in 0.1% CTAB (w/v) in pH 7.4 at 50 rpm; S_4 - Formulation A in 0.1% CTAB (w/v) in pH 7.4 at 75 rpm; M_1- Formulation B in 0.1% CTAB (w/v) in pH 6.5 at 50 rpm; M_2 - Formulation B in 0.1% CTAB (w/v) in pH 6.5 at 75 rpm; M_3 - Formulation B in 0.1% CTAB (w/v) in pH 7.4 at 50 rpm; M_4 - Formulation B in 0.1% CTAB (w/v) in pH 7.4 at 75 rpm; N_1 - Formulation C in 0.1% CTAB (w/v) in pH 6.5 at 50 rpm; N_2 - Formulation C in 0.1% CTAB (w/v) in pH 6.5 at 75 rpm; N_3 - Formulation C in 0.1% CTAB (w/v) in pH 7.4 at 50 rpm; N_4 - Formulation C in 0.1% CTAB (w/v) in pH 7.4 at 75 rpm

Table 4: Statistical evaluation of dissolution results for formulations A, B and C at different stirring speeds in 0.1% CTAB (w/v) in pH 6.5 (900 ml) and 0.1% CTAB (w/v) in pH 7.4 phosphate buffer (900 ml) medium (n=6)*

Formulations	Time	0.1% CTAB (w/v) in pH 6.5 Phosphate buffer				0.1% CTAB (w/v) in pH 7.4 Phosphate buffer			
		% Cumulative drug release (%CDR) (Mean ± SD)* (n=6)		*t* - test	*P* value	% Cumulative drug release (%CDR) (Mean ± SD)* (n=6)		*t* - test	*P*-value
		50 rpm	75 rpm			50 rpm	75 rpm		
A	0	0	0			0	0		
	5	70.0±1.4	76.3±2.9			77.9±3.3	83.1±3.4		
	10	78.6±3.7	84.0±2.3			83.8±2.6	89.9±1.2		
	15	88.7±1.7	93.2±2.4			90.1±2.9	93.3±0.7		
	30	92.9±1.1	96.3±2.3	5.1	0.004	95.0±1.9	96.2±1.4	2.53	0.052
	45	96.0±0.8	98.4±1.9			98.2±1.0	98.4±0.9		
	60	99.0±1.1	100.3±1.0			100.2±0.3	100.3±0.2		
B	0	0	0			0	0		
	5	81.5±1.4	82.5±3.7			82.3±3.6	84.4±1.9		
	10	85.9±0.9	89.4±2.9			88.2±3.1	89.8±2.6		
	15	88.9±1.2	95.0±0.9	3.4	0.019	94.5±0.8	95.1±1.3	3.17	0.025
	30	93.0±1.0	97.2±1.0			96.5±1.0	97.4±0.6		
	45	97.0±0.7	99.2±0.4			98.3±1.2	98.8±0.7		
	60	100.1±0.3	100.6±0.3			100.5±0.4	100.6±0.4		
C	0	0	0			0	0		
	5	81.7±1.1	89.37±3.2			84.6±2.4	82.2±2.8		
	10	86.7±0.7	89.35±3.1			89.3±1.6	88.4±3.0		
	15	89.5±0.5	94.91±1.1	3.5	0.017	93.4±1.2	93.1±1.8	2.17	0.082
	30	93.2±1.5	96.85±1.0			96.2±1.4	95.8±1.4		
	45	96.5±2.0	98.81±0.5			98.6±0.8	98.3±1.1		
	60	100.2±0.5	100.5±0.3			100.6±0.3	100.4±0.4		

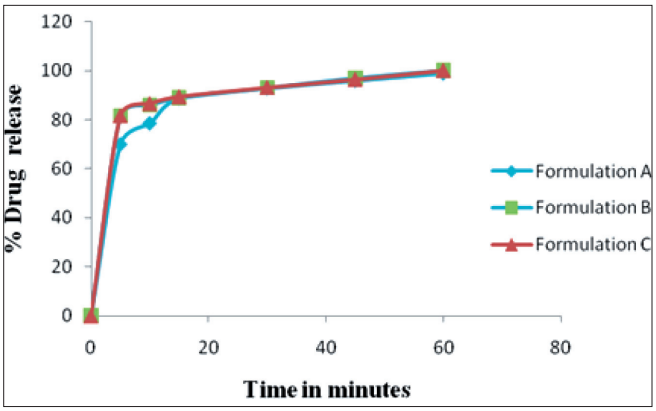

Figure 2: Mean Dissolution profiles of three marketed glyburide tablets formulations at 50 rpm at pH 6.5 (Number of replicates of study conducted = 6)

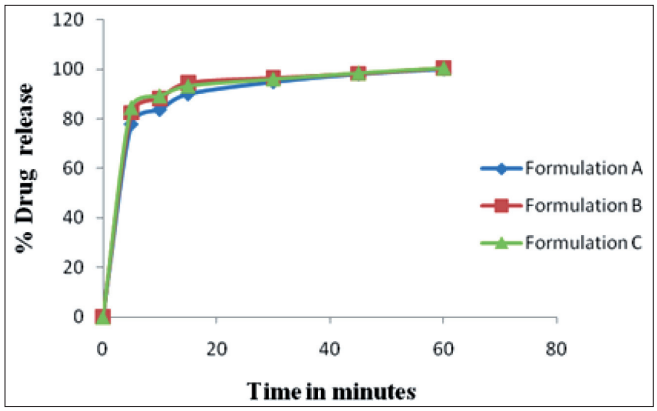

Figure 3: Mean Dissolution profiles of three marketed Glyburide tablets formulations at 75 rpm at pH 6.5 (Number of replicates of study conducted = 6)

Table 4 contains the statistical evaluation of the % cumulative drug release (%CDR) at 50 and 75 rpm for tablets A, B, and C in 0.1% CTAB (w/v) in pH 6.5 and 7.4, respectively, using the Student's *t*-test at the 5% significance level. The *P*-value less than or equal to the delineated significance level (0.05) indicates that there is a statistically significant difference in the drug release in formulations at varying speeds of rotation. A significant difference in %CDR was found for formulation A in 0.1% CTAB (w/v) pH 6.5, formulation B in 0.1% CTAB (w/v) pH 6.5 and 7.4, respectively, and formulation C in 0.1% CTAB (w/v) in pH 6.5 with varying speeds, while no statistically significant difference was found for formulation A and formulation C in 0.1% CTAB (w/v) pH 7.4 with varying speeds. The maximum significant difference was found with Formulation A at varying speeds (*P*-value 0.004).

Table 5 shows a comparison of the dissolution profiles of marketed products using the similarity factor *f*2 at different stirring speeds in pH 6.8 phosphate buffer. The mean of *f2* factor for each condition compared to other conditions

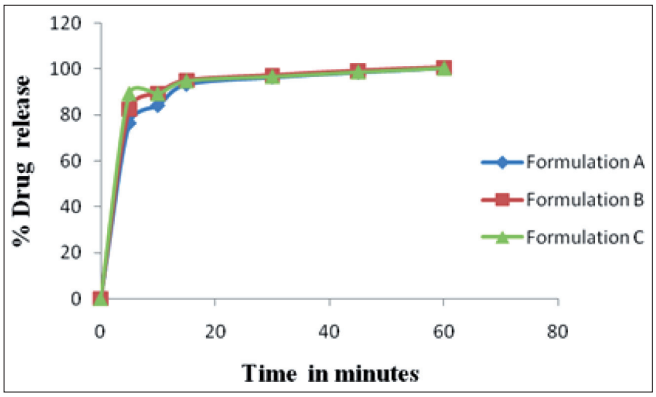

Figure 4: Mean dissolution profiles of three marketed glyburide tablet formulations at 50 rpm at pH 7.4 (Number of replicates of study conducted=6)

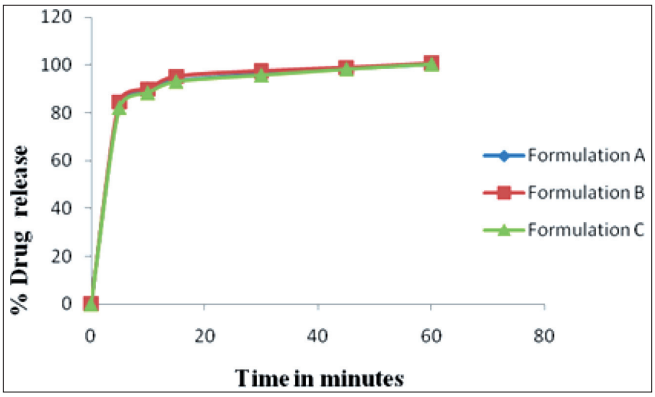

Figure 5: Mean dissolution profiles of three marketed glyburide tablet formulations at 75 rpm at pH 7.4 (Number of replicates of study conducted = 6)

Table 5: Similarity factor, *f2*, between different dissolution conditions for glyburide formulations

	S1	S2	S3	S4	M1	M2	M3	M4	N1	N2	N3	N4
S1	N/A	49.05	49.67	36.72	43.10	36.56	38.12	34.8	42.16	31.37	35.77	38.79
S2	49.05	N/A	70.63	52.15	55.01	53.36	56.21	49.44	54.78	42.27	50.20	56.40
S3	49.67	70.63	N/A	52.84	64.94	51.95	55.29	48.91	62.95	43.01	51.29	57.45
S4	36.72	52.15	52.84	N/A	57.28	80.14	80.35	77.94	59.88	58.95	85.65	84.21
M1	43.10	55.01	64.94	57.28	N/A	53.24	57.02	51.65	90.77	47.22	56.40	61.40
M2	36.56	53.36	51.95	80.14	53.24	N/A	84.84	82.57	55.08	57.86	75.74	76.75
M3	38.12	56.21	55.29	80.35	57.02	84.84	N/A	75.67	59.21	56.92	76.27	86.37
M4	34.84	9.44	48.91	77.94	51.65	82.57	75.67	N/A	53.51	64.52	81.18	70.85
N1	42.16	54.78	62.95	59.88	90.77	55.08	59.21	53.51	N/A	48.50	58.68	64.16
N2	31.37	42.27	43.01	58.95	47.22	57.86	56.92	64.52	48.50	N/A	64.41	55.92
N3	35.77	50.20	51.29	85.65	56.40	75.74	76.27	81.18	58.68	64.41	N/A	77.49
N4	38.79	56.40	57.45	84.21	61.40	76.75	86.37	70.85	64.16	55.92	77.49	N/A
Mean	39.64	53.59	55.36	66.01	58.00	64.38	66.02	62.82	59.06	51.90	64.82	66.34

were calculated and showed at the bottom of each column in Table 5. Lowest mean *f2* value in table indicated the largest difference between dissolution conditions. Results showed that smallest *f2* values for formulations B and C were seen when compared to Formulation A in pH 6.5 phosphate buffer containing 0.1% CTAB (w/v) at 50 rpm stirring speed, as the lowest mean *f2* value in the table is 39.64.

The above data shows that 0.1% CTAB (w/v) in pH 6.5 phosphate buffer using USP II paddle apparatus at 50 rpm stirring speed may provide more discriminating condition for glyburide tablets in future studies.

The design for conducting recovery study is shown in Table 6. The spiked samples at 50, 100 and 150% levels have shown better recovery of 98.3, 98.82 and 97.28%, respectively, as shown in Table 7. This states that the method was accurate and can be used for further studies.

Table 8 contains a summary of the stability data for solutions used for solubility studies. The absolute difference between the concentrations of drug stored at 2–8°C and the same solution at room temperature over the period of 7 days was found to be less than 3.0% for all media. Table 9 contains the summary of the stability data for the different recovery level's samples with compared to the amount of drug (initial)

Table 6: Design for conducting recovery studies in 900 ml dissolution medium

Recovery (Theoretical)				
Range/ Percentage	API (mg)	Placebo (mg)	Medium (ml)	µg/ml
50	2.5	95	900	2.78
100	5.0	95	900	5.55
150	7.5	95	900	8.33

Table 7: Mean recovery data of glyburide formulation samples at three levels (n= 6)*

Recovery level	% Recovery (mean ± SD) *	% RSD
Recovery 50%	98.317±1.12	1.143
Recovery 100%	98.823±0.95	0.962
Recovery 150%	97.280±1.08	1.108
	Overall Mean of % Recovery	98.140
	Overall SD	1.190
	Overall % RSD	1.212

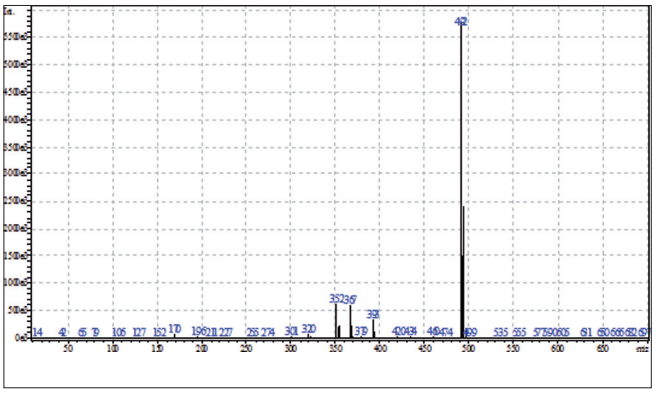

Figure 6: Mass spectrum of pure glyburide

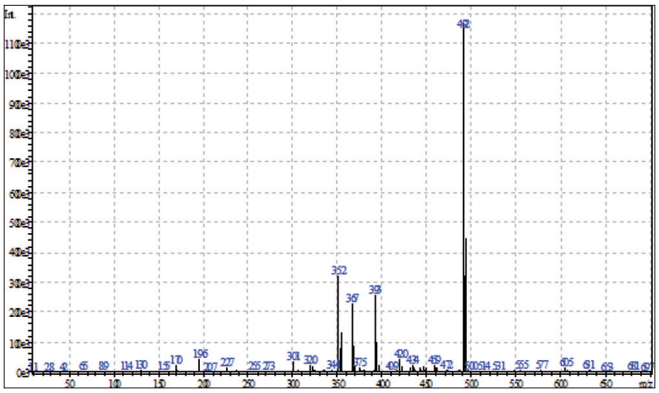

Figure 7: Mass spectrum of spiked glyburide with excipients (placebo) in dissolution media

Table 8: Stability study data (percentage of absolute difference) of glyburide standard solutions in various solubility Media after 7 days (n=3)*

Medium	% Absolute difference (Value should be less than 3)
Double distilled water	1.75
0.1% CTAB (w/v) in DD water	1.60
0.25% CTAB (w/v) in DD water	2.66
0.5% CTAB (w/v) in DD water	1.54
1% CTAB (w/v) in DD water	2.14
2% CTAB (w/v) in DD water	2.12
0.1% CTAB (w/v) in phosphate buffer pH 7.4	1.65
0.25% CTAB (w/v) in phosphate buffer pH 7.4	2.71
0.5% CTAB (w/v) in phosphate buffer pH 7.4	1.30
1% CTAB (w/v) in phosphate buffer pH 7.4	1.95
2% CTAB (w/v) in phosphate buffer pH 7.4	1.59
0.1% CTAB (w/v) in phosphate buffer pH 6.5	0.67
0.25% CTAB (w/v) in phosphate buffer pH 6.5	2.41
0.5% CTAB (w/v) in phosphate buffer pH 6.5	1.41
1% CTAB (w/v) in phosphate buffer pH 6.5	0.50
2% CTAB (w/v) in phosphate buffer pH 6.5	2.20

Table 9: Stability study data at different percentage of recovery levels after 3 hrs, 6 hrs and 1 week (n=6)*

Stability at different % Recovery Levels	% Recovery (mean ± SD)*	% RSD
Stability at 50% level after 3 hours	97.99±1.27	1.30
Stability at 100% level after 3 hours	98.71±0.75	0.76
Stability at 150% level after 3 hours	99.11±0.28	0.28
Stability at 50% level after 6 hours	97.24±1.39	1.40
Stability at 100% level after 6 hours	97.51±0.86	0.86
Stability at 150% level after 6 hours	97.64±0.86	0.88
Stability at 50% level after 1 week	96.57±1.08	1.12
Stability at 100% level after 1 week	97.22±0.64	0.66
Stability at 150% level after 1 week	97.15±0.79	0.81

added during spiking after 3 hrs, 6 hrs and 1 week; which are well within the limits.

In Figures 6 and 7 the Comparison of mass spectrum of pure glyburide and spectrum of accuracy samples of glyburide spiked with Placebo showed that, both the spectra are same. This stated that the method was specific and has not caused the degradation of the drug.

CONCLUSIONS

Dissolution testing plays a very important role as an *in vitro* test for evaluating drug products. In the present study, an attempt has been made to develop and validate a new dissolution procedure for glyburide which can provide the discriminatory results when different formulations of the same drug, in similar dosage forms with same dose were being compared. The use of 900 ml of pH 6.5 phosphate buffer containing 0.1% CTAB at 37±0.5°C, at paddle speed of 50±5 rpm for 60 mins has produced maximum discriminatory effect and can be applied for dissolution testing of glyburide for future studies. Moreover it has overcome the problems related with the use of pH 9.6 alkaline borate (0.05 M) buffer (as per USP 30 NF 27) for the dissolution studies of glyburide, as the pH 6.5 is well within human gastrointestinal physiology and also can be able to minimize the problems related to use of basic pH (9.6 borate buffer) which may cause column degradation.

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