STABILITY INDICATING ASSAY METHOD DEVELOPMENT AND VALIDATION OF GLASDEGIB BY RP-HPLC

S. Sravani*[1], P. Anusha[2] and B. Tarakeswari Bai[3]
Department of Chemistry, Maris Stella College, Vijayawada, AP, India.

Abstract
A validated stability indicating RP-HPLC method for glasdegib was developed by separating its degradation products on a C_{18} (150x4.6mm, 3.5µm) waters symmetry column using 1ml Tri Ethyl Amine of pH=7.0 adjusted with Ortho Phosphoric acid and acetonitrile in simple isocratic at a flow rate of 1.0 ml/min. The column effluents were monitored by a photodiode array detector set at 268nm. The method was validated in terms of specificity, linearity, accuracy, precision, detection limit, quantification limit and robustness. Forced degradation of Glasdegib was carried out under acidic, basic, peroxide, reduction, thermal, photo and hydrolysis conditions. The proposed method is validated as per ICH Q2 (R1) guidelines.

***Index Terms*-Glasdegib, Method Validation, RP-HPLC.**

INTRODUCTION

Glasdegib is an FDA [1] approved cancer drug [2, 3] developed by Pfizer. It is a small molecule inhibitor of sonic hedgehog [4, 5], which is a protein [6] over expressed in many types of cancer [7, 8]. It inhibits the sonic hedgehog receptor smoothened (SMO), as do most drugs in its class.

Four phase II clinical trials are in progress. One is evaluating the efficacy [9] of glasdegib in treating myelofibrosis [10, 11] in patients who were unable to control the disease with ruxolitinib [12,13,14]. Another is a combination trial of glasdegib with decitabine, daunorubicin, or cytarabine for the treatment of acute myeloid leukemia [15, 16]. The third is for the treatment of myelodysplastic syndrome [17, 18] and chronic myelomonocytic leukemia [19, 20]. The fourth administers glasdegib to patients at high risk for relapse [21, 22] after stem cell transplants in acute lymphoblastic [23, 24] or myelogenous leukemia.

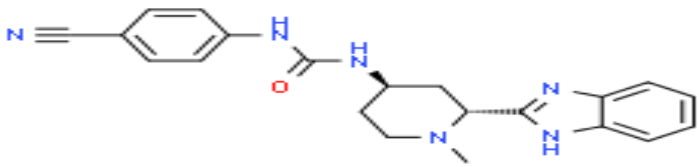

Fig 1:Structure of Glasdegib

MATERIALS AND REQUIREMENTS

Instrument:
HPLC, make: Waters alliance e-2695 chromatographic system consisting of quaternary pump, PDA detector-2998 and chromatographic software Empower-2.0 was used.

Reagents:
Acetonitrile (HPLC grade), Ortho Phosphoric acid (HPLC grade), Water (HPLC grade), Tri ethyl amine.
Mobile Phase Preparation:
Mobile Phase-A: Acetonitrile .
Mobile Phase-B:Ortho phosphoric acid
Diluent Preparation: Mix Mobile Phase-A and Mobile phase-B in 20:80 v/v.

Optimization of mobile phase:

Different trials have done, different buffers and different mobile phases were used to develop the method. In all trials peaks are not separated properly. Finally for the proposed method all the peaks are separated and the entire suitability conditions are within the limit.

Chromatographic conditions:

The chromatographic system was carried out in symmetry C_{18}, (150x4.6mm, 3.5µm) column. Flow rate was maintained at 1.0ml/min injection volume is 10µl and sample and column temperatures are ambient. Wavelength detection is maintained at 268nm.

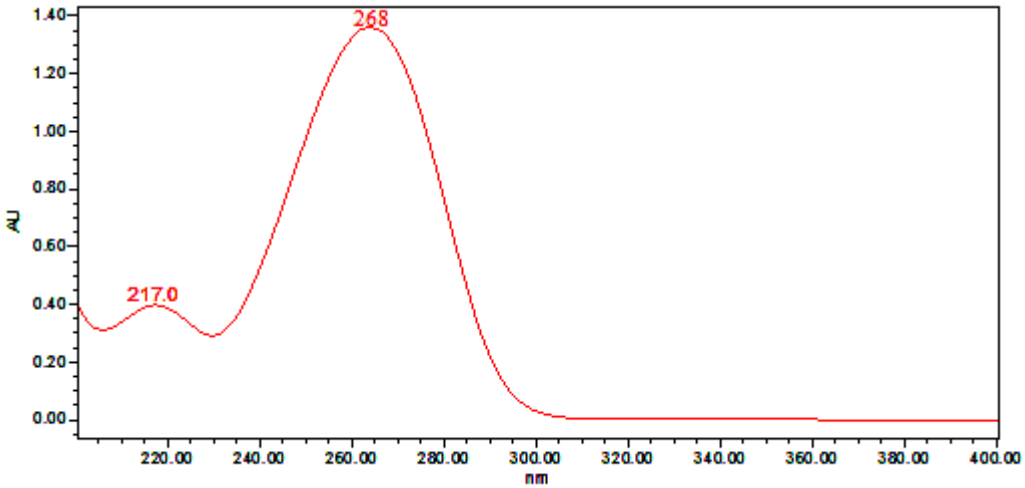

Fig. 2: PDA Spectrum

Standard Solution:

Weigh accurately 50mg of Glasdegib. These working standards were transferred into a 100ml volumetric flask, add 70ml of diluent sonicated for 20min to dissolve the contents make up to the mark with diluent. Further dilute 5ml of above solution to 50ml with diluent.

Sample Solution:

Transfer 350mg of Glasdegib equivalent weight of sample into a 100ml volumetric flask diluted to volume with diluent. Further dilute 5ml of above solution to 50ml with diluents. Filter through 0.45µ nylon syringe filter.

RESULTS AND DISCUSSION

Validation of proposed method

The method was validated for parameters like system suitability, specificity, linearity, LOD, LOQ, Precision, Accuracy, Robustness and Ruggedness as per ICH guidelines [17-18].

System Suitability

The HPLC system was stabilized for 60min to get a stable baseline. Six replicate injections of satandard solution were injected. The results are summarized below table 1.

Table 1: System Suitability data

System Suitability parameter	Acceptance criteria	Drug Name
		Glasdegib
% RSD	NMT 2.0	0.98
USP Tailing	NMT 2.0	1.06
USP Plate Count	NLT 3000	3600

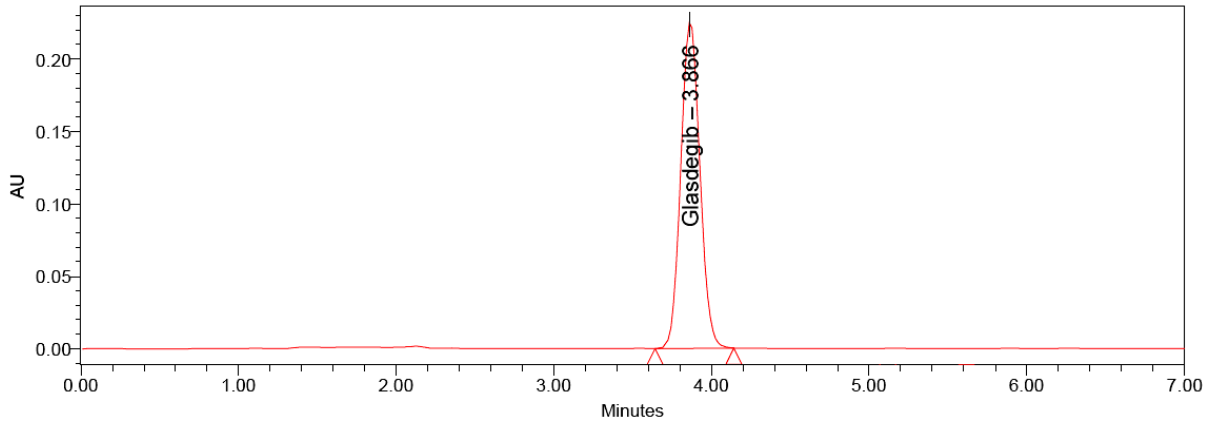

Fig. 4: Chromatogram for system suitability

Specificity

There is no interaction of peaks in blank and standard, sample, placebo chromatograms in the total runtime of chromatogram. Hence it proves that method is specific.

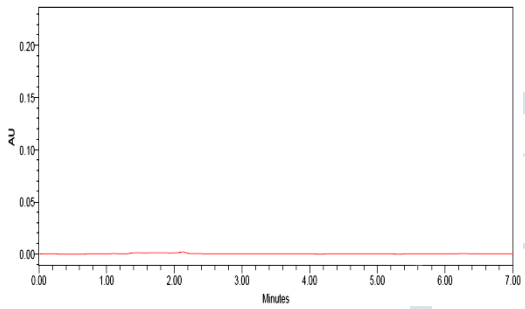

Fig. 5: Chromatogram for Blank

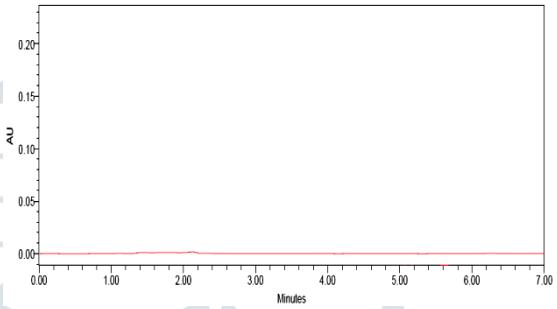

Fig. 6: Chromatogram for Placebo

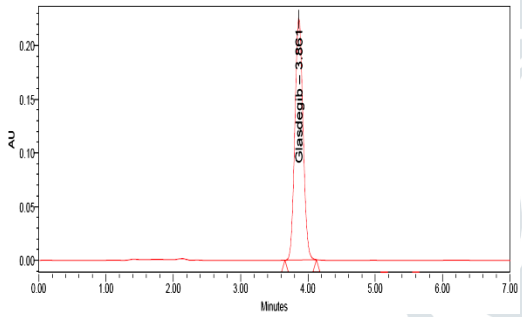

Fig.7: Chromatogram of standard

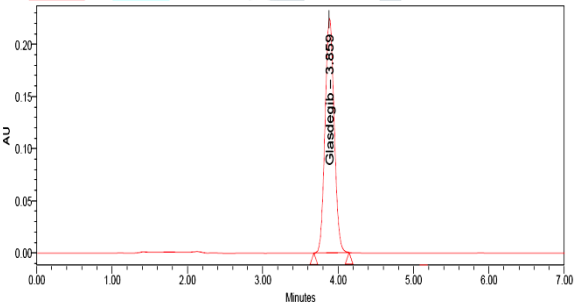

Fig. 8: Chromatogram for sample

Linearity

The linearity was observed in the concentration range of 3.5-52.5µg/ml of Glasdegib. The regression equation is Y= 68070x+3461.17 and correlation coefficient was found to be 0.9997.

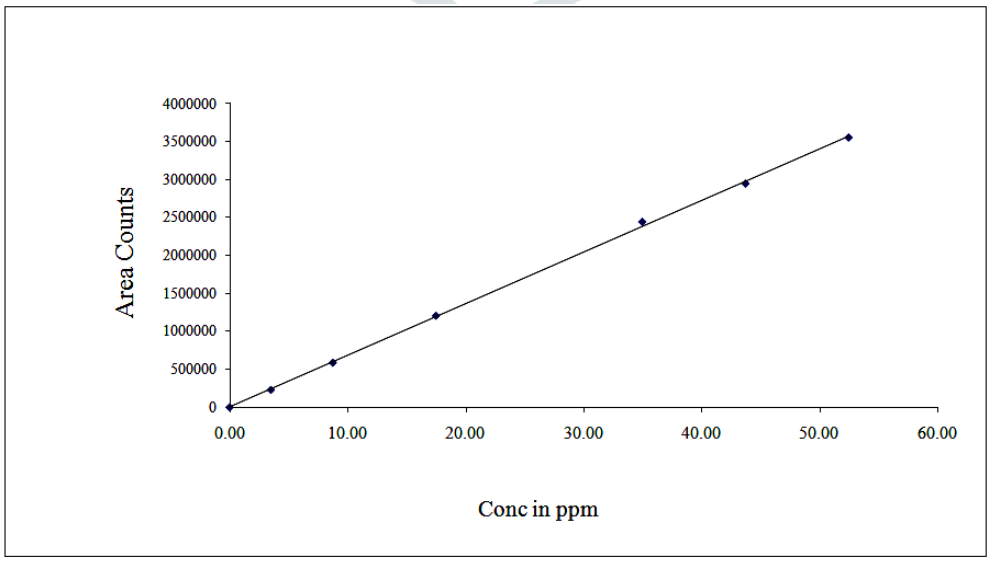

Fig.9:Linearity plot for Glasdegib

Accuracy

Injecting samples in triplicate at 50%, 100% and 150% of the target concentration. The recovery results should be NLT 95% and NMT 105%.

Table 2: Accuracy results of Glasdegib

S. No.	% Level	% Recovery	Avg. % Recovery
1	50	99.6	99.5
2		99.5	
3		99.4	
4	100	99.6	99.9
5		100.3	
6		99.7	
7	150	100.0	99.9
8		100.2	
9		99.4	

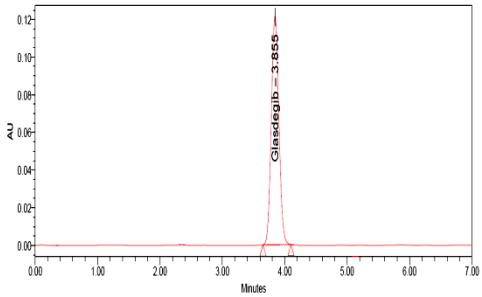

Fig. 10: Chromatogram for Accuracy 50%

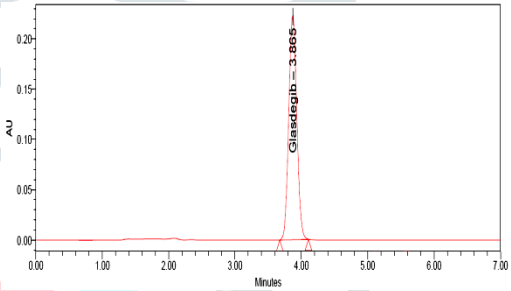

Fig. 11: Chromatogram for Accuracy 100%

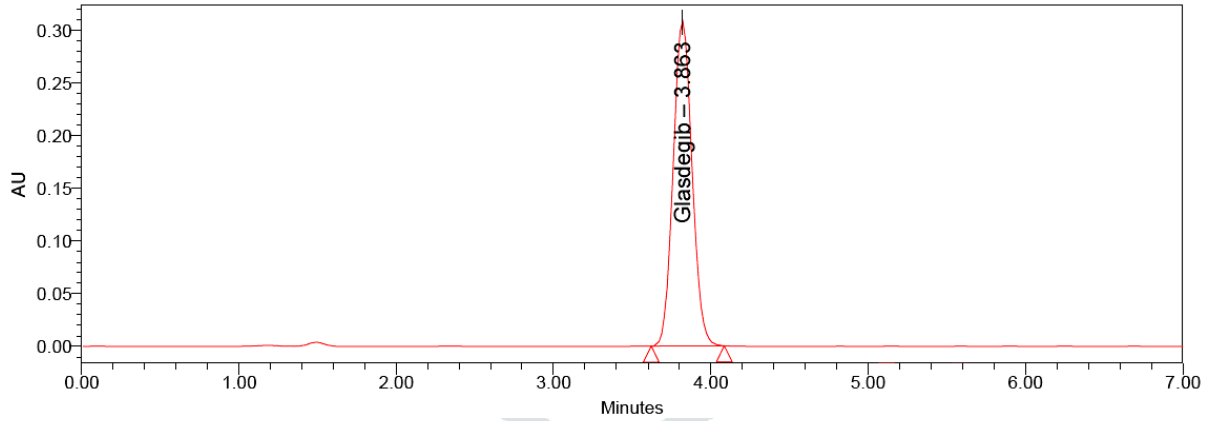

Fig. 12: Chromatogram for Accuracy 150%

Precision

Method Precision

Method Precision was investigated by the analysis of six separately prepared samples of the same batch. From these six separate sample solution was injected and the peak areas obtained used to calculate mean and percentage RSD values.

Intermediate Precision

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different HPLC system, analyst, column. The value of percentage of RSD was below 2% exhibits the ruggedness of the developed method. The results are given in table 4.

Table 4: Method Precision and Intermediate Precision results

Analyte	Amount present	Intra-day Precision	Inter-day Precision
		% RSD	
Glasdegib	350	0.39	0.06

Robustness

Robustness of the method was found to be %RSD should be less than 2%. Slightly variations were done in the optimized method parameters like flow rate (±20%), Organic content in mobile phase (±10%). The results are given in table 5.

Table 5: Robustness results

Drug Name	Flow Plus	Flow Minus	Organic Plus	Organic Minus
	% RSD			
Glasdegib	0.07	0.31	0.33	0.09

Stability

The stability of Glasdegib in solution was determined by sample solution stability initial to 24h at different time intervals at room temperature. There is no significant deviation of purity.

Table 6: Results of solution stability

Stability	% Lable claim Glasdegib	% Deviation Glasdegib
Initial	99.8	0.00
6h	99.8	0.00
12h	100.2	0.40
24h	99.6	-0.20

CONCLUSION

All the factors lead to the conclusion that the proposed method is simple, specific, accurate, precise and reproducible. Statistical analysis proves that the method is suitable for the analysis of Glasdegib.

ACKNOWLEDGEMENT

We are thankful to our parents, lecturers and my friends for supporting this research work.

REFERENCES

1. Zuckerman, Diana M.; Brown, Paul; Nissen, Steven E. Medical Device Recalls and the FDA Approval Process. Archives of Internal Medicine, 2011: 171 (11): 1006–11.
2. Martens, UM, ed. 11 5-Azacytidine/Azacitidine. Small molecules in oncology. Recent Results in Cancer Research. 184. Heidelberg: Springer, 2010: pp. 159–170.
3. Robak, Tadeusz; Korycka, Anna; Robak, Ewa. Older and New Formulations of Cladribine. Pharmacology and Clinical Efficacy in Hematological Malignancies. Recent Patents on Anti-Cancer Drug Discovery, 2005: 1 (1): 23–38.
4. Echelard Y, Epstein DJ, St-Jacques B, Shen L, Mohler J, McMahon JA, McMahon AP. Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity, Cell. 1993: 75 (7): 1417–30.
5. Riddle RD, Johnson RL, Laufer E, Tabin C. Sonic hedgehog mediates the polarizing activity of the ZPA. Cell. 1993: 75 (7): 1401–16
6. Ritchie DW.. Recent progress and future directions in protein-protein docking. Current Protein & Peptide Science, 2008: 9 (1): 1–15.
7. Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical Research, 2008: 25 (9): 2097–116.
8. Jayasekara H, MacInnis RJ, Room R, English DR. Long-Term Alcohol Consumption and Breast, Upper Aero-Digestive Tract and Colorectal Cancer Risk: A Systematic Review and Meta-Analysis. Alcohol and Alcoholism, 2016: 51 (3): 315–30.
9. Colquhoun D. Binding, gating, affinity and efficacy. The interpretation of structure–activity relationships for agonists and of the effects of mutating receptors. British Journal of Pharmacology, 1998: 125 (5): 923–948.
10. Efferi, Ayalew. Primary myelofibrosis: 2014 update on diagnosis, risk-stratification, and management. American Journal of Hematology, 2014: 89 (9): 915–925.

11. Trow, TK; et al. A 71-Year-Old Woman With Myelofibrosis, Hypoxemia, and Pulmonary Hypertension, Chest. 2010: 138 (6): 1506–10.
12. Mesa RA. Ruxolitinib, a selective JAK1 and JAK2 inhibitor for the treatment of myeloproliferative neoplasms and psoriasis. IDrug. 2010: 13 (6): 394–403
13. Harrison, C.; Kiladjian, J. J.; Al-Ali, H. K.; Gisslinger, H.; Waltzman, R.; Stalbovskaya, V.; McQuitty, M.; Hunter, D. S.; Levy, R.; Knoops, L.; Cervantes, F.; Vannucchi, A. M.; Barbui, T.; Barosi, G.. JAK Inhibition with Ruxolitinib versus Best Available Therapy for Myelofibrosis. New England Journal of Medicine, 2012: 366 (9): 787–798.
14. Hobbs, GS; Rampal RK. JAK2 Mutations and JAK Inhibitors in the Management of Myeloproliferative Neoplasms. Contemporary Oncology, 2015: 7 (1): 22–28.
15. Dohner, H; Weisdorf, DJ; Bloomfield, CD. Acute Myeloid Leukemia. The New England Journal of Medicine, 2015: 373 (12): 1136–52.
16. Kayser, S; Levis, MJ, FLT3 tyrosine kinase inhibitors in acute myeloid leukemia: clinical implications and limitations, Leuk Lymphoma, 2014: 55 (2): 243–255,
17. List A, Kurtin S, Roe DJ, et al.. Efficacy of lenalidomide in myelodysplastic syndromes. N. Engl. J. Med, 2005: 352 (6): 549–57.
18. Kantarjian HM, O'Brien S, Shan J, et al. Update of the decitabine experience in higher risk myelodysplastic syndrome and analysis of prognostic factors associated with outcome. Cancer 2007: 109 (2): 265–73.