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STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF GLASDEGIB IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Glasdegib in pharmaceutical dosage form. Chromatographic separation of Glasdegib was achieved on Waters Alliance-e2695, by using Waters Symmetry C_{18}, 150x4.6mm, 3.5µm column and the mobile phase containing 0.1% TEA adjusting pH=2.5 with OPA & Acetonitrile in the ratio of 90:10% v/v. The flow rate was 0.5 ml/min; detection was carried out by absorption at 268nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Glasdegib is NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Glasdegib.

Keywords: Stability, HPLC and Glasdegib.

INTRODUCTION

Glasdegib is a small-molecule hedgehog signaling inhibitor selected under the group of the benzimidazoles. In early research, benzimidazoles attracted large interest as they represented a class of inhibitors with a low molecular weight, potent inhibitory activity and lacking unstable functionality. The great lipophilicity of this group of compounds brought interest to further modification. Glasdegib is chemically 1-[(2*R*, 4*R*)-2-(1*H*-benzimidazol-2-yl)-1-methylpiperidin-4-yl]-3-(4-cyanophenyl)urea with molecular formula $C_{21}H_{22}N_6O$ shown in Fig No:1 and molecular weight is 374.448 g/mol [1,2]. Glasdegib is a potent and selective inhibitor of the hedgehog signaling pathway that acts by binding to the smoothened (SMO) receptor. The hedgehog signalling pathway is involved in maintenance of neural and skin stem cells. In this pathway, the binding of specific ligands to the trans membrane receptor patched (PTCH1) allows the activation of the transcriptional regulators GL11, GL12 and modulation of the gene expression through SMO-mediated signaling. The aberrant activation of the hedgehog pathway is thought to be implicated in the pathogenesis of chronic myeloid leak .As per literature survey no HPLC methods are reported for the estimation of Glasdegib.[5,6,7] The objective of the study is to develop accurate precise repeatable and reproducible method for simultaneous estimation of Glasdegib and to validate according to ICH guidelines and to perform the forced degradation studies.[8,9,10]

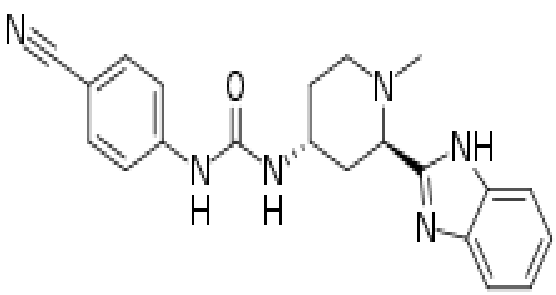

Fig. 1: Structure of Glsdegib

MATERIALS AND METHODS

The reference sample of glasdegib was obtained as a gift samples and the tablet containing glasdegib 25mg was procured from local market. Water (HPLC grade) from Rankem and acetonitrile (HPLCgrade),orthophosphoricacid (ARgrade) methanol(Rankem), triethylamine (Rankem) from Merck Limited, 0.45 µm Nylon filter was from Zodiac life sciences were used.

Instrumentation

Waters HPLC 2695 system equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer, PG Instruments T60 with special bandwidth of 2 mm and 10 mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of glasdegib solutions, Electronics Balance-Denver pH meter -BVK enterprises, India Ultrasonicator-BVK enterprises.

Chromatographic conditions

Waters symmetry C_{18} (150.x.4.6.mm, 3.5µ) column. Temperature was maintained ambient, mobile phase used was Acetonitrile and0.1%TEA (10:90v/v) and flow rate was maintained at 0.5 ml/min. Diluents used throughout the method was Water: Acetonitrile (50:50 v/v) and the run time was 7 minutes. All the samples and mobile phase were degassed for 30 minutes and filtered by ultrasonic filtration by using 0.45 µm Nylon (N66) 47 mm membrane filter. Detection was carried out at 268nm using PDA detector with an injection volume of 10µL. By using the above optimized conditions method was developed.

Preparation of Buffer

Buffer: (0.1%TEA)

Take 1ml of Tri ethyl amine and adjust its pH=2.5 with Ortho Phosphoric Acid and dissolved in 1litre of HPLC grade water. Filter through 0.45µ nylon filter.

Preparation of Mobile Phase

Mobile phase was prepared by mixing 0.1% TEA and Acetonitrile taken in the ratio 90:10. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram

Preparation of standard stock solution

Accurately weigh and transfer 7mg of Glasdegib working standard into a 20 ml clean dry volumetric flask add Diluents and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents. (35ppm of Glasdegib).

Sample Solution Preparation

Accurately weighed and transfer equivalent to 7mg of Glasdegib sample into a 20mL clean dry volumetric flask add Diluents and sonic ate it up to 30mins to dissolve, and centrifuge for 30min. to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron Injection filter (Stock solution). Further pipette 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. (35ppm of Glasdegib).

METHOD VALIDATION

Specificity

Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drug was specific

SYSTEM SUITABILITY

Tailing factor for the peak due to Glasdegib in Standard solution should not be more than 2.0
Theoretical plates for the Glasdegib peak in Standard solution should not be less than 2000.

Formula for Assay

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where

AT= average area counts of test (sample) preparation.

AS= average area counts of standard preparation.

WS= Weight of working standard taken in mg.

DS= Dilution of working standard in ml.

DT= Dilution of test (sample) in ml.

WT= Weight of test (sample) taken in mg.

P= Percentage purity of working standard

LC= Label Claim mg/ml.

Linearity

The linearity of the proposed method was determined by quantitative dilution of the standard solution of glasdegib to obtain solution in concentration range of 3.50µg/ml-

52.50µg/ml. A graph of peak area versus concentration in µg/ml was plotted for the drug. The slope intercept and correlation coefficient of regression line.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ represent the concentration of analyte that would yield to signal-to-noise ratio of 3 for LOD and 10 for LOQ. LOD and LOQ were calculated using following formula,

$$LOD = 3.3\ \sigma / S$$

$$LOQ = 10\ \sigma / S$$

where, σ = standard deviation of response (peak area) and

S = average of slope of the calibration

Method precision

In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 35ppm of Glasdegib).

System precision

System precision is checked by using standard chemical substance to ensure that the analytical system is working properly. In this peak area and % of drug of six determinations is measured and % RSD should be calculated.

Accuracy

The accuracy of this method was performed at three different levels (50%, 100%, 150%), by the addition of a known amount of standard to the sample at each level. Each level was repeated three times (n=3).

Robustness

Robustness is the measure of optimized method capacity to remain unaffected by small, but deliberate variations in method parameters such as mobile phase flow rate (±0.2 mL/min), wavelength nm (±1 nm), and column oven temperature (±1°C).

Solution Stability

The solution stability of glasdegib in diluents was determined by storing sample

Solution in tightly capped volumetric flask at room temperature for 24hrs. The amount of

Glasdegib was measured at different time intervals like 6, 12, 18 and 24 hrs and results obtained were compared with freshly prepared of glasdegib solution.

RESULTS AND DISCUSSION

Chromatographic conditions

Waters symmetry C_{18} (150 x 4.6.mm, 3.5µparticle size) column. Temperature was maintained ambient, mobile phase used was : Acetonitrile and0.1%TEA (10:90v/v) and flow rate was maintained at 0.5 ml/min. Diluent used throughout the method was Water: Acetonitrile (50:50 v/v) and the run time was 7 mins. All the samples and mobile phase were degassed for 30 mins and filtered by ultrasonic filtration by using 0.45 µm Nylon (N66) 47 mm membrane filter. Detection was carried out at 268nmnm using PDA detector with an injection volume of 10 µL. By using the above optimized conditions method was developed .Shown in Fig. 2 and Table 1.

Specificity

Specificity of an analytical method is ability to measure specifically the analyte of interest without interference from blank and known impurities. For this purpose blank chromatogram, standard chromatogram and sample chromatogram were recorded. The chromatogram of blank shows no response at the retention times of drugs which confirms the response of drug was specific**.**

Linearity

The linearity of the proposed method was determined by quantitative dilution of the standard solution of glasdegib to obtain solution in concentration range of 3.50µg/ml-52.50µg/ml. A graph of peak area versus concentration in µg/ml was plotted for the drug. The slope, intercept, and correlation coefficient of regression line were determined. Shown in Table 2.

Method precision

In method precision, a homogenous sample of single batch should be analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and calculate the % RSD

The precision of the instrument was checked by repeatedly injecting (n=6) .solutions of 35ppm of Glasdegib). Table 3.

System precision

System precision was determined by measuring the peak area of six replicate injections of standard solution. The value of %RSD was found to be <2, which ensure the analytical system is working properly. The results of system precision are tabulated in Table No:4**.**

Accuracy

The accuracy of this method was determined by calculating percent recovery of glasdegib in formulation at three different levels (50%, 100%, and 150%). The % recovery obtained was found to be in the range of 100.36to100.54%. The accepted limits of mean recovery is 100.25% and obtained results were within the acceptable range, which indicate recovery values were good, affirming the accuracy of the developed method. The results are summarized in Table 5.

Robustness

The method was found to be robust when minor changes were made in optimized chromatographic conditions such as oven temperature (±5°C), mobile phase flow rate (±0.1 mL/min), and ratio of mobile phase (±5mL). It was observed that there was no marked change in analytical data of the drugs which indicates good reliability during normal usage. The results are shown in Table 6.

Solution Stability

The solution stability of glasdegib in diluents was determined by storing sample solution in tightly capped volumetric flask at room temperature for 24hrs. The amount of glasdegib was measured at different time intervals like 6, 12, 18 and 24 hrs and results obtained were compared with freshly prepared of glasdegib solution

Acceptance Criteria: The %RSD values for the assay of the solution stability experiments were calculated and should not more than 2.0%.

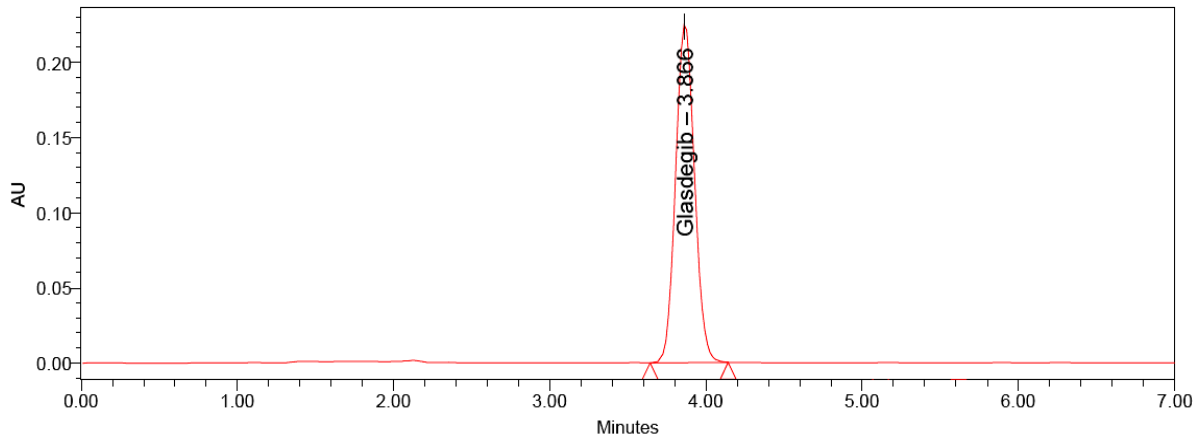

Fig. 2: Optimized chromatogram of Glasdegib

Table 1: Optimized Chromatographic conditions of Glasdegib

Parameters	Observation
Instrument used	Waters HPLC with auto sampler and PDA detector.
Injection volume	10µL
Mobile Phase	Acetonitrile and 0.1% TEA of pH=2.5 adjusted with OPA 10:90
Column	Waters Symmetry $C_{18,}$ 150x4.6mm, 3.5µ
Detection Wave Length	268 nm
Flow Rate	0.5 ml/min
Runtime	7min
Temperature	Ambient(25° C)
Mode of separation	Isocratic mode

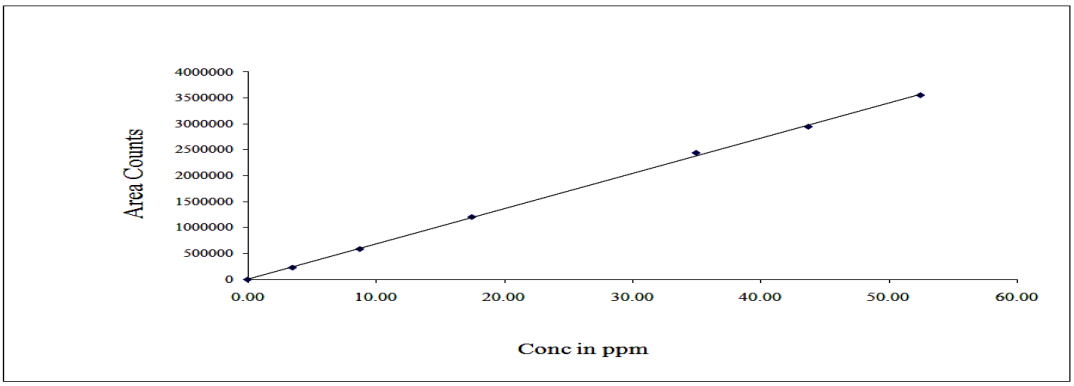

Fig. 3: Calibration curve of Glasdegib

Table 2: Results of linearity of Glasdegib

S.NO	Glasdegib	
	Conc.(µg/ml)	Peak area
1	3.50	229582
2	8.75	587786
3	17.50	1207676
4	35.00	2447475
5	43.75	2951689
6	52.50	3559389
Regression equation	y = 68070x +3461	
Slope	68070.61	
Intercept	3461.17	
R^2	0.99977	

Table 3: Method Precision of Glasdegib

Injection	Area for Glasdegib
Injection-1	2416385
Injection-2	2416795
Injection-3	2418957
Injection-4	2463125
Injection-5	2415316
Injection-6	2417854
Average	2424738.7
Standard Deviation	18846.799
%RSD	0.78

Table 4: System Precision of Glasdegib

Injection	Area for Glasdegib
Injection-1	2413679
Injection-2	2413255
Injection-3	2468957
Injection-4	2495875
Injection-5	2469856
Injection-6	2413625
Average	2445874.7
Standard Deviation	36738.471
%RSD	1.7

Table 5: Accuracy results of Glasdegib

%Concentration(at specification Level)	Area	Amount of sample Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1364852	25.1	17.5	100.36	100.25
100%	2463157	50.1	35	99.95	
150%	3746275	75.2	52.5	100.54	

Table 6: Robustness results of Glasdegib

Parameter	Glasdegib				
	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change (ml/min)	Less flow(0.4ml)	4.975	2315648	1.04	3579
	Actual(0.5ml)	3.845	2463157	1.04	3545
	More flow(0.6ml)	3.161	2567841	1.05	3492
Organic Phase change	Less Org (9:91)	4.876	2369854	1.11	3493
	Actual(10:90)	3.847	2463975	1.12	3528
	More Org(11:89)	3.068	2563145	1.05	3496

CONCLUSION

The developed HPLC method for the estimation of selected drug is simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming. The sample recoveries were in good agreement with their respective label claims and they suggested non interference of formulation excipients in the estimation and can be used in laboratories for the routine analysis of selected drugs. Since the system validation parameters of HPLC method used for estimation of selected drug in pure and have shown satisfactory, accurate and reproducible results (without any interference of excipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose. The present work concluded that stability indicating assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the placebo and degradation products. Hence these can be used for routine analysis of Glasdegib.

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