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ASSAY METHOD DEVELOPMENT AND VALIDATION OF GLYBURIDE ACTIVE PHARMACEUTICAL INGREDIENT BY REVERSE PHASE HPLC

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ABSTRACT

A simple, specific, linear, sensitive, precise, efficient Reverse phase HPLC method was developed and validated for the Assay determination of Glyburide Active Pharmaceutical ingredient (API.) The proposed method involves use of Ascentis Express C18; 7.5cm x 4.6mm, 2.7µcolumn and mobile phase comprising of mixture of 0.1%Triethyl amine with pH adjusted to 3 with Ortho phosphoric acid and Acetonitrile (45:55) v/v. The column temperature is maintained at 50ºC and flow rate at 1 ml/minute. Detector used is a UV (Ultra violet- Visible) detectorat wavelength of 254 nm. The retention time of active ingredient is about 2 minutes.The method was validated for specificity, system suitability, linearity, precision androbustness. Method is specific as no interference was observed at Retention time of Principle peak due to Glyburide. The linear regression analysis data for the calibration plots showed a linear relationship over the concentration range of 26.13-78.38 ppm for active ingredient. Percentage RSD of active ingredient was found to below 2%. Statistical analysis showed that the method is repeatable and selective for the Assay determination of GluburideActive Pharmaceutical ingredient (API.)

Keywords *– Glyburide, Reverse Phase HPLC, Validation, Assay*

1. INTRODUCTION

Glyburide is an oral blood glucose-lowering drug of sulfonylurea class. The chemical name of Glyburide is 1-[[p-[2-(5-chloro-o-anisamido)ethyl]phenyl]-sulfonyl]-3-cyclohexaylurea. Molecular weight is 493.99. Glyburide appears to lower the blood glucose acutely by stimulating release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islets. The mechanism by which glyburide lowers glucose during long term administration has not been clearly established. With chronic administration in Type II diabetic patients, the blood glucose lowering effect persists despite a gradual decline in insulin secretory response to drug[1].

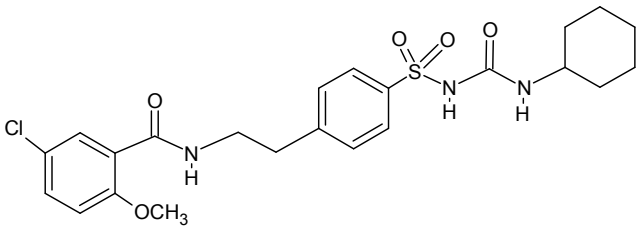

Fig.1 : Chemical Structure of Glyburide

Evaluation of available literature reveals that Literature survey reveals methods have been reported for assay determination of Glyburide drug substance as well as Glyburide containing finished drug product. However there was no method available with short run time for Assay evaluation[2-6]. So an attempt was made to develop and validate asimple, rapid reverse-phase high performance liquidchromatographic method with short run time for the estimation of Glyburidein active ingredient.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

GlyburideSample and standardwere gifted by commercial source. Triethyl amine (S.D fine), Acetonitrile (Runa chem), Ortho Phosphoric acid (S.D fine), water (Mili -Q) were obtained from Merck chemicals. Distilled water was prepared using a Milli-Q system (Millipore). Nylon syringe filters (0.45 µm) werefrom Millipore.

2.2 Equipment

Chromatographic separation was achieved using HPLC System (Agilent 1100 series) containing PDA detector. The output signal was monitored and processed using Chemstation Software®. The analytical balance used was from Sartorius, Model-BSA224SCW.

2.3 Selection of UV Wavelength

Structure evaluation of Active ingredient confirmed presence of multiple number of chromophores e.g. Unsaturated double bonds as part of Benzene ring as well as presence of Carbonyl group. Based on these structural features we decided to adopt UV technique for detection purpose.

Further literature survey of available data in Public domain confirms reported UV max to be at about 254 nm. Hence we decided to adopt the same as wavelength of detection for Assay determination of this Active ingredient.

2.4 HPLC Analytical Conditions

The proposed method involves use of Ascentis Express C18; 7.5cm x 4.6mm, 2.7µcolumn and mobile phase comprising of mixture of 0.1%Tritehtyl amine with pH adjusted to 3 with Ortho phosphoric acid and Acetonitrile (45:55) v/v. The column temperature is maintained at 50ºC and flow rate at 1 ml/minute. Detector used is a UV (Ultra violet- Visible) detector at wavelength of detection for Assay determination is 254 nm. The retention time of active ingredient is about 2 minutes.

Sample and standard preparationwas done in a Mobile phase.

2.5 Preparation of standard solution

Weighed accurately about 10 mg of Glyburideworking standard and into 10 ml volumetric flask. 5 ml of diluent was added, sonicated for 2 minutes and made up to volume with the diluent. Further 1.0 ml of this solution was diluted to 20.0 ml with diluent.

2.6 Preparation of Sample solution

Weighed accurately about 10 mg of Glyburide working sample and into 10 ml volumetric flask. 5 ml of diluent was added, sonicated for 2 minutes and made up to volume with the diluent. Further 1.0 ml of this solution was diluted to 20.0 ml with diluent.

2.7 Method validation

The developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, Validation ofAnalytical Procedures: Q2(R1)[7], for the parameters like Specificity, system suitability, linearity and range, precision (repeatability), and robustness.

2.8 System suitability

System suitability testing is essential component of all the analytical techniques used in Pharmaceutical industry, and they are based on principle that equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. These tests need to be established for each individual analysis to confirm validity of testing.

System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

The systemsuitability test performed using the standard solution and results were recorded to find theadequate percentage relative standard deviation for area, retention time, Tailing factor and theoretical plates.

2.9 Specificity

No blank interference as observed at retention time of peak due to Glyburide.

2.10 Precision

The precision of an analytical method expresses the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Method precision was evaluated by injecting six different sample preparation.

The assay of these samples was determined. Precision of the method was evaluated by calculatingthe % RSD.

2.11 Linearity

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration (amount)of analyte in the sample. The linearity of detector response was determined by preparing a series of solution of the working standards of active ingredient over the range of 50% to 150% of targeted concentration. These solutions were injected into thechromatographic system and response area was recorded.

2.12Robustness

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.

A study was performed todetermine the effect of variation in the temperature. Standard solution prepared as per the test method and was injectedinto the HPLC system at 45°C temperature.

3. RESULTS AND DISCUSSION

The UV max of glyburide as reported in literature is 254 nm, so for quantification HPLC with UV detector was employed. To begin with development mobile phase comprising of water and Acetonitrile in the ratio of 50:50 v/v and zorbax SB C8 15cm*4.6mm*5 was used. Column oven temperature was 25ºC and flow rate of 1 ml was kept and 10 µl of 1000 ppm of glyburide in Acetonitrile was injected. It was observed that peak eluted at about 4 minutes and had tailing. As zorbax SB C8 column showed tailing Inertsil C18 column with 25cm x 4.6mm x 5µ was used, still peak shape was not improved. Then the diluent was changed to Acetonitrile: Water 50:50 v/v still tailing was observed.

Since glyburide showed tailing 0.1%TEA was introduced in Mobile phase and pH was adjusted to 3 with Ortho phosphoric acid. It was observed that peak tailing was reduced but the peak shape was broad and Retention time of peak was about 6 minutes. To reduce the peak broadening mixture of Acetonitrile and methanol was employed and sample was injected. Peak broadening was reduced and retention was about 4 minutes. As it is an Assay method so to shorter the run time it was decided to employ fused core column. So Ascentis Express C18 column with dimension of 7.5cm x 4.6mm, 2.7µ was used. It was observed that peak shape was good. Further optimization of chromatographic parameters was done to improve peak shape and reduce run time.

The finalized chromatographic conditions are shown in Table-1.

System suitability parameters proved that the proposed method suits for the Assay determination of estimation of Glyburide.

Chromatogram for assay determination of Glyburidewas found satisfactory on Ascentis Express C18; 7.5cm x 4.6mm, 2.7µcolumn. Drug peak was found symmetrical as observed from Tailing factor of the proposed method was satisfactory.

Representative chromatograms are shown in Fig.2 and 3.

System suitability parameters were given in Table-2. UV detection was set at 254.

The data of precision were given in Table-3 and 4. The percentage RSD value was less than 2%.

The data for similarity factor was provided in Table -5.

Sensitivity of the method was good and also linearity was observed over a wide concentration range of 26.13-78.38 ppm for Glyburide.

The correlation coefficients for Glyburide was found to bewithin the limits for r^2=0.9999. The linearity data were given in Table-6 and Fig.4.

Results of Robustness study are provided in Table no. 7.

So the developed method was said to be validated. By performing deliberate variation in temperature, it was observed that there were no marked changes obtained in the chromatograms, which demonstrated that the method developed is robust.

Table 1: Optimized chromatographic conditions for proposedHPLC method for Glyburide

Parameter	Chromatographic condition
Instrument	Agilent 1100 series
Column	Ascentis Express C18,7.5cm x 4.6mm, 2.7µ
Detector	Agilent UV detector 254 nm
Mobile phase	%Triethyl amine with pH adjusted to 3 with Ortho phosphoric acid and Acetonitrile (45:55) v/v
Diluent	Mobile phase
Flow rate	1 ml/minute
Column Temperature	50ºC
Injection volume	5 µl
Retention time of peak due to Levomilnacipran	About 2 minutes
Mode of Chromatography	Isocratic

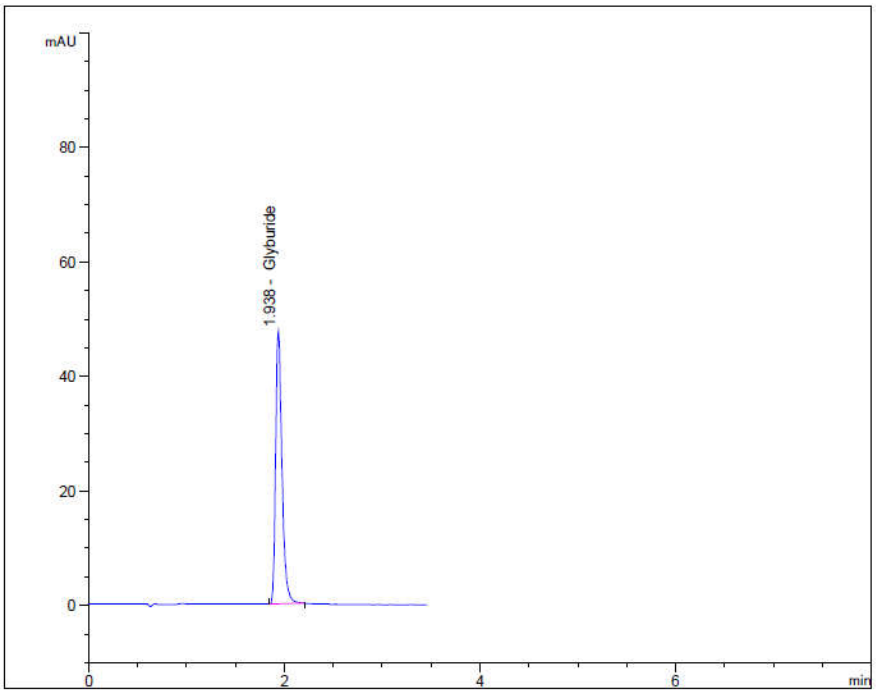

Fig. 2 : HPLC Chromatogram of Standard solution of Gluburide

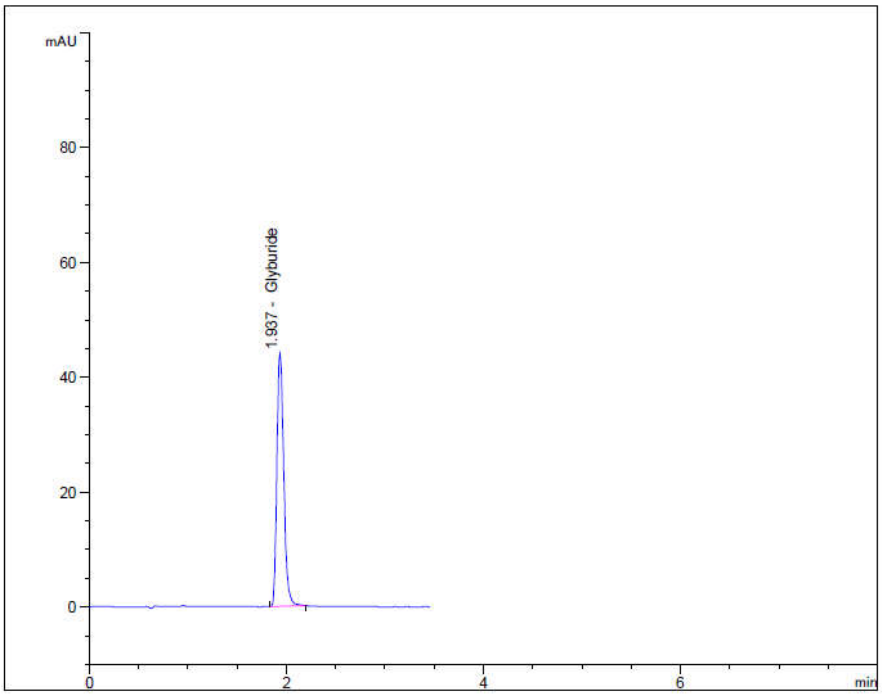

Fig. 3 : HPLC Chromatogram of Sample solution of Gluburide

Table 2: Results of System suitability

Parameter	For peak due to Glyburide
Retention time	About 2 minutes
Tailing Factor	1.32
Theoretical plates	4524

Table 3: Results of System precision

Replicate no. of Standard solution	Retention time	Area
1	1.938	215.40
2	1.941	214.90
3	1.942	217.10
4	1.943	216.30
5	1.940	217.00
6	1.940	216.50
Mean	1.94	216.20
Standard Deviation	0.00	0.88
% Relative Standard deviation	0.1	0.4

Acceptance criteria: Percentage RSD should not be more than 2%.

Table 4: Results of Method Precision

Sample No.	Assay
1	101.19
2	101.34
3	100.7
4	99.39
5	100.98
6	99.93
Mean	100.48

Table 5: Results of Similarity Factor

Standard Preparation	Weight	Retention time	Peak Area Response
Mean of Peak area response of 6 replicates of System precision	10.45	----------	216.20
Standard Preparation B	10.26 mg	1.942	214.30
Similarity Factor			0.99

Acceptance criteria: Similarity factor should be between 0.98 tom 1.02.

Table6: Results of linearity data

Linearity Level	Concentration (in ppm)	Mean Peak Area Response
L1	**26.13**	**107.3**
L2	**41.80**	**172.1**
L3	**52.25**	**216.2**
L4	**62.70**	**258.9**
L5	**78.38**	**326.8**

Inj: Injection

Figure.4: Calibration curves for Glyburide

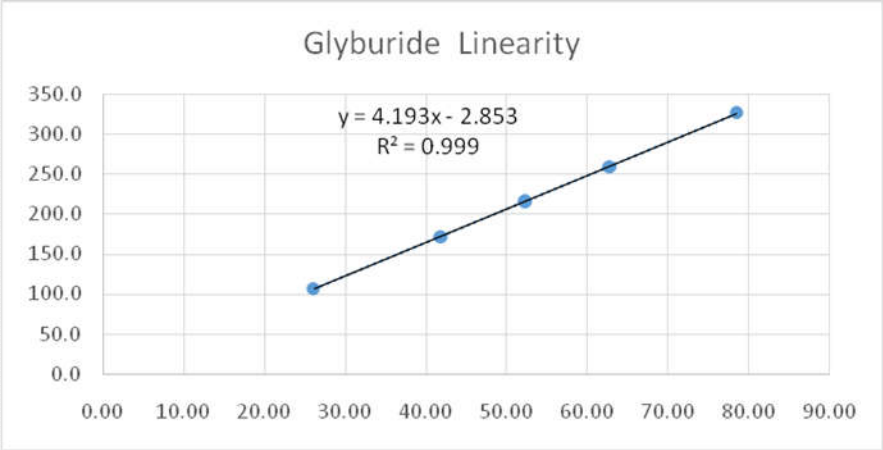

Table 7: Results of Robustness study

Parameter	Retention time of Glyburide Peak	Peak area response of Glyburide Peak
ROBUSTNESS for temperature changed to45 C	1.87	233.95

4. CONCLUSION

The Reverse Phase -HPLC method developed for Assay estimation of Glyburide is simple, specific, linear, sensitive, preciseand efficientand is suitable for its intended purpose.

The method developed has shorter run time, thus ensuring optimum utilization of the HPLC system. Further the proposed method is on HPLC with shorter run time obtained generally with advance and costly techniques. Also shorter run time ensures lesser consumption of solvents in turn reducing further cost per analysis and also generating lesser solvent waste. The shorter run time enables analysis of multiple batches in short duration thus enhancing the out-put of batch analysis.

The method was validated as per ICH guidelines, showing satisfactory data for all the method validation parameters tested. Hence, the proposed method can be employed for assessing the Assay determination of Glyburide.

5. ACKNOWLEDGEMENT

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