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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF INOTUZUMAB OZOGAMICIN IN BULK DRUG AND INJECTION DOSAGE FORM

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Keywords:

Inotuzumab Ozogamicin, RP-HPLC, Validation, Stability

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ABSTRACT: A simple, rapid, accurate, precise, and reproducible stability indicating RP-HPLC method for the estimation of Inotuzumab Ozogamicin in bulk and injection dosage form was developed and validated as per ICH guidelines. The separation was done using BDS C18 150 × 4.6 mm, 5 µ column. The mobile phase (Water and Acetonitrile 55:45% v/v) was pumped at 1.0 ml/min, and effluent was detected at 240 nm using a PDA detector. The retention time was 2.93 ± 0.1 min, and the method produced a linear response in the concentration range of 4.5-27 µg/ml (r^2- 0.9995). In recovery studies, %RSD from reproducibility was found to be below 2%. LOD and LOQ were 0.1 µg/ml and 0.34 µg/ml, respectively. The drug was subjected to different stress conditions such as acidic, alkaline, oxidative, photothermal, and hydrolysis. The drug showed more degradation in acidic conditions, and no degradation was observed in hydrolysis and photo conditions. The developed RP-HPLC method was found to be effective, sensitive, and specific for the estimation of Inotuzumab Ozogamicin in bulk and injection dosage form.

INTRODUCTION: Inotuzumab Ozogamicin **Fig. 1**, is an antibody-drug conjugate used for adults with relapsed/refractory B-cell precursor (BCP) acute lymphoblastic leukemia [1]. Inotuzumab ozogamicin is a humanized monoclonal antibody-drug conjugate that targets and binds to CD-22 (found on the surface of B cells). Once the drug attaches to the cancer cells, there is a release of something known as calicheamicin. Calicheamicin is very toxic to living cells; therefore, it is responsible for stopping further cell division and causing cancer cell death [2]. Inotuzumab Ozogamicin is chemically, N-acetyl-gamma-calicheamin 1, 2-dimethyl hydrazine.

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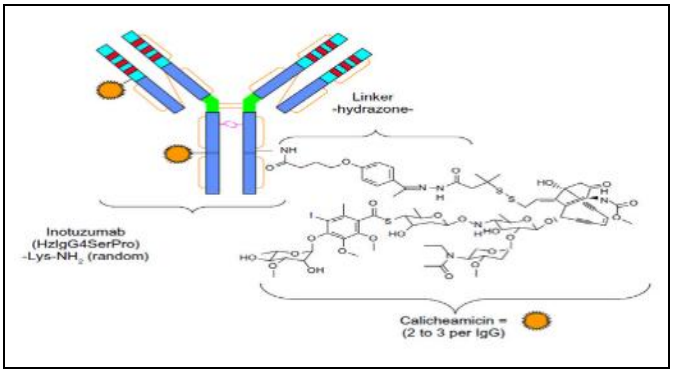

FIG. 1: STRUCTURE OF INOTUZUMAB OZOGAMICIN

It is white to fine off-white powder, insoluble in water and soluble in alcohol [3]. A literature survey revealed that no RP-HPLC based methods had been reported for the estimation of Inotuzumab Ozogamicin. The aim of the present work was to develop a novel, simple, rapid, sensitive, specific, accurate, precise, economical, and reliable RP-HPLC method for the estimation of Inotuzumab Ozogamicin in bulk and injection dosage form suitable for quality control analysis.

MATERIALS AND METHODS:

Chemicals: Inotuzumab Ozogamicin working standard was received as a gift sample, and sample injections (Label claim: 0.25 mg in 1 ml; Besponsa injection) were procured from Spectrum Labs Pvt. Ltd., Hyderabad.

HPLC grade acetonitrile, orthophosphoric acid, and water were purchased from Merck Specialities Private Ltd., Mumbai, and Rankem Laboratories, Haryana.

Instrument: Waters HPLC 2965 system with auto-injector and PDA detector integrated with Empower 2 Software was used for LC peak integration and Data processing.

Chromatographic Conditions: Chromatographic separation was performed on BDS C18 150 mm × 4.6 mm, 5 mm column at 30 °C. The Injection volume for standard and sample was 10 µL. The mobile phase used was water: Acetonitrile in the ratio 55:45(v/v), with a flow rate of 1 ml/min. samples were analyzed by using a PDA detector at wave-length 240 nm, and the run time is 6 min.

Diluent: HPLC Eater: Acetonitrile (55:45%v/v)

Preparation of Standard Stock Solution: About 1.8 mg of Inotuzumab Ozogamicin working standard was accurately weighed and transferred into 10 ml volumetric flask, dissolved in a diluent, filtered through 0.45 µm Ultipor N66 nylon filter and the volume was made up to the mark with the diluent to get 180 µg/ml of Inotuzumab Ozogamicin.

Preparation of Standard Solution: Inotuzumab Ozogamicin (18 µg/ml) was prepared from the standard stock solution by pipetting out 1 ml and made up to 10 ml with the diluent. Accurately 10 µl was injected into the HPLC system, and chromatogram was recorded.

Preparation of Sample Solution: Reconstitute each vial with 4 mL of Sterile Water for Injection to obtain a concentration of 0.25 mg/mL of a vial that delivers 3.6 mL (0.9 mg) transferred into 10 ml volumetric flask, 5 ml of diluents was added and sonicated for 25 min; further the volume was made up with diluent and filtered by HPLC filters. 2 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent (18 µg/ml of Inotuzumab Ozogamicin).

Validation of the Developed Method: The method developed was validated as per ICH guidelines [4] for linearity, accuracy, precision, LOD, LOQ, ruggedness, and specificity.

Accuracy: The accuracy of the method was determined by calculating % recovery. A known amount of Inotuzumab Ozogamicin was added to the placebo, and the amounts were estimated by measuring the peak area. The studies were carried in triplicate manner (50%, 100%, and 150%).

Specificity: It is the ability of an analytical method to measure the response of the analyte and have no interference from other extraneous components, and well-resolved peaks are obtained.

Linearity: Linearity solutions were prepared by pipetting out 2.5 – 15 ml from the Stock solution of Inotuzumab Ozogamicin into 6 different volumetric flasks and diluted to 10 ml with diluent to get 4.5-27 µg/ml.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ for Inotuzumab Ozogamicin were found to be 0.11 µg/ml and 0.34 µg/ml respectively based on signal to noise ratio where s/n for LOD is 2.95:1 (limit-3:1) and LOQ is 9.8:1(limit-10:1)

Robustness: Robustness of the method was determined by making slight changes in the composition of mobile phase ± 10%, flow rate by ± 0.1 ml/min, and temperature by ± 5 ºC. Retention time and chromatograms were determined for the drug.

System Suitability: The system suitability parameters like Retention time, USP theoretical plates, USP tailing, and peak area and peak height were calculated.

Stability: Stability of both the standard and sample solutions was tested during analysis up to 24 h at room temperature.

Degradation Studies:

Acid Degradation Studies: 1 ml of Inotuzumab Ozogamicin stock solution was exposed to 2N HCl and refluxed at 60 ºC for 30 min.

Alkali Degradation Studies: 1 ml of Inotuzumab Ozogamicin stock solution was exposed to 2N sodium hydroxide and refluxed at 60 ºC for 30 min.

Peroxide Degradation: To 1 ml of Inotuzumab Ozogamicin stock solution 1 ml of 20% Hydrogen peroxide (H_2O_2) was added and kept for 30 min.

Thermal Degradation Studies: The standard drug solution was placed in an oven at 105 ºC for 6 h to study dry heat degradation.

Photo Stability Studies: The photochemical stability of the drug was also studied by exposing the solution to UV light by keeping the beaker in the UV chamber for 7 days or 200 Watt-hours/m^2 in photostability chamber.

Water Degradation Studies: stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60 ºC.

RESULTS & DISCUSSION:

Optimized Chromatographic Conditions: To develop and establish a suitable RP-HPLC method for estimation of Inotuzumab Ozogamicin in bulk and injection dosage form, different preliminary tests were performed, and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in **Table 1**.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Parameter	Condition
HPLC	WATERS HPLC with PDA detector
Column	BDS C18 150 mm × 4.6 mm, 5 µm
Mobile phase	Water: Acetonitrile (55 : 45v/v)
Flow rate	1 ml/min
Detector wave length	240 nm
Column temperature	30 °C
Injection volume	10 µL
Run time	6 min
Diluent	Water: Acetonitrile (55 : 45v/v)

The final analysis was performed by using water: Acetonitrile (55:45v/v) at a flow rate of 1 ml/min. samples were analyzed at 240 nm detector wavelength and at an injection volume of 10 µL using BDS C18 150 mm × 4.6 mm, 5 µ, with a run time of 6 min. The proposed method was optimized to give a sharp peak, retention time of 2.93 min with minimum tailing for Inotuzumab Ozogamicin. The optimized chromatogram was obtained, as shown in **Fig. 2**.

The assay was performed for tablet formulation and the mean % purity obtained was 99.63%. The result was shown in **Table 3,** and the chromatogram of sample solution was shown in **Fig. 3** respectively.

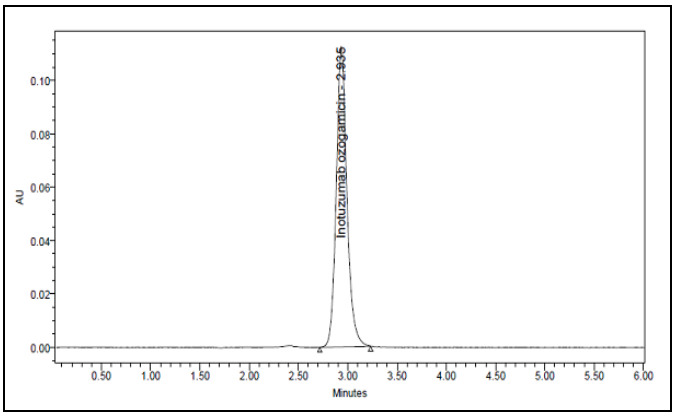

FIG. 2: STANDARD CHROMATOGRAM OF INOTUZUMAB OZOGAMICIN

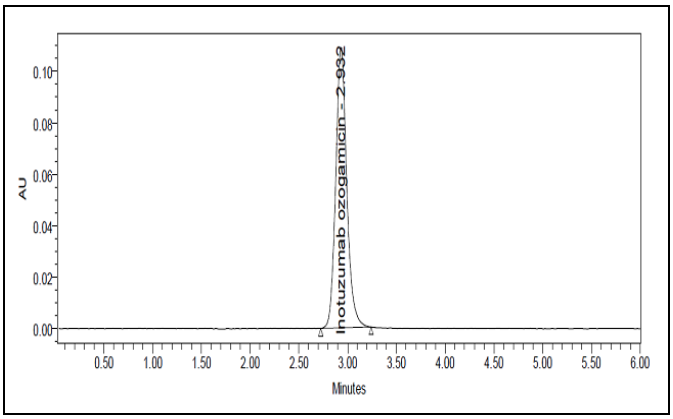

FIG. 3: SAMPLE CHROMATOGRAM OF INOTUZUMAB OZOGAMICIN

TABLE 2: SYSTEM SUITABILITY AND VALIDATION PARAMETERS OF THE DEVELOPED METHOD

Parameter	Inotuzumab Ozogamicin
Theoretical plates	3775
Tailing factor	1.13
Retention time (min)	2.93
Linearity range (µg/ml)	4.5-27 µg/ml
Regression equation Y = mx+c	y = 48052x + 5704.6
Slope (m)	48052
Intercept (c)	5704.6
Correlation coefficient	0.9995
Percent RSD	< 2
Precision Repeatability (n = 6)	0.382
Intermediate Precision (n = 6)	0.872
LOD (µg/ml)	0.11
LOQ (µg/ml)	0.34

TABLE 3: ASSAY RESULTS OF INOTUZUMAB OZOGAMICIN

Formulation	Label claim (mg/ml)	Amount found (mg) (n=3) Mean ± SD	Assay	% RSD
Besponsa	0.25 mg	0.249 ± 0.0023	99.63	0.4

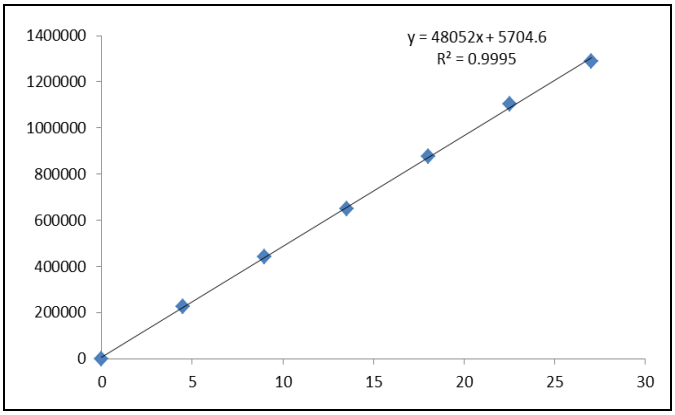

FIG. 4: LINEARITY CURVE OF INOTUZUMAB OZOGAMICIN

Validation: The developed method was found to be specific as no interference was observed in blank and placebo at the retention time of the drug.

The linearity was established (4.5-27 µg/ml) at six different concentrations each were injected in duplicates, and average areas were determined, and linearity equations were obtained as y = 48052x + 5704.6, correlation coefficient (R^2) was determined as 0.9995. The Linearity calibration curves were plotted, as shown in **Table 2** and **Fig. 4**.

TABLE 4: ACCURACY RESULTS OF INOTUZUMAB OZOGAMICIN

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	9	8.96	99.53	99.96%
	9	8.99	99.94	
	9	8.99	99.99	
100%	18	18.13	100.73	
	18	18.04	100.22	
	18	17.85	99.15	
150%	27	26.89	99.61	
	27	27.11	100.40	
	27	27.02	100.08	

TABLE 5: PRECISION RESULT OF INOTUZUMAB OZOGAMICIN

S. no.	Repeatability	Intermediate precision
1	881090	851207
2	880754	831649
3	881603	842314
4	876437	832551
5	875704	835538
6	873664	839219
Mean	878209	838746
S.D	3357.6	7311.2
%RSD	0.382	0.872

Three levels of Accuracy samples 50%, 100%, 150% were prepared, and triplicates of injections were given for each level of accuracy and mean %Recovery was obtained as 99.96% was shown in **Table 5**. %RSD for precision studies was calculated from the corresponding peak areas obtained by injecting six times a known concentration of Inotuzumab Ozogamicin was obtained as 0.382% and 0.872% for repeatability and intermediate precision respectively which is within limits (<2%). Hence the method was precisely shown in **Table 5**.

The values were evaluated based on the Relative standard deviation of response and slope of the calibration curve Inotuzumab Ozogamicin. The detection limit values were obtained as 0.11 µg/ml, and the Quantification limit was found to be 0.34 µg/ml, as given in **Table 1**.

TABLE 6: ROBUSTNESS DATA OF INOTUZUMAB OZOGAMICIN

S. no.	Parameter	Proposed	Modification	%RSD	Retention time(min)	Tailing factor
1	Flow Rate	1.0	1.1	0.4	2.683	1.12
	(± 0.1 ml/min)		0.9	0.6	3.239	1.13
2	Mobile Phase	55:45	45:55	1.4	2.573	1.12
	(± 10%) (W:A)		65:35	0.4	3.567	1.18
3	Temperature	30 °C	35 °C	1.0	2.919	1.12
	(± 5 °C)		25 °C	0.8	2.945	1.13

Robustness of the method was studied by changing the chromatographic conditions slightly, and results were presented in **Table 6**. From the method developed it was observed that there were no significant changes in the retention time and area of the chromatograms by making slight alterations in temperature, mobile phase composition, and flow rate. The %RSD was less than 1%, which demonstrated that the RP-HPLC method developed was robust. Degradation studies were performed with the formulation, and the degraded samples were injected.

Assay of the injected samples was calculated, and the % degradation is calculated shown in **Table 7** and **Fig. 5-10**.

TABLE 7: DEGRADATION DATA OF INOTUZUMAB OZOGAMICIN

S. no.	Degradation Condition	% Drug Degraded
1	Acid	6.84
2	Alkali	5.72
3	Oxidation	4.96
4	Thermal	3.74
5	UV	2.00
6	Water	2.00

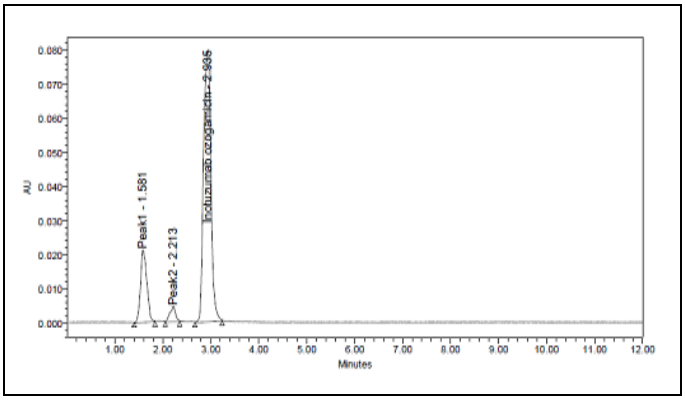

FIG. 5: ACID DEGRADATION CHROMATOGRAM OF INOTUZUMAB OZOGAMICIN

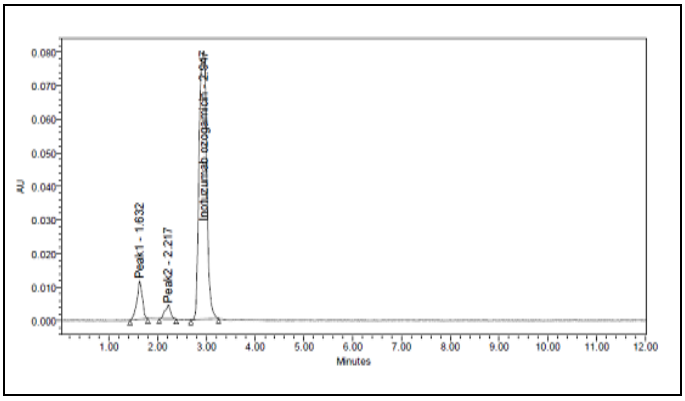

FIG. 6: BASE DEGRADATION CHROMATOGRAM OF INOTUZUMAB OZOGAMICIN

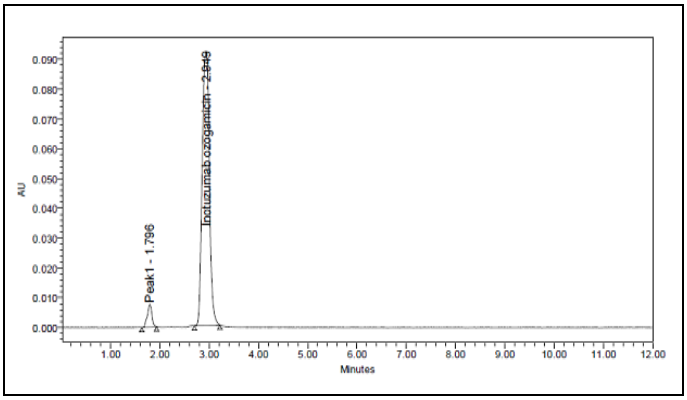

FIG. 7: PEROXIDE DEGRADATION CHROMATOGRAM OF INOTUZUMAB OZOGAMICIN

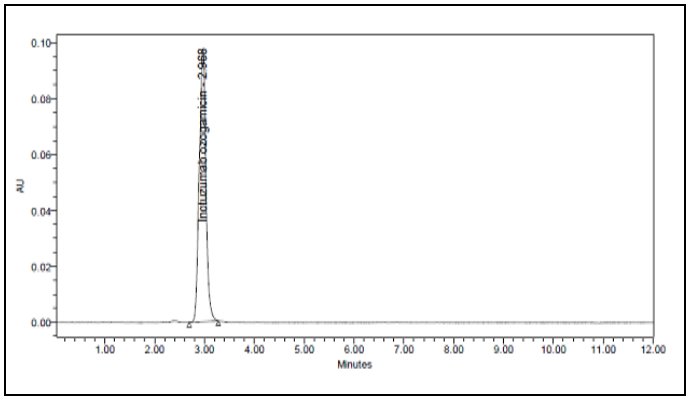

FIG. 8: THERMAL DEGRADATION CHROMATOGRAM OF INOTUZUMAB OZOGAMICIN

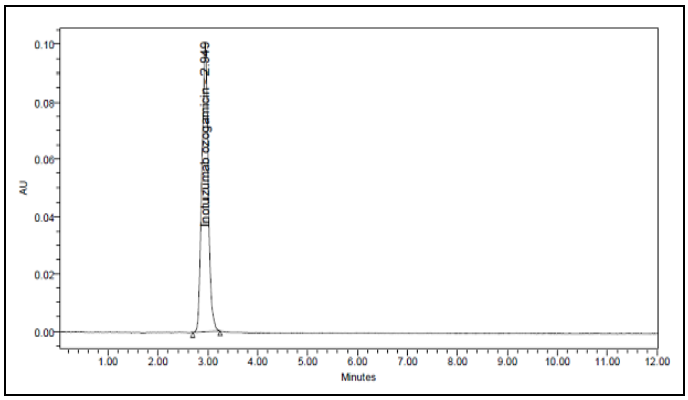

FIG. 9: UV DEGRADATION CHROMATOGRAM OF INOTUZUMAB OZOGAMICIN

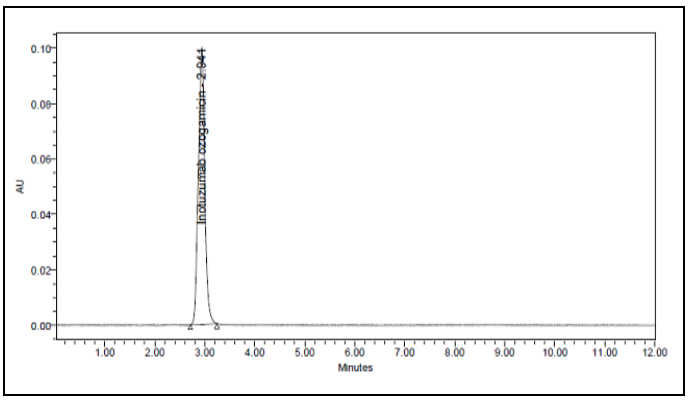

FIG. 10: HYDROLYTIC DEGRADATION CHROMATOGRAM OF INOTUZUMAB OZOGAMICIN

CONCLUSION: A simple, Accurate, precise method was developed for the estimation of Inotuzumab Ozogamicin in bulk and pharmaceutical dosage form. The retention time of was found to be 2.93 min. %RSD was found to be 0.382 and 0.872 for repeatability and intermediate precision, respectively.

% Recovery was obtained as 99.96%. LOD, LOQ values were obtained from regression equations of Inotuzumab Ozogamicin was 0.11 µg/ml and 0.34 µg/ml, respectively. Regression equation of Inotuzumab Ozogamicin is y = 48052x + 5704.6. Hence, the method developed was simple and economical that can be adopted in regular Quality control analysis in Industries.

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CONFLICT OF INTEREST: No

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