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Photochemically Assisted LC–MS Method Development and Validation for Stability-Indicating Determination of Glasdegib in Rat Plasma

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Abstract

A simple, precise, and cost-effective LC–MS method was successfully developed for the determination of Glasdegib in rat plasma. The method optimization was carried out by systematically varying key chromatographic parameters, including flow rate, injection volume, analyte concentration, and mobile phase composition, to achieve optimal sensitivity and resolution. A C18 Hypersil BDS column (150 mm × 4.6 mm, 3.5 µm particle size) was used for chromatographic separation, offering effective peak shape and repeatability. The mobile phase consisted of a mixture of acetonitrile and 0.1% formic acid in the ratio of 30:70 (v/v), ensuring proper ionization and improved analyte response. The flow rate was maintained at 0.7 mL/min, allowing adequate interaction between the analyte and stationary phase while minimizing analysis time. Detection was carried out at a wavelength of 250 nm, where Glasdegib exhibited significant absorbance, enhancing method sensitivity. Under these optimized conditions, Glasdegib showed a retention time of 3.115 minutes, indicating a rapid and efficient analytical run suitable for high-throughput analysis. By assessing metrics including linearity, accuracy, precision, specificity, and robustness, the created approach was validated in compliance with accepted practices. With high correlation values, the data showed outstanding linearity over the chosen concentration range. Studies on accuracy and precision verified the method's dependability and repeatability. Overall, the proposed LC–MS method is robust, rapid, and economical, making it highly suitable for routine quantitative analysis of Glasdegib in bulk drug substances, pharmaceutical formulations, and biological samples such as rat plasma.

Keywords: Glasdegib, LCMS, method development and validation, benzimidazole, formulation design

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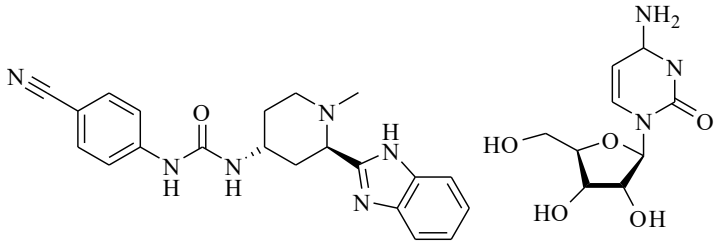
INTRODUCTION

The main objective of the progress of the LC-MS method for glasdegib is to accomplish a standardized chromatographic method that provides excellent elemental or isotopic nature of a sample, to elucidate the chemical structures of molecules, separation, sensitivity, and reproducibility [1–3]. This involves selecting appropriate chromatographic conditions including column type, temperature, mobile phase composition, flow rate, sample run time, and detection wavelength [4, 5]. High-performance liquid chromatography (HPLC) is commonly used as the LC component in LC–MS, typically operated in reverse-phase mode for pharmaceutical analysis due to its efficiency and compatibility with mass spectrometric detection [2–5].



Glasdegib Cytarabine

Figure 1. Chemical structures of glasdegib and cytarabine.

LC–MS offers high sensitivity, selectivity, and precision, making it a widely trusted and validated technique for bioanalytical applications [3, 4]. Glasdegib, for instance, can be reliably determined by LC–MS once separated from potential interferences, with quantification supported by robust detector settings and validated methods [1, 2].

Glasdegib6-10 is a benzimidazole moiety heterocyclic compound having urea scaffold chiral active molecule. Its molecular formula is C21H22N6O and molecular weight is 374 gr/mol. The chemical structure of glasdegib is shown in Figure 1. It is used as medicine for the treatment of AML (acute myeloid leukemia) in adults who are more than 75 years old. Generally, it is administered through oral and is used in combination with low-dose Cytarabine (chemical structure is shown in Figure 1) [4].

The main objective of the progress of the LCMS method for glasdegib is to accomplish a standardized chromatographic method that provides excellent elemental orisotopic nature of a sample, to elucidate the chemical structures of molecules, separation, sensitivity, and reproducibility[11–16]. This consists choosing proper chromatographic conditions, column type, temperature, mobile phase composition, flow rate, sample run time and detection wavelength. In LC-MS, HPLC is typically utilized as LC. The normal phase mode or reverse phase mode of adsorption is the basis for separation in HPLC [17–20]. The pharmacological molecules are easily detected liquid chromatography mass spectroscopy (LC-MS) because of its great sensitivity, selectivity, and precision, hence, it is a commonly employed and trusted analytical method. Glasdegib is determined by LC-MS soon after the drug is segregated from impeding substances and the drug molecule is quantified with the help of a proper detector [21].

Here we would like to create an authentic LC-MS method for glasdegib quatification in pharmaceutical formulations. Further, we wish also validation studies such as accuracy, precision, linearity, specificity, and robustness of the method with the reference to international standards [22, 23]. By developing a constructive analytical technique for the measurement of glasdegib, the reported technique will advance the body of knowledge by facilitating quality control in the pharmaceutical sector.

EXPERIMENTAL

Chemicals

The reference sample was provided as Glasdegib samples from Biocon, Bangalore. The Merck Chemical Division in Mumbai provided HPLC-grade acetonitrile, HPLC-grade methanol, and all other chemicals. Throughout the investigation, HPLC-grade water from a Milli-Q water purification system was utilized.

METHOD DEVELOPMENT

Instrumentation

Chromatography was carried out using a SCIEX QTRAP 5500 mass spectrometer with class AB SCIEX software and a Waters 2695 HPLC equipped with a high-speed auto sampler, column oven, and degasser.

Preparation Standard Stock Solutions

120 ng/ml of Glasdegib Stock Solution: After transferring 6 mg of Glasdegib into a 10 ml volumetric flask, the diluent (0.1 ml to 10 ml) was added to dilute the solution. Fill a 10 ml volumetric flask with 0.2 ml of the aforementioned solution, then top it off with diluents.

Internal Standard Stock Solution (120 ng/ml) is prepared by completely dissolving 6 mg of gilteritinib in water, transferring it into a 10 ml volumetric flask, and then further diluting it by adding 0.1 ml to 10 ml of diluent.

- *Preparation of Standard Solution (30ng/ml of Glasdegib):* We have transferred standard stock solution (500 µl) to 2 ml centrifuged tube. Plasma (200 µl), internal standard (500 µl), CH3CN (300 µl), and diluent (500 µl) were added to the prior solution and centrifugation was done for 20 min. Later, the supernatant liquid has been filtered and collected in HPLC vial.
- *Linearity solution preparation:* We have made linearity solutions with concentrations ranging from 7.5 ng to 60 ng per ml of Glasdegib.
- *Extraction procedure:* Plasma (200 µL) and diluent (500 µL) were mixed well in round bottom flask. Later, Acetonitrile (300 µL) was added to get precipitation. The entire reaction mixture was centrifuged for 15 to 20 minutes at 4000 RPM. Filter the supertentent solution, collect the resulting compound in HPLC vial.
- *Buffer Preparation:* Transferred 1ml formic acid into 1 lt water and stir well. Filtered through 0.22µ membrane filter paper.
- *Standardized Chromatographic Conditions:* The spectra of this solution was obtained by scanning under the UV range between from 200 to 400 nm. Other parameters were also optimized and the results are shown in Table 1. Mass spectrometer conditions are provided in the Table 2.

Table 1. Chromatographic conditions for determination of Glasdegib.

S. No	Parameter	Condition
1	Stationary phase	C18, Hypersil BDS, 150mm x 4.6mm, 3.5µm
2	Mobile phase	Mix Acetonitrile and 0.1% Formic acid in the ratio of 30+70
3	Diluent	Mobile phase
4	Injection Volume	10 µL
5	Column Temperature	Ambient
6	Flow rate	1 mL/min
7	Sample Temperature	Ambient
8	Run time	7 min
9	Detection Wavelength	250 nm

Table 2. Mass spectrometer conditions of Glasdegib.

S.N.	Parameter	Condition
1	Collision energy	15 V
2	Ion spray voltage	5500 V
3	Source temperature	550oC
4	Drying gas temperature	120-250°C
5	Collision gas	Nitrogen
6	Drying gas flow stream	5 mL/min
7	Declustering potential	40 V
8	Entrance potential	Entrance potential: 10V
9	Exit Potential	7 V
10	Dwell time	1 sec

Analytical Method Validation

Linearity: Linearity can be determined either directly or proper mathematical transformation. It refers to an analytical approach that provides through the observed concentrations of analytes in test samples and theoretical concentrations of analytes in measured samples. The calibration curve of linearity is given by the graph drawn between area under curve vs. concentration [11, 12].

Accuracy

If the experimental values are similar to the theoretical components, then the method is considered to accurate. Recovery exploration studies were performed with standard drug solutions having 80%, 100% and 120% levels. If the results are within the acceptance criteria i.e. 100% ± 20%, then the method will have enough capacity to detect analytes in samples [13,14].

Limit of Detection (LOD) and Limit of Quantifica tion (LOQ)

LOD is identified under the given experimental parameters and settings. It has generally lower concentration of analyte that can be studied more accurately and precisely under given set of experimental circumstances. This procedure is equipped with more quantitatively by changing outcomes that depending on the tool, and technique[15, 16].

Robustness

To evaluate the durability of sample, we have to change the procedure parameters like retention time and concentration. The robustness of method was checked by altering the wavelength by 2 nm and the flow rate by 0.2 ml/min.

RESULTS AND DISCUSSION

System Suitability

To know system suitability, a set of reference standards tested before to the analytical run. The results are provide in Table 3. %CV of Glasdegib and istd area ratio was identified as 0.73 which is under accepted criteria i.e. should be ≤ 5.00 %. Therefore, system is suitable for analyzing Glasdegib drug sample. Chromatogram curve of system suitability is present in Figure 2 [17, 18].

Specificity and Screening of Biological Matrix

We have checked specificity and screening of biological matrix by analyzing blank rat plasma samples at the retention time of both Glasdegib and ISTD. Table 4 presents the results, which are under LLOQ. i.e. ≤20.00 for analyte and ≤5.00 for blank ISTD.

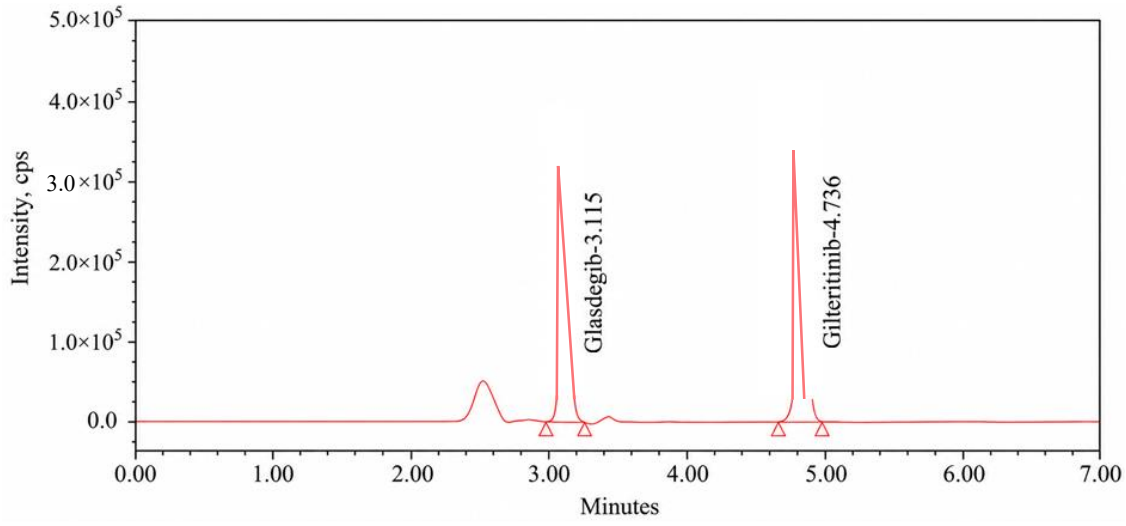


Figure 2. Chromatogram of system suitability.

Table 3. Optimization results system suitability.

Samlple Name MQC (30 ng/ml)	Analyte area (cps)	Analyte RT (min)	ISTD Area (50 ng/ml)	ISTD RT (min)	Area ratio
MQC-1	3.227×105	3.115	3.454×105	4.736	0.9343
MQC-2	3.263×105	3.120	3.445×105	4.729	0.9472
MQC-3	3.217×105	3.113	3.472×105	4.733	0.9266
MQC-4	3.244×105	3.116	3.460×105	4.737	0.9376
MQC-5	3.251×105	3.110	3.480×105	4.734	0.9342
MQC-6	3.232×105	3.113	3.439×105	4.738	0.9398
Mean	3.239×105	3.115	3.458×105	4.735	0.9366
SD	0.017	0.003	0.016	0.003	0.00685
%CV	0.52	0.11	0.45	0.07	0.73

Table 4. Specificity and screening of biological matrix of Glasdegib.

S.N.	Sample ID	Intensity(cps)		% Interference		Pass/Fail
		Drug	*ISTD*	*Drug*	*ISTD*	
1.	Std Blk 1	0	0	0	0	Pass
2.	LLOQ 1 (3 ng/ml)	0.345×105	3.474×105	0	0	Pass
3.	Std Blk 2	0	0	0	0	Pass
4.	LLOQ 2 (3 ng/ml)	0.373×105	3.432×105	0	0	Pass
5.	Std Blk 3	0	0	0	0	Pass
6.	LLOQ 3 (3 ng/ml)	0.336×105	3.428×105	0	0	Pass
7.	Std Blk 4	0	0	0	0	Pass
8.	LLOQ 4 (3 ng/ml)	0.341×105	3.411×105	0	0	Pass
9.	Std Blk 5	0	0	0	0	Pass
10.	LLOQ 5 (3 ng/ml)	0.357×105	3.439×105	0	0	Pass
11.	Std Blk 6	0	0	0	0	Pass
12.	LLOQ 6 (3 ng/ml)	0.353×105	3.469×105	0	0	Pass

Table 5. Linearity results of Glasdegib.

Final conc. in ng/ml	RES	Area response ratio
0	0	0.0
7.50	0.885	0.255
15.00	1.643	0.475
22.50	2.493	0.724
30.00	3.241	0.937
37.50	4.056	1.169
45.00	4.862	1.400
60.00	6.467	1.881
Slope	0.0306	
Intercept	0.02422	
R2 Value	0.99975	

Linearity

We have tested the linearity of Glasdegib molecule having the concentration ranging between 0 to 60 ng/ml. The results are documented in Table 5. The linearity was accomplished by plotting concentration vs area under the curve, and it is given in Figure 3. Linearity regression coefficient was found to be $R^2 = 0.99975$ which was under acceptable limit [19].

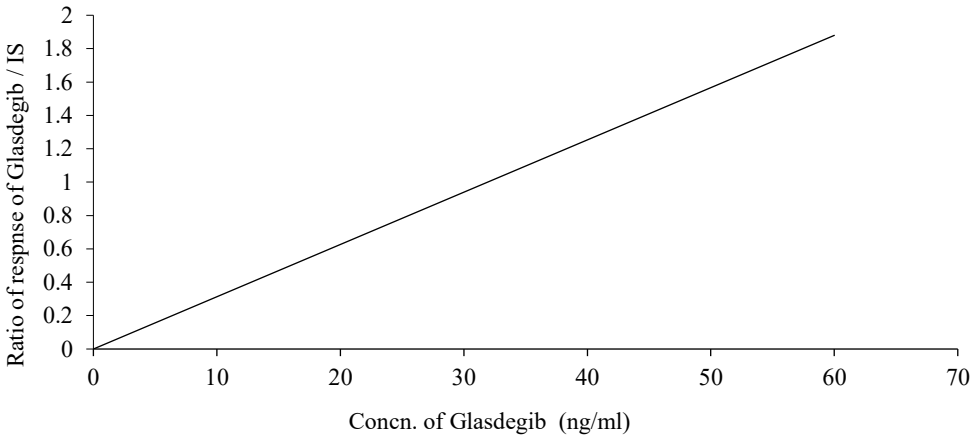


Figure 3. Calibration plot of linearity for Glasdegib.

Table 6. Matrix effect results of Glasdegib.

S.N.	Plasma lot No.	HQC	LQC
		Nominal concentration(ng/ml)	
		45.0	*15.0*
		Analyte peak area	
1.	Lot 1	4.840×105	1.622×105
		4.858×105	1.610×105
		4.833×105	1.631×105
2.	Lot 2	4.851×105	1.620×105
		4.829×105	1.603×105
		4.842×105	1.612×105
3.	Lot 3	4.836×105	1.630×105
		4.863×105	1.606×105
		4.849×105	1.631×105
4.	Lot 4	4.861×105	1.614×105
		4.854×105	1.619×105
		4.871×105	1.608×105
5.	Lot 5	4.864×105	1.627×105
		4.843×105	1.610×105
		4.860×105	1.632×105
6.	Lot 6	4.835×105	1.613×105
		4.856×105	1.629×105
		4.834×105	1.606×105
n		18	18
Mean		4.849×105	1.618×105
SD		0.013	0.010
%CV		0.26	0.62
% Mean Accuracy		99.93%	99.91%
No. of QC Failed		0	0

Matrix effect: Rat plasma that had been chromatographically filtered was used to evaluate the planned matrix effect approach. After determining the matrix effect values, acceptable limits were compared. Table 6 presented the findings. LQC and HQC samples made from various biological matrix lots should have a mean accuracy of backcalculated concentration between 85.00 and 115.00 percent, which is consistent with our observed findings [20].

Precision and accuracy: Six replicates containing Glasdegib at four distinct QC levels were examined in order to assess the intra-assay precision and accuracy. Four tiers of QC samples were analyzed on four separate runs to evaluate the inter-assay precision. After determining the values, they were compared to their recognized bounds (Table 7). Accuracy within 85–115% of the actual values is one of the requirements for data acceptability. The accuracy is within ±15% of the relative standard deviation (RSD) [21].

The precision and accuracy numbers, which should be between 80 and 120% for accuracy and less than 20% of RSD, did not, however, fall within recognized bounds.

The %RSD values for intra- and interday variability were frequently low, i.e., 1% in both cases, indicating that the suggested method for estimating at all concentration levels is the best.

LOD and LOQ: As tabulated in Table 8, the LOD and LOQ values used to determine the proposed method's sensitivity were 0.01 and 0.03, respectively. Chromatogram of LOD and LOQ are expressed in Figure 4 and Figure 5, respectively [22, 23].

Table 7. Precision and accuracy results of Glasdegib.

Acquisition Batch ID	**Date**	**HQC**	**MQC**	**LQC**	**LLQC**
		Nominal Concentration (ng/ml)			
		45.0	**30.0**	**15.0**	**3.0**
		Analyte peak area			
		4.877×105	3.235×105	1.626×105	0.328×105
		4.856×105	3.250×105	1.614×105	0.325×105
		4.864×105	3.224×105	1.609×105	0.314×105
		4.848×105	3.258×105	1.604×105	0.321×105
		4.835×105	3.220×105	1.614×105	0.316×105
		4.859×105	3.231×105	1.641×105	0.321×105
n		6	6	6	6
Mean		4.857x105	3.236×105	1.618×105	0.321×105
SD		0.014	0.015	0.013	0.005
% CV		0.29	0.46	0.87	1.64
% Mean Accuracy		99.97%	99.91%	99.91%	99.10%

Table 8. LOD and LOQ results of Glasdegib.

Drug name	**LOD (S/B) value**	**LOQ (S/B) value**
Glasdegib	3	10

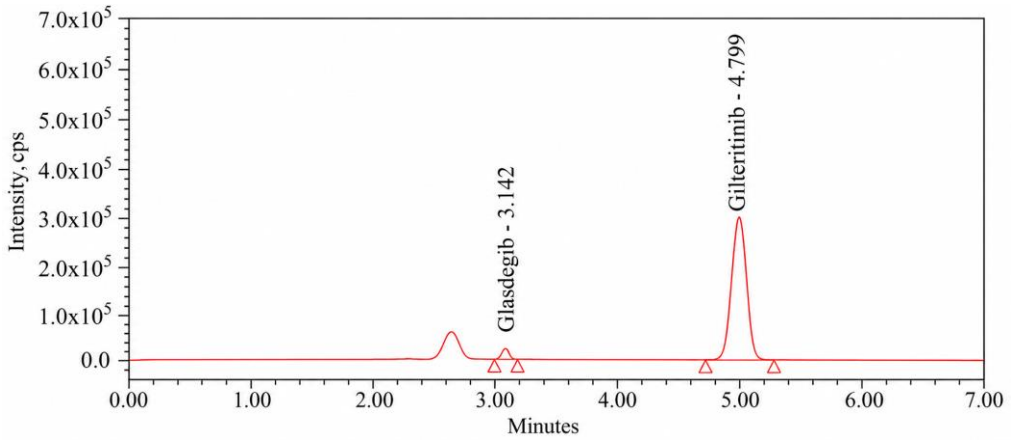


Figure 4. LOD chromatogram.

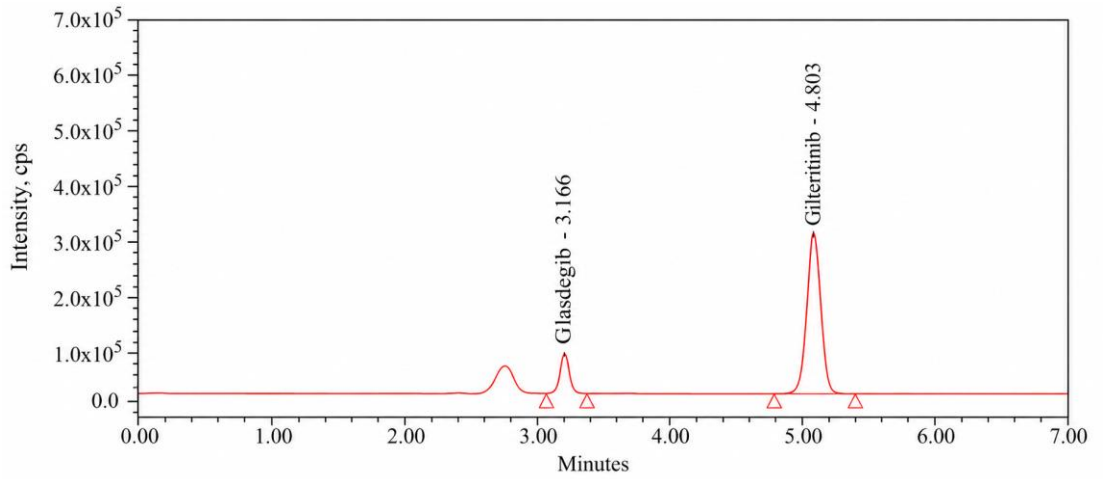


Figure 5. LOQ chromatogram.

CONCLUSIONS

In this paper, we have accomplished method development and validation using LCMS technique for the determination of Glasdegib in rat plasma. It is a general, linear, accurate, simple and exact method for determination of any drug molecules. The mobile phase solvents and analytical technique settings were responsible for the remarkable Glasdegib resolution. The major advantage of this approach is very quick run time and low retention time (roughly 3.5 minutes). We also confirmed that our reported approach was successfully validated using ICH recommendations.

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Author Contributions

"Conceptualization, R.R. and R.T.; methodology, S.V.S.S.; validation, S.V.S.S.; investigation, U.M.; resources, B.D.; data curation, B.D.; writing—original draft preparation, R.T.; writing—review and editing, R.R. All authors have read and agreed to the published version of the manuscript."

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Conflicts of Interest

“The authors declare no conflict of interest.”

Abbreviations

The following abbreviations are used in this manuscript

Abbreviation	Definition
HQC	High quality control
MQC	Medium quality control
LQC	Low quality control
LLQC	Lower limit quality control
LLOQ	Lower limit of quantification
SD	Standard deviation
LBAs	Liquid chromatography based assays
CV	Coefficient of variation

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