ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TISOTUMAB VEDOTIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY USING RP-HPLC

M. Prasanthi[1*] Dr. A. Krishna Manjari Pawar[2]

1Research Scholar, Department of Pharmaceutical Analysis, A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India – 530003.

2Associate Professor, Department of Pharmaceutical Analysis, A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India- 530003.

Address for correspondence: M. Prasanthi Email: daveprasanthi @ gmail.com

Mobile: 8074522254

ABSTRACT

Tisotumab vedotin is approved to treat cervical cancer that is recurrent or metastatic. It is used in women whose cancer got worse during or after treatment with chemotherapy. The main aim of this research is to develop an RP-HPLC for quantification of Tisotumab Vedotin in pharmaceutical dosage form. This method is designed to be specific, simple, pricise, & economical featuring a lesser runtime making it ideal for routine quality control applications. Method was achieved on Waters Alliance -e 2695.by using Waters x-bridge C18 (150X4.6mm,3.5µ) column and the mobile phase containing CAN and 0.1% formic acid in the ratio of 70:30% v/v. Flow rate was 1.0ml/min. Detection was carried out by absorption at 223nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Tisotumab Vedotin were NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurement less than 2.0. A validated and efficient RPHPLC method that ensures Accuracy, Consistency, system Suitability speed, sensitivity, and cost- effectiveness.

Key words: Tisotumab vedotin, RPHPLC Assay method & antineoplastic

1. **Introduction:** Tisotumab vedotin is a type of targeted therapy called an antibody - drug conjugate. It is made of a monoclonal antibody chemically linked to a cancer killing drug. Tisotumab vedotin is approved to treat cervical cancer that is recurrent or metastatic. It is used in women whose cancer got worse during or after treatment with chemotherapy. It is primarily works by inducing cytotoxic effects on TF- expressing tumours. Tisotumab vedotin binds to TFs expressed expressed on cervical tumours which leads to the internalization of the antibody- drug conjugate-TF complex. Once internalized, MMAE from the drug-target complex is released via proteolytic cleavage. MMAE is a microtubule-

disrupting agent that disrupts the microtubule network of actively dividing cells, leading to cell cycle arrest and apoptotic cell death. Tisotumab vedotin, sold under the brand name Tivdak, is an antibody-drug conjugate used to treat cervical cancer. It is a combination of tisotumab, a monoclonal antibody against tissue factor and monomethyl auristatin E (MMAE), a potent inhibitor of cell division. It is administered by infusion into a vein.

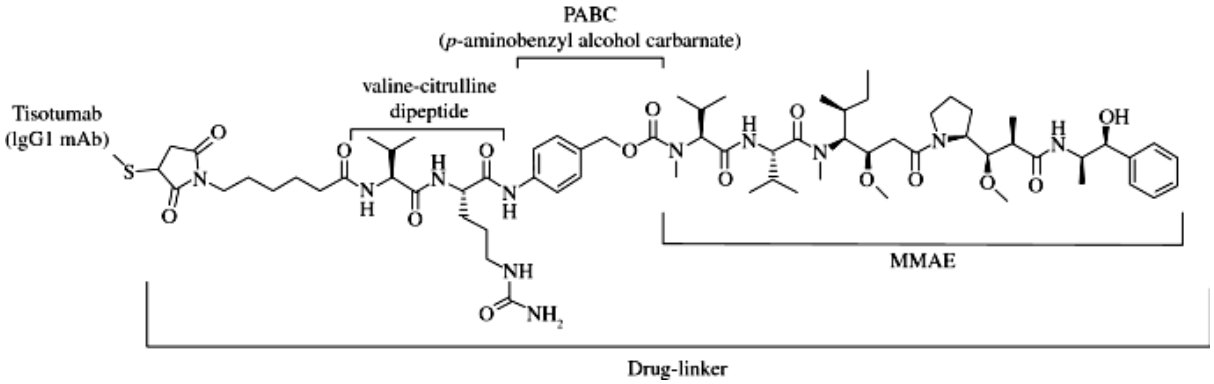


Figure: 1 Chemical Structure of Tisotumab vedotin

2. Experimental part:

2.1. List of Apparatus used:

Table: 1 Apparatus used

S. No	Name	Model	Manufacturer
1.	HPLC	Alliance	Waters
2.	pH meter	-	Eutech
3.	Weighing balance	-	Sartouris
4.	UV/VIS spectrophotometer	-	UV-1700
5.	Pipettes, beakers and Burettes	-	Borosil
6.	Ultra sonicator	UCA 701	Unichrome
7.	Pump	Isocratic model	-

2.2 Reagents & Chemicals:

Table: 2 Reagents & Chemicals

S.NO	Name	Grade	Manufacturer
1.	Acetonitrile	HPLC	Rankem
2.	Water (Milli Q)	HPLC	In house production

3.	Formic acid	HPLC	Analytical reagent

2.3 Chromatographic conditions (Optimized)

Table: 3 Chromatographic conditions

Column	Waters X-Bridge C18 (150x4.6mm, 3.5µm)
Movable phase	Acetonitrile: 0.1% Formic acid (70:30)
Wavelength	223 nm
Flow rate	1ml/min
Injection volume	10µl
Run time	5min
Observation	This method is suitable for validation

2.4 General preparations

Preparation of 0.1% Formic acid buffer solution:

1ml of Formic acid is 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% Formic acid and ACN taken in the ratio 30:70. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram.

Preparation of standard solution

Accurately weigh and transfer 4mg of Tisotumab Vedotin working standard into a 10 ml clean dry volumetric flask add Diluent and sonicate 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 diluent. (40ppm of Tisotumab Vedotin)

Sample Solution Preparation:

Accurately weighed and transfer 4mg of Tisotumab Vedotin sample into a 10mL clean dry volumetric flask add diluent and sonicate it up to 30 min to dissolve, and centrifuge for 30min 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 10ml volumetric flask and dilute up to the mark with diluent (40ppm of Tisotumab Vedotin).

Procedure:

Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Tisotumab Vedotin peak and calculate the %Assay by using the formulae.

3. RESULTS AND DISCUSSION

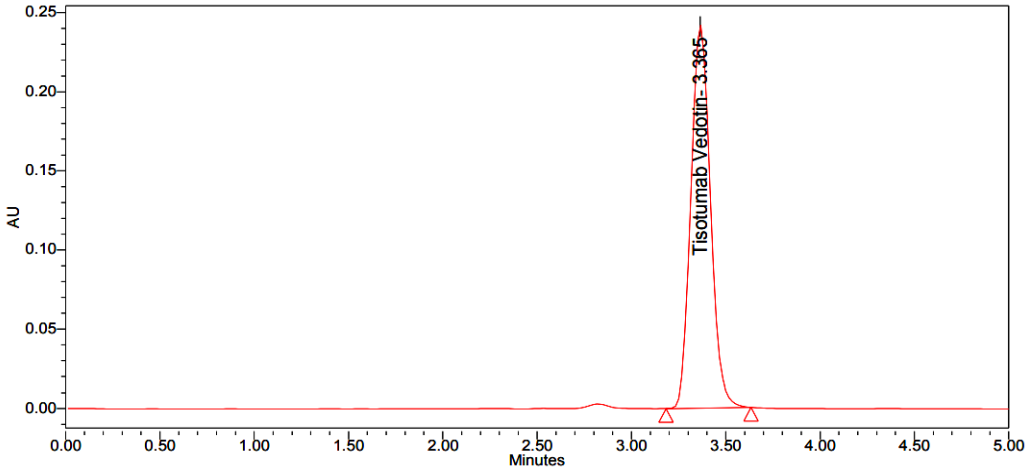


Fig No-2: Chromatogram of Trial-6

Table: 4

Name	Retention Time	Area	USP Tailing	USP Plate Count
	3.365	2429162	1.13	7916

Table 5: Optimized chromatographic conditions

PARAMETERS	OBSERVATION
Instrument used	Waters Alliance e-2695 HPLC
Injection volume	10µl
Mobile Phase	ACN and 0.1% Formic acid (70:30)
Column	Waters X-Bridge C18 (150mmx4.6, 3.5µm)
Detection Wave Length	223 nm
Flow Rate	1 mL/min

Runtime	5 min
Temperature	Ambient(25° C)
Mode of separation	Isocratic mode

3.1 System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Table: 6 System suitability parameters for Tisotumab Vedotin

S. no	Parameter	Tisotumab Vedotin
1	Retention time	3.368
2	Plate count	7925
3	Tailing factor	1.18
4	Resolution	----
5	%RSD	0.23

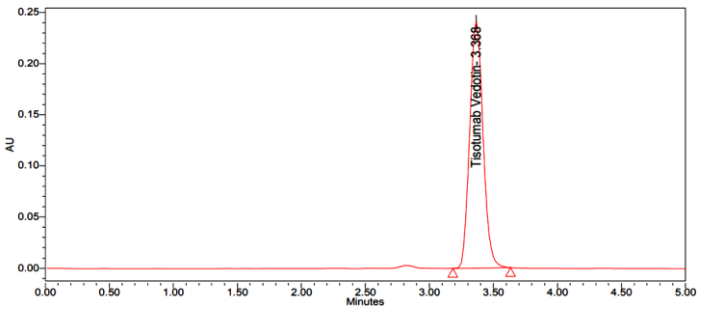


Fig No.3: Chromatogram of standard

3.2 Specificity:

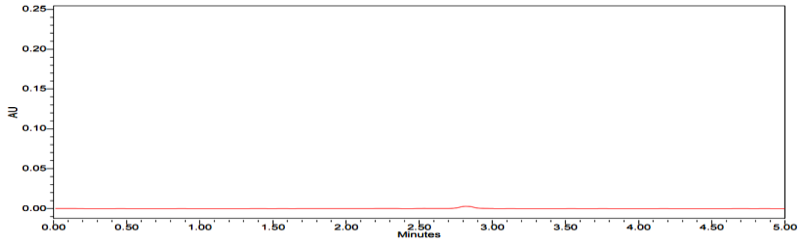


Fig No.4: Chromatogram of blank

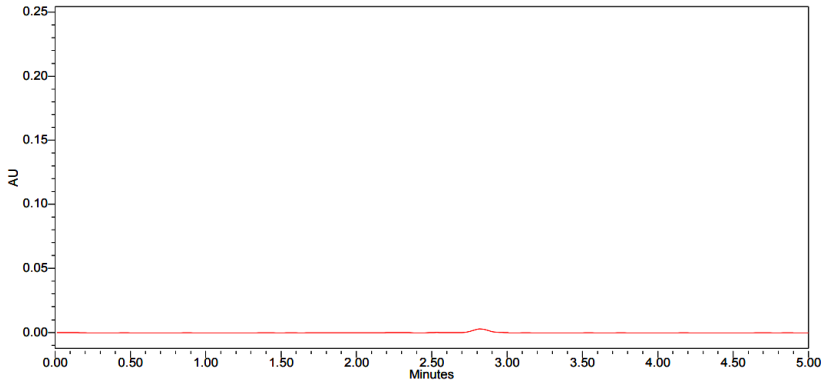


Fig No.5: Chromatogram of placebo

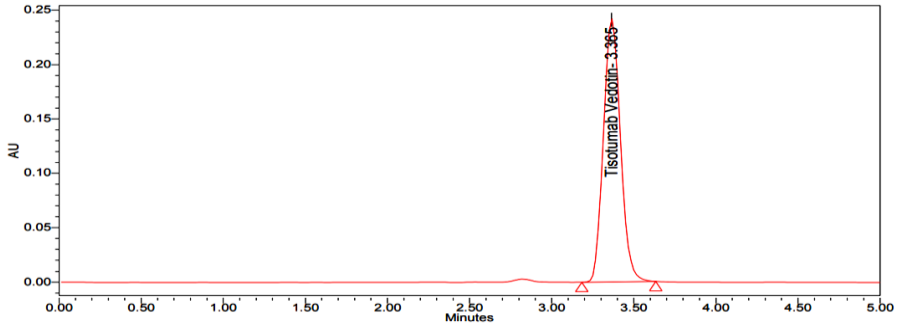


Fig. No. 6: Optimized chromatogram

3.3 PRECISION:

System Precision:

Table 7: System precision table of Tisotumab Vedotin

S. No	Concentration Tisotumab Vedotin (µg/ml)	Area of Tisotumab Vedotin
1.	40	2429162
2.	40	2433584
3.	40	2427715
4.	40	2438640
5.	40	2422551
6.	40	2428795
Mean	2430075	
S.D	5483.146	
%RSD	0.23	

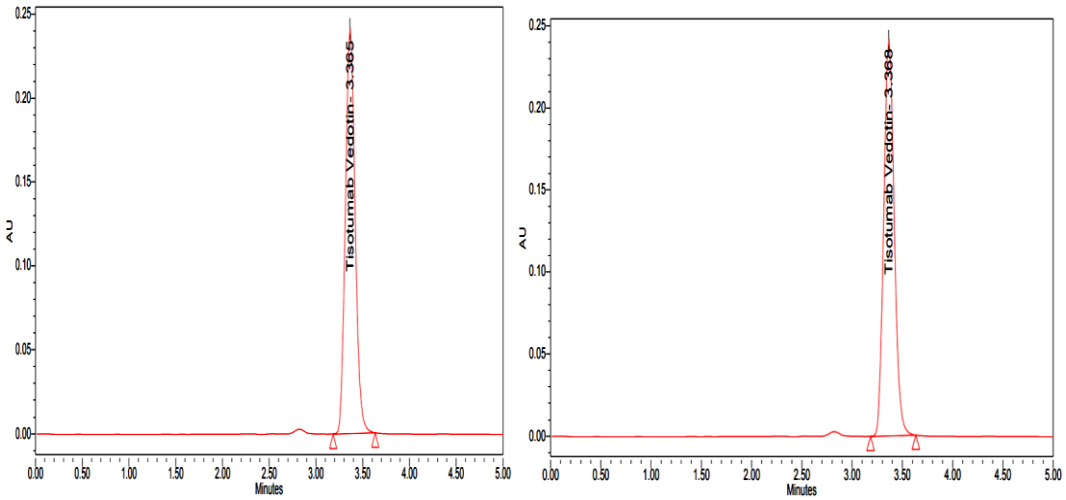


Fig. 7: System precision chromatogram-1 Fig. 8: System precision chromatogram-2

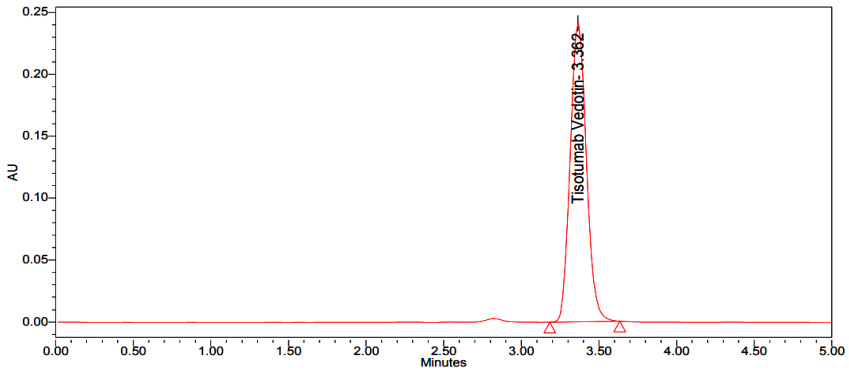


Fig. 9: System precision chromatogram

3.4 Linearity:

Table No.8: Results of linearity for Tisotumab Vedotin

S.NO	Tisotumab Vedotin	
	Conc.(µg/ml)	Peak area
1	10.00	631124
2	20.00	1215605
3	30.00	1837242
4	40.00	2460358
5	50.00	3076123
6	60.00	3624515
Regression equation	y=597053.60x+11664.57	
Slope	60743.91	
Intercept	12677.86	
R^2	0.99988	

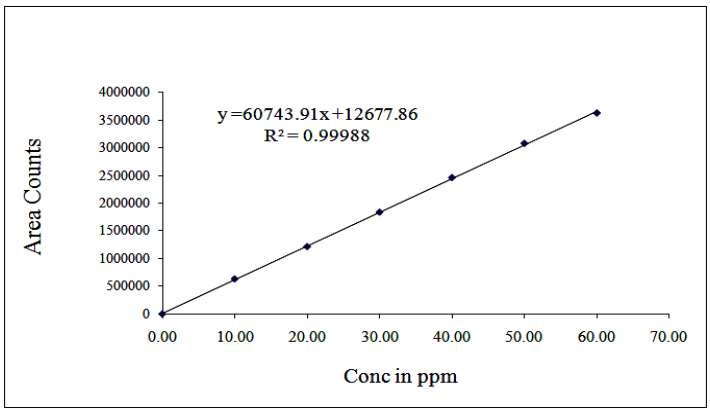


Fig No.10: Calibration curve for Tisotumab Vedotin at 223nm

3.5 Assay

Table No.9: Assay of Tisotumab Vedotin

Brand	Drug	Area	Average sample area	Std. wt. (mg)	Sample wt. (mg)	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
-	Tisotumab Vedotin	2419546 2462901	2441224	4	4	1	99.9	4.02	100.5

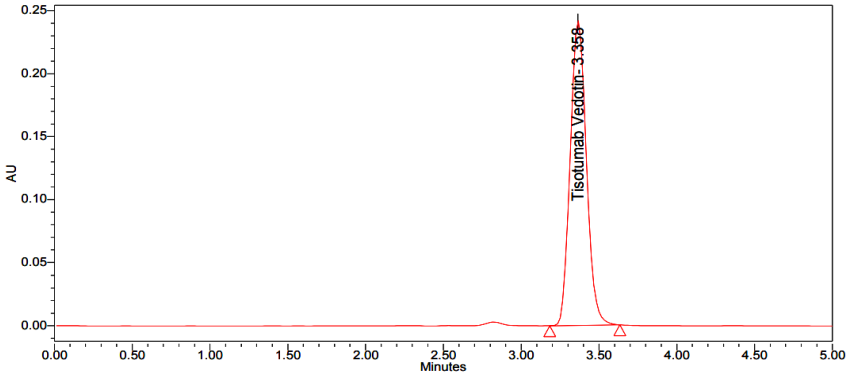


Fig. No. 11: Chromatogram of Assay-1

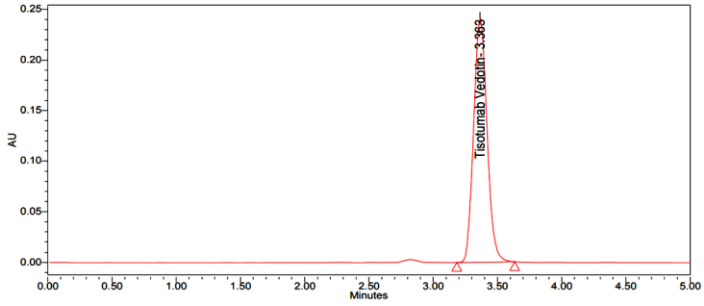


Fig. No. 12: Chromatogram of Assay-2

3.6 Repeatability:

Table No.10: Method Precision for Tisotumab Vedotin by HPLC method

S. No.	Area for Tisotumab Vedotin
1	2421541
2	2450185
3	2473161
4	2440535

5	2419774
6	2451628
Average	2442804
Standard Deviation	20206.603
%RSD	0.83

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%**.**

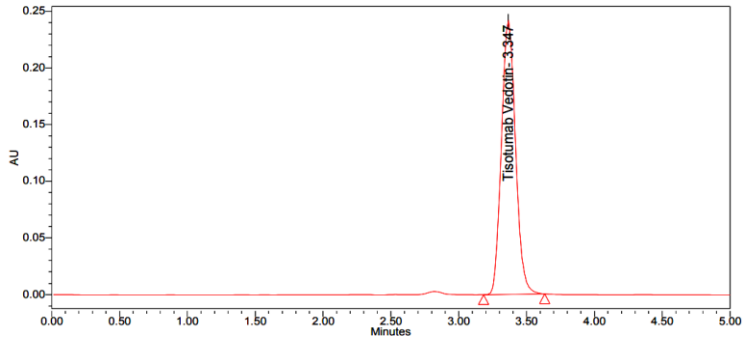


Fig. 13: Repeatability chromatogram-6

3.6. Intermediate precision (Day_ Day Precision):

Table No11: Intermediate Precision (Day variation) for Tisotumab Vedotin by HPLC method

Injection	**Area**	
	Day-1	**Day-2**
1	2441065	2458124
2	2467198	2431647
3	2452315	2460975
4	2435422	2444032
5	2416944	2452713
6	2422887	2418927
Average	2439305	2444403
Standard Deviation	18628.043	16394.102
%RSD	0.76	0.67

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%.

3.7 Accuracy:

Table No.12: Accuracy results of Tisotumab Vedotin by HPLC method

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean %Recovery	%RSD
50%	1212458	2.0	2.00	99.8	99.9	0.37
	1218364	2.0	2.01	100.3		
	1209410	2.0	1.99	99.5		
100%	2412887	4.0	4.00	99.3	100.4	1.03
	2463184	4.0	4.05	101.4		
	2441224	4.0	4.02	100.5		
150%	3650511	6.0	6.01	100.1	100.8	0.58
	3692322	6.0	6.08	101.3		
	3676740	6.0	6.05	100.9		

3.8 Robustness:

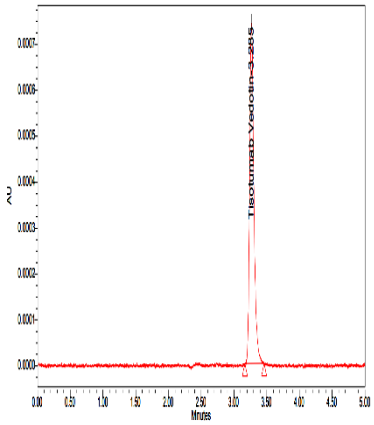
Table No.13: Robustness results of Tisotumab Vedotin by-HPLC

Parameter	Tisotumab Vedotin				
	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change (mL/min)	Less flow(0.9ml)	3.703	2256140	1.16	8037
	Actual(1ml)	3.365	2429162	1.13	7916
	More flow(1.1ml)	3.067	2517556	1.08	7853
Organic Phase change	Less Org (63:37)	3.677	2120487	1.21	8085
	Actual(70:30)	3.368	2433584	1.18	7925
	More Org (77:23)	3.148	2647602	1.12	7821

3.9 LOD and LOQ (µg/ml):

Table No.14: Sensitivity parameters (LOD & LOQ) by HPLC

Name of drug	LOD (µg/ml)	S/N	LOQ (µg/ml)	S/N
Tisotumab Vedotin	0.12	3	0.40	10



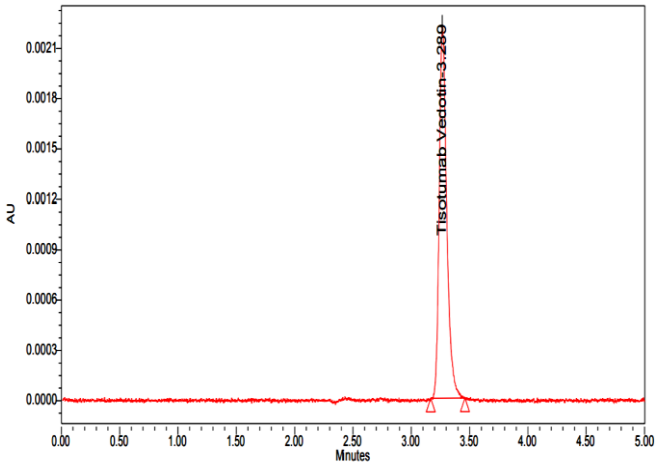


Fig No.14: Chromatogram for LOD **Fig No.15: Chromatogram for LOQ**

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