Method Development and Validation for Estimation of Glyburide (GLIBENCLAMIDE) In Pharmaceutical Formulations and Rat Plasma by RP-HPLC

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Abstract - A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for determination of glyburide in pure form and marketed pharmaceutical dosage forms. A column having Kromasil ODS 3V (250×4.6mm, 5µ) in isocratic mode with mobile phase containing acetonitrile (ACN): phosphate buffer (60:40v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 238 nm. The method has been validated for linearity, accuracy and precision, robustness and limit of detection and limit of quantitation. The retention time (min) and linearity range for glyburide was (2.262 min) and (10-50 µg/ml), respectively. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.02µg/ml and 0.05µg/ml for glyburide. The developed method was found to be accurate, precise and selective for determination of glyburide in pharmaceutical dosage forms. The short retention time allows the analysis of a large number of samples in a short period of time and, therefore, should be cost effective for routine quality control in the pharmaceutical industry.

Index Terms - Glyburide, RP-HPLC, Validation and method development.

1.INTRODUCTION

High performance liquid chromatography (HPLC) is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitate the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as parts per trillion (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals.

During the development of modern liquid chromatography, advances have been driven by both instrumentation and chemistry. The technique as now practiced produces elegant separations that have been developed through chemical manipulation of the stationary and mobile phases, which are then effected and detected by modern instrumentation [1].

Glibenclamide, also known as glyburide, is a medication used to treat diabetes mellitus type 2. It is recommended that it be taken together with diet and exercise. It may be used with other antidiabetic medication. It is not recommended for use by itself in diabetes mellitus type 1 [2].

Glyburide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It belongs to the sulfonylurea class of insulin secretagogues, which act by stimulating β cells of the pancreas to release insulin. Sulfonylureas increase both basal insulin secretion and meal-stimulated insulin release. Medications in this class differ in their dose, rate of absorption, duration of action, route of elimination and binding site on their target pancreatic β cell receptor [3].
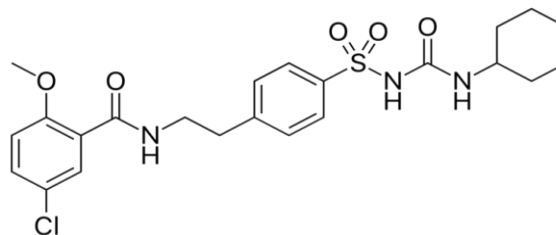

Figure 1: Structure of Glyburide

The medication works by binding to and inhibiting the ATP-sensitive potassium channels (K_{ATP}) inhibitory regulatory subunit sulfonylurea receptor 1(SUR1) in pancreatic beta cells. This inhibition causes cell membrane depolarization,

opening voltage-dependent calcium channels. This results in an increase in intracellular calcium in the pancreatic beta cell and subsequent stimulation of insulin release [4].

2. MATERIALS AND METHODS

2.1. Materials:
Pharmaceutical grade pure glyburide gift sample was procured from Sri Krishna pharmaceuticals Ltd, Hyderabad, India. Marketed formulation Tablets with dose of 5 mg of glyburide was procured from local market ((Diaβeta®, 5mg)). HPLC grade acetonitrile was procured from Merck specialties private limited, Mumbai. Potassium dihydrogen orthophosphate was procured from Sd fine-Chem ltd; Mumbai.

2.2. Methods:
2.2.1. Method development and optimization of chromatographic conditions:
2.2.1.1. Selection of chromatographic condition
The proper selection of the method depends upon the nature of the sample, its molecular weight and solubility. The drugs selected in the present study are polar in nature and hence reversed phase chromatography method may be used. The reversed phase HPLC was selected for the separation because of its simplicity and suitability [5].

2.2.1.2. Selection of detection wavelength:
The sensitivity of method that uses UV- Visible detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected.
Standard solutions of glyburide were scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was recorded. From the overlain spectrum, 238 nm was selected as the detection wavelength for the present study [6].

2.2.1.3. Selection of mobile phase:
The mobile phase tried was methanol and water, methanol and acetonitrile, buffer and water in various proportions. Finally, the mobile phase was optimized to acetonitrile: phosphate buffer in proportion 60:40v/v respectively.

2.2.1.4. Optimization of flow rate:

The method was performed with flow rates 0.6ml, 0.8ml and 1ml/min. Flow rate of 1ml/min was found to be ideal as it gave sharp peak.
Based on the above study, the following chromatographic conditions were selected for the simultaneous estimation of drugs in multi component dosage forms.

2.2.1.5. Preparation of Phosphate buffer:
Accurately weighed 136.09 gms of potassium dihydrogen ortho phosphate was taken into a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 6.2 with NaOH. The optimized mobile phase ratio buffer and methanol (40:60 ratio).

2.2.1.6. Preparation of mobile phase:
Accurately measured 400 ml (40%) of above buffer and 600 ml of acetonitrile HPLC grade (60%) were mixed and degassed in an ultrasonic water bath for 5 minutes and then filtered through 0.45 µ filter under vacuum filtration [7].

2.2.2. Estimation of drug in marketed formulation by developed RP-HPLC method:
2.2.2.1. Preparation of Solutions:
A. Standard Preparation:
Accurately weigh and transfer 10 mg of glyburide sodium working standard into a 10mL clean dry volumetric flask add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

B. Sample Preparation:
Accurately weigh 20 tablets average weight and take the powder weight equivalent to 10 mg of glyburide into a 10mL clean dry volumetric flask add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
Further pipette 0.3ml of glyburide of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [8].

2.2.2.2. Assay procedure
20µL of the standard and sample solutions of glyburide was injected into the HPLC system and the

chromatograms were recorded. Amount of drug present in the tablet was calculated using the peak areas [9].

Amount of drug in tablet was calculated using following formula:

% Label claim = Asp/Ast X Dst/Dsp X A/Lc X P

Where,

Asp = Area for sample solution.

Ast = Area for standard solution.

Dst = Dilution factor for standard.

Dsp = Dilution factor for sample.

Lc = Label claim.

A = Average weight.

P = Potency

2.2.3. Validation

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical parameters. After development of RP-HPLC method for estimation of glyburide, validation of the method was carried out according to ICH guidelines

2.2.3.1. System suitability:

A Standard solution of glyburide working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from five replicate injections [10].

2.2.3.2. Linearity:

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range [11].

2.2.3.3. Specificity:

ICH defines specificity as the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc.

2.2.3.4. Precision:

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported.

Intra-day precision:

Intra-day precision also called as repeatability/method precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions.

Intermediate precision:

Intermediate precision of the analytical method was determined by performing method precision on another day by different analysts under same experimental condition. Assay of all six replicate sample preparations was determined and mean %assay value, standard deviation & %RSD was calculated [12].

2.2.3.5. Accuracy:

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

a) Standard addition method: To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

b) Percentage method: For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively [13].

The mean % recovery of the glyburide at each level should be not less than 95.0% and not more than 105.0%.

2.2.3.6. Limit of detection and limit of quantification:

The sensitivity of measurement of glyburide by use of the proposed method was estimated in terms of the Limit of Detection (LOD) and the Limit of Quantitation (LOQ). The LOD and LOQ were calculated by the use of the equations:

$$\text{LOD} = 3.3 \times \frac{\sigma}{s} \qquad \text{LOQ} = 10 \times \frac{\sigma}{s}$$

Where, σ is the standard deviation of intercept of calibration plot and S is the average of the slope of the corresponding calibration plot [14].

2.2.3.7. Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. For the determination of a method's robustness, deliberate change in the Flow rate was made to evaluate the impact on the method.

Effect of variation in flow rate:

A study was conducted to determine the effect of variation in flow rate. The standard and test solutions of 100% concentration was prepared & injected into the HPLC system by keeping flow rates 0.9 ml/min& 1.1 ml/min. The effect of variation of flow rate was evaluated.

Effect of variation in mobile phase composition:

A study was conducted to determine the effect of variation in mobile phase ratio by changing the ratio of organic solvent i.e., phosphate buffer: acetonitrile by ±2ml. Standard & test solutions of 100% concentration were prepared and injected into the HPLC system and the chromatograms were recorded. The retention times, tailing factors & %RSD values were calculated [15].

2.2.4. Stress degradation studies

Stress degradation studies were performed as per the ICH guidelines Q1A (R2) stability testing of new drug substances and products, using the proposed validated analytical method.

2.2.4.1 Acid degradation studies:

To 1ml of stock solution glyburide, 1ml of 2N HCl was added and refluxed for 30min at 60^0c. The resultant solution was diluted to obtain 30 µg/ml solutions and 20 µl was injected into the system and the chromatograms were recorded to detect the stability of sample.

2.2.4.2. Alkali degradation studies:

To 1ml of stock solution of standard drug and sample glyburide, 1ml of 2N NaOH was added and refluxed for 30min at 60^0c. The resultant solution was diluted to obtain 30 µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample [16].

2.2.4.3. Oxidation:

To 1ml of stock solution of standard drug and sample of glyburide, 1ml of 20% H_2O_2 was added and refluxed for 30min at 60^0c. The resultant solution was diluted to obtain 30 µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample [17].

2.2.4.4. Dry degradation studies:

The standard drug and sample solution of glyburide, was placed in oven at 105^0c for 6hrs to study. For HPLC study, resultant solution was diluted to obtain 30 µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample [18].

2.2.4.5. Photo stability studies:

The photochemical stability of the drug was also studied by exposing the 100 µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, resultant solution was diluted to obtain 30 µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to detect the stability of degraded sample [19].

2.2.5. Rat serum studies of glyburide

2.2.5.1. Animals

Male Albino rats (200 -250 g) were purchased from Sainath agencies, Hyderabad, Telangana. The animals were maintained on a 12 hr light–dark cycle (light on from 8:00 to 20:00 h) at ambient temperature of 25 ± 2 °C and 50 ± 15% relative humidity. Rats were fed with a commercial pellet diet and water *ad libitum*. They were fasted overnight prior to the experiment and during the experiment, the food is withdrawn but not the water. The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee [20].

2.2.5.2. Extraction procedures

Albino rats were administered with glyburide as mentioned above. Blood samples (0.2 ml) were collected through retro-orbital plexus under mild ether anesthesia at a time period of 0, 1, 2, 3, 4, 8, 12 and 24 hr following drug administration using sodium citrate (3.8%) as an anticoagulant. Plasma was separated immediately by centrifugation at 5000 rpm for 15 min and stored at -20° C until analysis. At the time of

analysis, the stored plasma was used for extraction as described above [21 & 22].

To 100 µL of plasma samples, 20 µL of internal standard (ketoconazole) from 100 µg/ml of working solution was added and 400 µL of methanol was added, the resultant solution was mixed for 2 minutes on cyclomixer at room temperature and centrifuged at 5000 rpm for 15 min and the supernatant was separated and the supernatant is evaporated to dryness on water bath, the residue was dissolved in 100 µL of mobile phase and after filtration through 0.2 µm syringe filter, 20 µL of the solution was used for the HPLC analysis [23 & 24].

Grouping of Animals:
Male albino rats were grouped as follows:
Group-I : Control (5% Sodium carboxy methyl cellulose 10ml/kg).
Group- II : Glyburide (10mg/kg for single day) [25].

3. RESULTS AND DISCUSSION

3.1. Selection of detection of wavelength
The standard solution of glyburide was scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was recorded. From the overlain spectrum, 238 nm was selected as the detection wavelength for the present study was depicted in figure (1).

The mobile phase was optimized to acetonitrile: phosphate buffer in proportion 60:40v/v respectively. The composition of mobile phase used for the achieved optimal separation of glyburide and internal standard (ketoconazole) without interference from the other components in plasma samples. Flow rate of 1ml/min was found to be ideal as it gave sharp peak.

3.2. Method development
Based on drug solubility and P^{ka} value following conditions has been used to develop the method estimation of glyburide in pharmaceutical dosage forms. The chromatogram was shown in figure 2.

Optimized chromatographic conditions:
Flow rate : 1.0 ml/min
Column : Kromasil ODS 3V (250×4.6mm, 5µ)
Detector wave length : 238nm
Column temperature : 30°C
Injection volume : 10L

Run time : 10 min
Diluent : Mobile phase used as a diluent.

3.3. Estimation of drug in marketed formulation by developed RP-HPLC method
The standard and sample solution of glyburide was injected into the HPLC system and the chromatograms were recorded. The retention time of glyburide was found to be 2.262 min (figure 2). The marketed formulation of glyburide (Diaβeta®, 5mg) was estimated. The amount of drug present in tablet was calculated.

The % assay of glyburide was found to be 99.50 %. Thus, % assay results were found to be within the limits i.e., 98-102% for the drug. Hence the developed method can be routinely used for the estimation of glyburide in the marketed formulations. The percentage of drug content (assay) %, of standard and test results are given in table 1 & 2 and figures 3 & 4.

3.4. Validation
3.4.1. System suitability
The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from five replicate injections, the results are given in table 3. All the system suitability parameters were satisfied, thus the method passed the system suitability test.

3.4.2. Linearity:
Serial dilutions of glyburide (10-50µg/ml) were injected into the column and detected at a wavelength set at 238 nm. The calibration curve was obtained by plotting the concentration vs. peak area. Concentration range 10-50µg/ml was found to be linear with r^2=0.9986. The linearity curve depicted in figure 5 and data represented in table 4.

3.4.3. Precision:
The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported. The results are shown in table 5 & 6.
The % RSD of peak areas of six samples was calculated. The method precision was performed on

glyburide formulation. The % RSD of the assay value for six determinations should not be more than 2.0%.

3.4.4. Accuracy:

The mean % recovery of the glyburide at each level should be not less than 95.0% and not more than 105.0%. The data should be represented in table 7 and figures depicted 6, 7 & 8.

3.4.5. Limit of detection and Limit of quantification:

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio (LOD). The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio (LOQ). LOD was found to be 0.02μg/ml & LOQ was found to be 0.05μg/ml for glyburide. Results were shown in below table 8.

3.4.6. Robustness:

Gyburide peak in the chromatogram passed the system suitability criteria. %RSD of peak areas of glyburide was not more than 2.0% for variation in mobile phase composition. From the above data, it was concluded that the method was robust. The results are shown in table 9.

3.5. Stress degradation studies

Glyburide undergoes degradation in acidic, oxidation, alkaline, dry heat and UV. More degradation was found for oxidation. As per ICH guidelines peak purity angle should be less than peak purity threshold. Hence, method of the analysis of glyburide in tablet dosage form shows that the degradation product doesn't interfere with the analytical determination. The results are shown in table 10 & 11, degradation of chromatograms are shown in 9, 10, 11, 12 & 13.

3.6. Rat serum studies:

The concentration of glyburide in extracted plasma was determined and the graph was plotted by taking time in hrs on X- axis and corresponding average concentration (μg/ml) values of drug in plasma samples on Y-axis. Our study showed that the glyburide showed has a significant effect in treated rats and compared with control group. The results are shown in figure 14 and data represented in table 12 & 13.

4.CONCLUSION

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for estimation of glyburide in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. The mobile phase acetonitrile: phosphate buffer (60:40) , Kromosil C 18 (250×4.6mm, 5μ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 238 nm. As the acetonitrile content was increased glyburide got eluted with good peak symmetric properties. The retention times for glyburide were found to be 2.262 min. From the present study we can conclude that there is significant effect of glyburide was observed in rat plasma studies for 24 hrs time period and compared with control.

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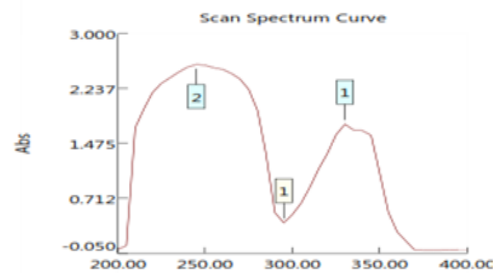
Titles of Figures:
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Figure 13: Chromatogram for U.V degradation studies
Figure 14: Graph showing time Vs concentration profile of glyburide and control

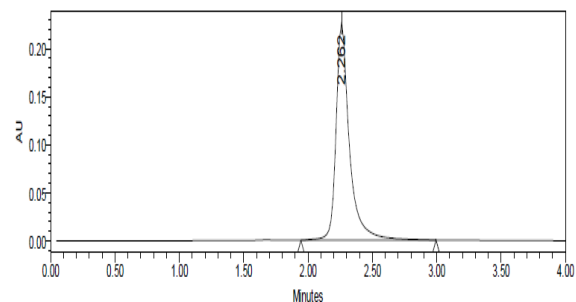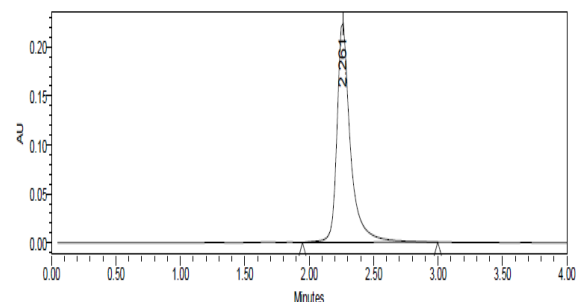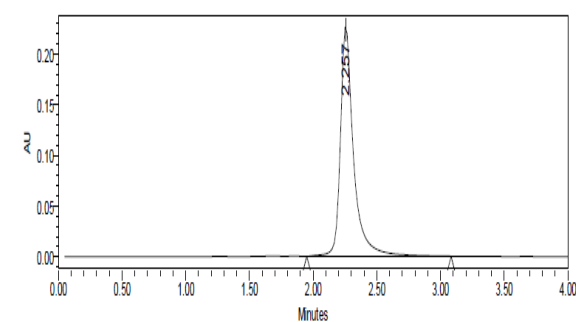Figure 1: Over line spectrum of glyburide

Figure 2: Chromatogram of glyburide

Figure 3: Chromatogram of standard

Figure 4: Chromatogram of test

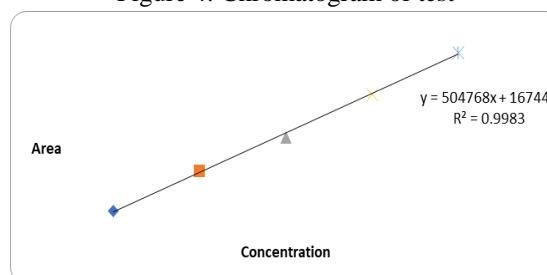$y = 504768x + 167444$
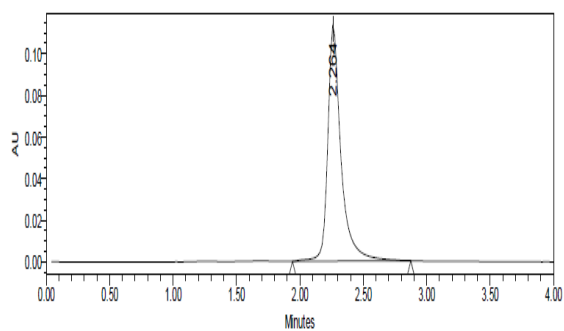$R^2 = 0.9983$

Figure 5: Linearity graph of glyburide

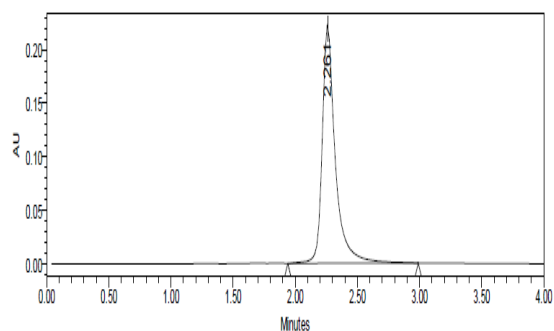Figure 6: Chromatogram of Accuracy 50%

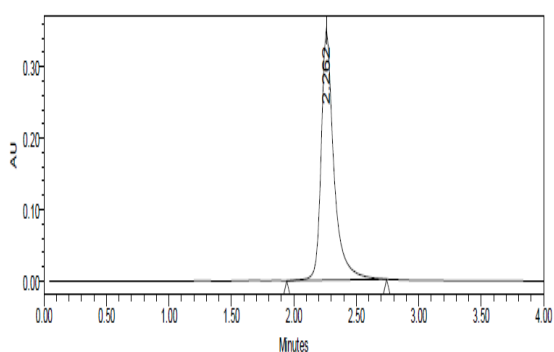Figure 7: Chromatogram of Accuracy 100%

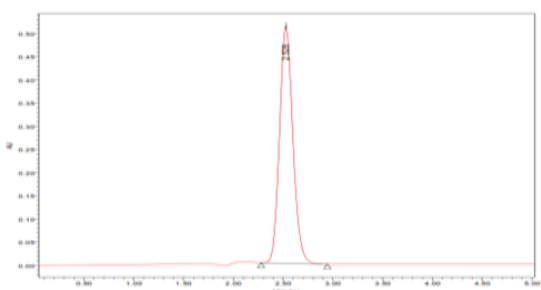Figure 8: Chromatogram of Accuracy 150%

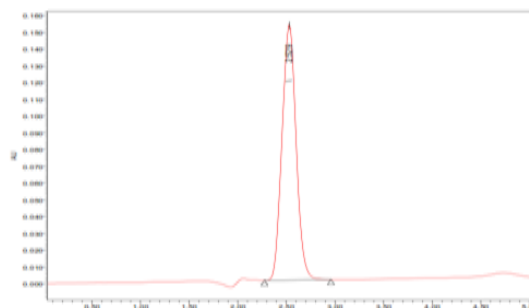Figure 9: Chromatogram for sample acid degradation

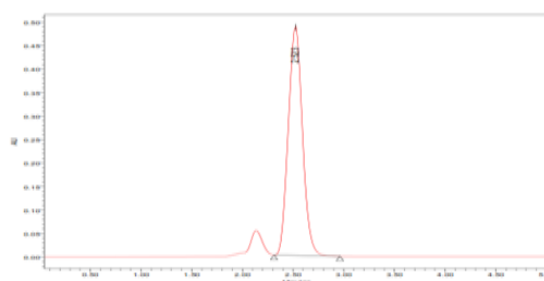Figure 10: Chromatogram for sample alkali degradation

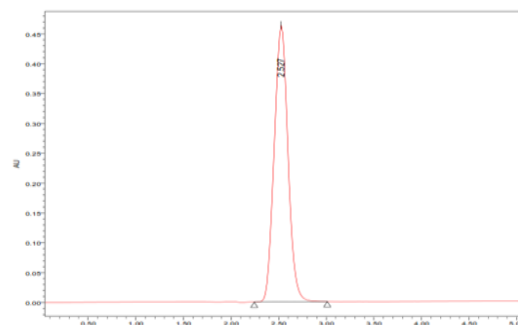Figure 11: Chromatogram for sample peroxide degradation

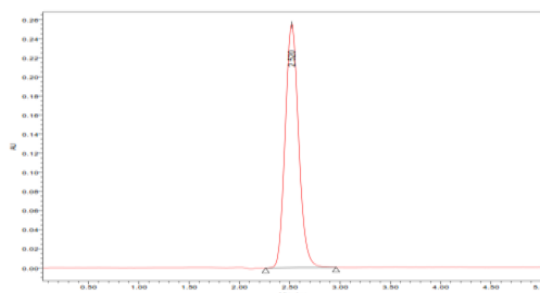Figure 12: Chromatogram for thermal degradation studies

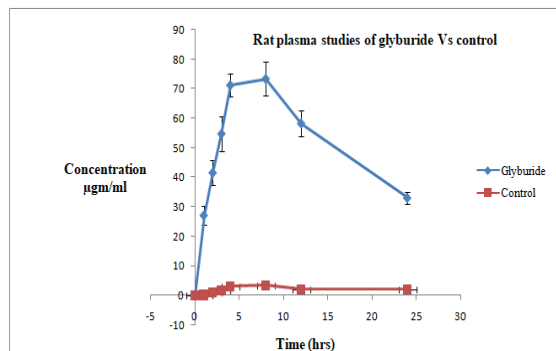Figure 13 : Chromatogram for U.V degradation studies

Figure 14: Graph showing time Vs concentration profile of glyburide and control

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Table 1: Peak results of standard & test chromatograms for Assay

Parameter	Standard	Test
	Glyburide	Glyburide
Retention time	2.261	2.257
Peak Area	1637535	1635162
USP Plate Count	2804	2810
Tailing Factor	1.5	1.5

Table 2: Assay results of glyburide

Parameters	Glyburide
Standard peak area	1637535
Test peak area (mean)	1635162

Average Weight	368.0 mg
Label claim	50 mg
% Purity of Standard	99.80
Amt obtained	49.75 mg
% Assay	99.50 %

Table 3: Results of system suitability test for glyburide

Injection	Retention time (Rt)	Peak Area	Plate count	Tailing factor
1	2.262	1631295	2804	1.52
2	2.210	1630511	2745	1.42
3	2.224	1636464	2810	1.50
4	2.220	1628557	2846	1.36
5	2.195	1635684	2812	1.52
6	2.205	1635162	2800	1.64
Mean	-	1632946	-	-
SD	-	3246.5	-	-
% RSD	-	0.19	-	-

Table 4: Preparation of working standard solutions for Linearity of glyburide

S.No	Concentration	Area
1	10ppm	682741
2	20ppm	1201305
3	30ppm	1627183
4	40ppm	2180552
5	50ppm	2716958
Correlation coefficient		0.998

Table 5: Intra-day precision data for glyburide

S.No.	Concentration (µg/ml)	Glyburide	
		Retention time (Rt)	Peak Area
1	30	2.270	1631295
2	30	2.267	1630511
3	30	2.262	1636464
4	30	2.269	1628557
5	30	2.264	1635684
6	30	2.269	1632047
Avg			1632426
SD			3065.2
%RSD			0.18

Table 6: Intermediate precision data for glyburide

S.No.	Concentration (µg/ml)	Day 1 Glyburide		Day 2 Glyburide	
		Retention on time	Peak Area	Retention on time	Peak Area

1	30	2.262	1639701	2.261	1631295
2	30	2.260	1645897	2.260	1630511
3	30	2.262	1640705	2.264	1636464
4	30	2.262	1637036	2.263	1628557
5	30	2.261	1638609	2.262	1635684
6	30	2.261	1639725	2.262	1632047
Avg			1640279		1632426
SD			3022.9		3065.2
%RSD			0.18		0.18

Table 7: Accuracy study of glyburide

% of concentration Level (n=3)	Conc found (µg/ml)	Conc Obtained (µg/ml)	%Recovery	Mean recovery	Statistical Analysis
50%	5	4.86	99.4	99.6	%RSD= 0.293
50%	5	4.87	99.99		
50%	5	4.82	99.54		
100%	10	9.68	100.6	100.2	%RSD= 0.84
100%	10	9.72	99.25		
100%	10	9.8	100.75		
150%	15	14.7	101.806	100.5	%RSD= 1.14
150%	15	14.81	101.313		
150%	15	14.89	99.605		

Table 8: LOD and LOQ results of glyburide

Glyburide		
Conc.(x) (µg/ml)	Peak Areas (y)	Statistical Analysis
10	682741	LOD: 0.02µg/ml
30	1627183	LOQ: 0.05µg/ml

Table 9: Robustness data for glyburide

Std. Replicate	Variation in flow rate		Variation in Mobile phase composition	
	Flow Rate	Flow Rate	Buffer: Acetonitrile	Buffer: Acetonitrile
	0.9ml/min	1.1ml/min	(70:30)	(50:50)
1	1635162	1605421	1637535	1625474
2	1634257	1612457	1639478	1624587
Mean	1634710	1608939	1638507	1625037
SD	639.9	4975.2	1373.9	627.2
%RSD	0.03	0.3	0.08	0.03
Retention time	2.995	1.827	1.821	3.936
Tailing factor	1.3	1.4	1.3	1.2
Theoretical plates	2396.0	2218	3353	2384

Table 10: Results of stress degradation studies of glyburide

S.no	Stress conditions	Time	%Assay	% Degradation
1	Acid degradation	30min	92.03 %	7.96 %
2	Base degradation	30min	93.09 %	6.90 %
3	Peroxide degradation	30min	92.03 %	7.96 %
4	Dry heat degradation	6hrs	93.44 %	6.55 %
5	U.V degradation	7days	92.58 %	7.41 %

Table 11: Peak purity analysis of glyburide

Stress conditions	Drug	Purity angle	Purity threshold
Acid degradation	Glyburide	0.250	1.339
Base degradation	Glyburide	0.252	0.378
Peroxide degradation	Glyburide	0.262	0.923
Dry heat degradation	Glyburide	0.180	0.255
U.V degradation	Glyburide	0.253	0.268

Table12: Concentration and time profile of glyburide after oral administration

Time (hr)	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean	SD
0	0	0	0	0	0	0	0
1	21.32	23.65	22.58	33.67	34.18	27.08	2.16735

2	38.25	39.67	40.15	45.86	43.61	41.508	2.92416
3	44.89	49.84	57.64	59.75	61.41	54.706	3.65129
4	69.18	76.15	70.78	69.43	70.39	71.186	3.98614
8	62.92	61.36	83.79	79.86	78.81	73.348	5.71479
12	48.89	58.95	55.35	67.37	60.59	58.23	3.95713
24	31.46	32.19	36.95	31.49	32.97	33.012	5.29438

Table13: Concentration and time profile of control after oral administration

Time (hr)	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean	SD
0	0	0	0	0	0	0	0
1	0.04	0.08	0.15	0.18	0.16	0.122	0.45
2	1.51	1.59	0.41	0.89	0.79	1.038	0.75
3	1.68	1.72	1.54	1.18	1.64	1.552	0.68
4	2.82	3.95	2.79	2.98	2.49	3.006	0.57
8	2.42	2.88	3.78	3.91	3.85	3.368	0.48
12	1.57	1.89	2.51	2.65	1.65	2.054	0.68
24	2.69	2.47	1.63	1.85	1.72	2.072	0.35