EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Coden USA: EJBPBO
Volume: 12
Issue: 12
Year: 2025

DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS OF ENFORTUMAB VEDOTIN

Dr. J. N. Suresh Kumar*, M. Kasthuri, Sd. Salma

Department of Pharmaceutical Analysis, Narasaraopeta Institute of Pharmaceutical Sciences, Kottappakonda Road, Yellamanda(p), Narasaraopet (m), Palnadu(Dt), 522601.

***Corresponding Author: Dr. J. N. Suresh Kumar**

Department of Pharmaceutical Analysis, Narasaraopeta Institute of Pharmaceutical Sciences, Kottappakonda Road, Yellamanda(p), Narasaraopet (m), Palnadu(Dt), 522601.

DOI: https://doi.org/10.5281/zenodo.17746531

How to cite this Article: Dr. J. N. Suresh Kumar*, M. Kasthuri, Sd. Salma. (2025). DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS OF ENFORTUMAB VEDOTIN. European Journal of Biomedical and Pharmaceutical Sciences, 12(12), 273–282.

Article Received on 31/10/2025 **Article Revised on 21/11/2025** **Article Published on 01/12/2025**

ABSTRACT

Enfortumab vedotin (EV) is an antibody-drug conjugate that's applied to the treatment of urothelial carcinoma, or bladder cancer. EV was a microtubule-disrupting chemotherapeutic drug and human monoclonal antibody. The current research study used to develop the RP-HPLC technique with a photodiode array detector to set up a reliable, stability-indicating, rapid method for drug determination. By utilizing this technique, the final chromatographic conditions were achieved with a column Waters Kromasil C18 (150x4.6mm, 3.5µm), and a mobile phase made with ACN+ 0.1% Perchloric acid (40:60), respectively by adopting isocratic elution. EV was detected at 271nm, and a satisfactory separation was achieved within 5.0 minutes of runtime with a flow rate of 1.0 mL/min. The method is linear from 25-150 µg/mL for EV (R^2= 0.99957). Results of precision for EV (%RSD< 0.2%) were found within acceptable limits. Furthermore, the prescription drug was exposed to oxidation, reduction, photolysis, hydrolysis (acid, basic and neutral) and heat degradation. There is evidence that the suggested technique can stability indicating of the drug by separating it from the degradation products that result from its stress study. The efficiency of the suggested stability-indicating method is demonstrated by the forced degradation study's results. The analysis of the cited medicine, EV, was accomplished successfully using the validated RP-HPLC method.

KEYWORDS: Enfortumab vedotin, Antibody –drug conjugate, Tailing factor and Degradation.

I. INTRODUCTION

Urothelial carcinoma, the most common form of bladder cancer, remains a significant clinical challenge, particularly in its advanced stages where therapeutic options are limited and prognosis is poor. Recent advances in targeted therapies have led to the development of antibody–drug conjugates (ADCs), which offer a novel approach by combining the specificity of monoclonal antibodies with the cytotoxic potency of chemotherapeutic agents. Among these, **Enfortumab vedotin** has emerged as a promising treatment for patients with locally advanced or metastatic urothelial carcinoma who have progressed after platinum-based chemotherapy and immune checkpoint inhibitors.[1]

Enfortumab vedotin is an ADC that targets **Nectin-4**, a transmembrane protein highly expressed in urothelial tumors. Upon binding to Nectin-4, the conjugate is internalized into the cancer cell, where it releases **monomethyl auristatin E (MMAE)**—a potent microtubule-disrupting agent that induces cell cycle arrest and apoptosis. This targeted delivery mechanism enhances antitumor efficacy while minimizing systemic toxicity.

Clinical trials have demonstrated significant response rates and manageable safety profiles, leading to regulatory approval and widespread clinical adoption. Moreover, ongoing studies are exploring its use in combination with immunotherapies and its potential application in other Nectin-4–expressing malignancies.

This paper aims to explore the pharmacological profile, clinical efficacy, safety considerations, and future directions of Enfortumab vedotin in the evolving landscape of urothelial carcinoma treatment.[2-3]

The proposed method have considerable advantages over the existing methods, VIZ. Chromatographic method, with respect to accuracy, selectivity, sensitivity, range of determination, speed and simplicity.

II. MATERIALS AND METHODS[4-16]

Materials and Instruments: The following are the list of instruments/Equipments, chemicals/reagents and standards to perform the HPLC Analysis of the drug Enfortumab Vedotin.

Equipments

Table 1: List of Equipments.

S. No	Instruments /Equipment / Apparatus	Model / Manufacturer
1.	HPLC	Alliance / Waters
2.	pH meter	pH 700 / Eutech
3.	Weighing balance	BSA224S-CW / Sartorius
4.	UV-Visible Spectrophotometer	UV-1700
5.	Pipettes, beakers and Burettes	Class-A / Borosil
6.	Ultra Sonicator	UCA 701 / Unichrome
7.	Pump	Isocrtic model

Chemicals and Reagents

Table 2: List of Chemicals used.

S. No	Name	Grade	Manufacturer /Suppliers
1.	Acetonitrile	HPLC	Merck
2.	Water (Milli Q)	HPLC	In house
3.	Perchloric acid	AR	Merck
4.	Ortho phosphoric acid	AR	Merck

Working Standard: Working Standard of Enfortumab Vedotin: 100ppm

HPLC Instrumentation & Conditions

The HPLC system employed was HPLC WATERS with Empower Software with Isocratic with UV-Visible Detector. Standard Preparation for UV-Spectrophotometer Analysis: The Standard Stock Solutions – 10 mg of Enfortumab Vedotin standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Acetonitrile and 0.1% Perchloric acid. Further dilutions were done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with Acetonitrile and 0.1% Perchloric acid to get 100ppm concentration. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Enfortumab Vedotin, so that the same wave number can be utilized in HPLC UV PDA detector for estimating the Enfortumab Vedotin.[16-17]

Different Trials for Chromatographic Conditions

Table 3: Different Chromatographic Conditions.

Column Used	Mobile Phase	Flow rate	Wave length	Observation	Results
Waters X-Terra RP-18 (150mmx4.6mm, 3.5µm)	ACN+ 0.1% OPA (80:20)	1ml/min	200-400nm	Retention time is not within the limit	Method rejected
Waters X-Terra RP-18 (150mmx4.6mm, 3.5µm)	ACN+ 0.1% OPA (75:25)	1ml/min	271 nm	Base line is not sufficient	Method rejected
Waters X-Terra RP-18 (150mmx4.6mm, 3.5µm)	ACN+ 0.1% Perchloric acid (70:30)	1ml/min	271 nm	Blank Peak is observed	Method rejected
Kromasil C18 (150x4.6mm, 3.5µm)	ACN+ 0.1% Perchloric acid (60:40)	1ml/min	271 nm	Peak shape is not good	Method rejected
Kromasil C18 (150x4.6mm, 3.5µm)	ACN+ 0.1% Perchloric acid (50:50)	1ml/min	271 nm	Broad peak is observed	Method rejected
Kromasil C18 (150x4.6mm, 3.5µm)	ACN+ 0.1% Perchloric acid (40:60)	1ml/min	271 nm	Proper Peak	Method Accepted

Preparation of 0.1% Perchloric acid buffer solution
1ml of Perchloric acid is dissolved in 1 litre of HPLC water and filter through 0.45µ membrane filter paper.

Preparation of Mobile Phase
Mobile phase was prepared by mixing Acetonitrile: 0.1% perchloric acid taken in the ratio 40:60. 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 10mg of Enfortumab 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. (100ppm of Enfortumab Vedotin).

Sample Solution Preparation
Accurately weighed and transfer 10mg of Enfortumab 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. 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 diluent (100ppm of Enfortumab Vedotin).

Method Validation Studies: The developed analytical method was validated as per the ICH Q2 (R1) guidelines.[18-21]

System Suitability
The system suitability parameters like retention time, number of USP theoretical plates, USP tailing, peak area, and peak height were evaluated. A standard mixture of Enfortumab Vedotin was injected six times to determine the system suitability of the developed method.

Specificity
Specificity was determined by injecting blank and placebo samples. No peaks were observed at the retention times of Enfortumab Vedotin.

Linearity and Range
Calibration curves of the three drugs were prepared at a concentration range of 25-150 µg/ml (six concentration levels) versus the peak area. The linearity was determined using the method of least square regression analysis.

Precision
The precision of an analytical method was studied by performing repeatability, intra-day, and inter-day precision as per the ICH guidelines.

Accuracy
The accuracy of the developed method was determined by calculating the recovery of the three drugs. A fixed concentration of each drug was taken (Enfortumab vedotin) was taken and the respective reference standard was added at 50%, 100%, and 150% levels. Each level was repeated three times, and the percent recovery and percent relative standard deviation were calculated to estimate the accuracy of the developed method.[22-24]

Robustness
The robustness of the developed method was studied by analyzing the effect of slight variation in the pH of mobile phase (± 0.1 units), change in flow rate (± 0.1 ml/min) and change in mobile phase composition (± 2%) on the retention time, tailing factor, theoretical plates and resolution.

Detection Limit
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation Limit
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.[25-27]

III. RESULTS AND DISCUSSION

Standard Preparation for UV-Spectrophotometer Analysis

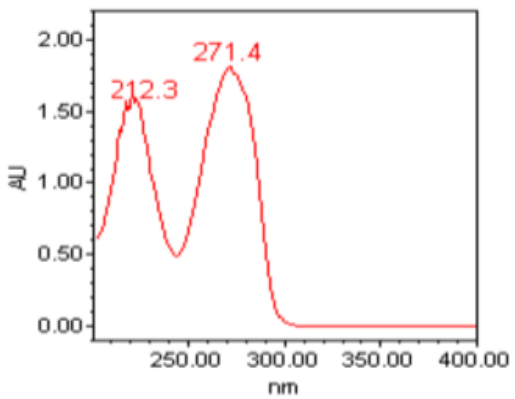

Fig 1: UV- PDA - Spectrum of Enfortumab vedotin.

Observation: While scanning the Enfortumab vedotin solution we observed the maxima at 271 nm.

Table 4: Optimization of Analytical Method.

Column Used	Mobile Phase	Flow rate	Wave length	Injection Volume	Run time
Kromasil C18 (150x4.6mm, 3.5µm)	ACN+ 0.1% Perchloric acid (40:60)	1ml/min	271 nm	10 µl	5 min

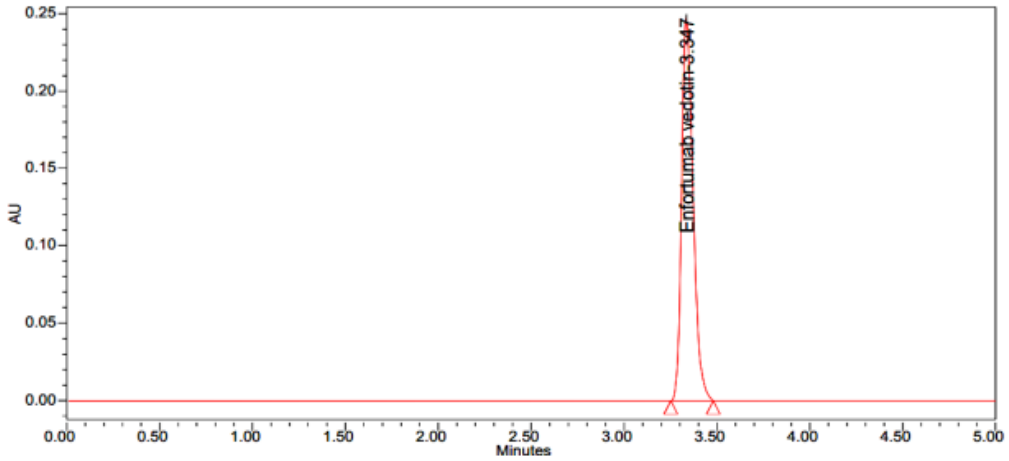

Fig. 2: Optimized Chromatogram for Enfortumab Vedotin.

The selected and optimized mobile phase6 was ACN+ 0.1% Perchloric acid (40:60) and conditions optimized were flow rate (1.0 ml/minute), wavelength (271nm), Run time was 05 mins. Here the peak has shown better theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the Enfortumab Vedotin drug.

Analytical Method Validation

Validation of a method is the process by which a method is tested by the developer for reliability, accuracy and preciseness of its intended purpose. The proposed method is validated as per ICH guidelines.[28-30]

System Suitability Test

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table 5 & Fig 3.

Table 5: Data of System Suitability Test.

S.No	RT	USP Plate Count	USP Tailing	% RSD
1.	3.347	12874	0.96	0.21

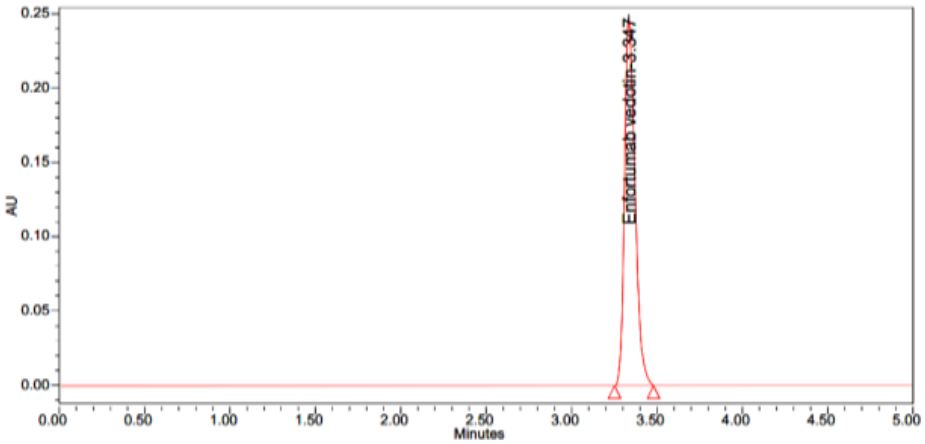

Fig 3: Chromatogram of standard.

Acceptance Criteria: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2. All the system suitable parameters were passed and were within the limits.

ACCURACY

Recovery study

To determine the accuracy8 of the proposed method, recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of pure drug of

Enfortumab Vedotin were taken and 3 replications of each has been injected to HPLC system. The results were shown in table-6.

Table 6: Recovery study.

%Concentration (at specification Level)	Average Area	Average Amount Added (mg)	Average Amount Found (mg)	Average % Recovery	Mean %Recovery
50%	1371822	5	4.99	99.8	100.2
	1385478	5	5.04	100.8	
	1373937	5	5	100	
100%	2751471	10	10.01	100.1	99.9
	2747241	10	10	100	
	2738687	10	9.97	99.7	
150%	4098617	15	14.91	99.4	99.8
	4077314	15	14.84	98.9	
	4161436	15	15.14	100.9	

Observation: From the Accuracy Method, we observed that the mean %Recovery of the drug is 99.686 which are within the range of 99-101%.

Precision

Repeatability The precisio9 of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug Enfortumab Vedotin (API). The percent relative standard deviation was calculated for Enfortumab Vedotin. The results are shown in table-7.

Table 7: Precision study.

S. No.	Concentration Enfortumab vedotin (µg/ml)	Area of Enfortumab vedotin
1.	100	2748271
2.	100	2747520
3.	100	2738478
4.	100	2754328
5.	100	2753809
6.	100	2746728
Mean	2748189	
S.D	5764.790	
% RSD	0.21	

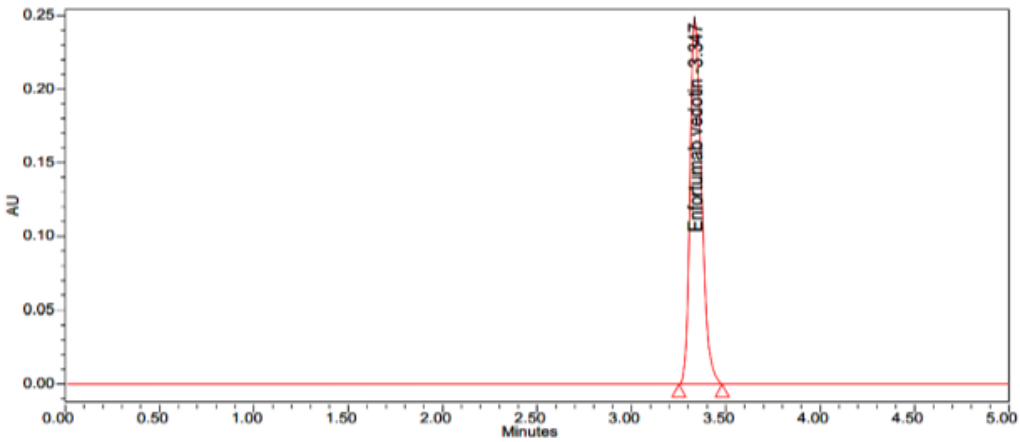

Fig. 4: System precision chromatogram Enfortumab Vedotin.

Observations: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for the drug. % RSD obtained as 0.21 for Enfortumab vedotin.

As the limit of Precision was less than "2" the system precision was passed in this method.

Linearity and Range

To evaluate the linearity serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 25-150 µg/ml. The prepared solutions were sonicated. From these solutions, 10µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Table 8: Linearity Concentrations of Enfortumab Vedotin.

S. No	Concentration (µg/ml)	Peak area
1.	25	681818
2.	50	1388822
3.	75	1943374
4.	100	2745546
5.	125	3350828
6.	150	4061815
Regression equation	y=26971.70x+1723.04	
Slope	26971.70	
Intercept	1723.04	
R2	0.99957	

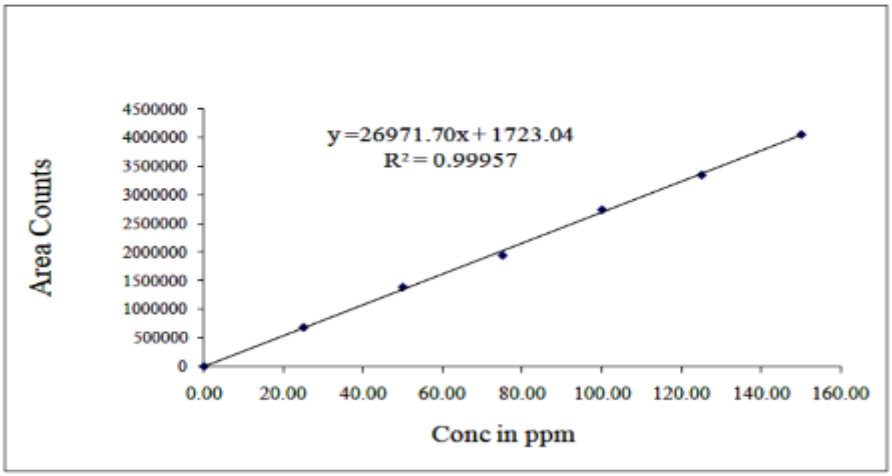

Fig 5: Calibration Curve of Enfortumab Vedotin at 271 nm.

Observation: We observed that the calibration curve showed good linearity in the range of 25-150 µg/ml for Enfortumab Vedotin with correlation coefficient (R2) of 0.99957. A typical calibration curve13 has the regression equation of y=26971.70x+1723.04 for Enfortumab Vedotin

Specificity

Specificity of the pharmaceutical analysis is the ability to measure accurately and specifically the concentration of API, without interference from other active ingredients, diluents, mobile phase. Solutions of mobile phase, sample solution, standard solution were injected into liquid chromatography. Retention times of samples and standard were compared.

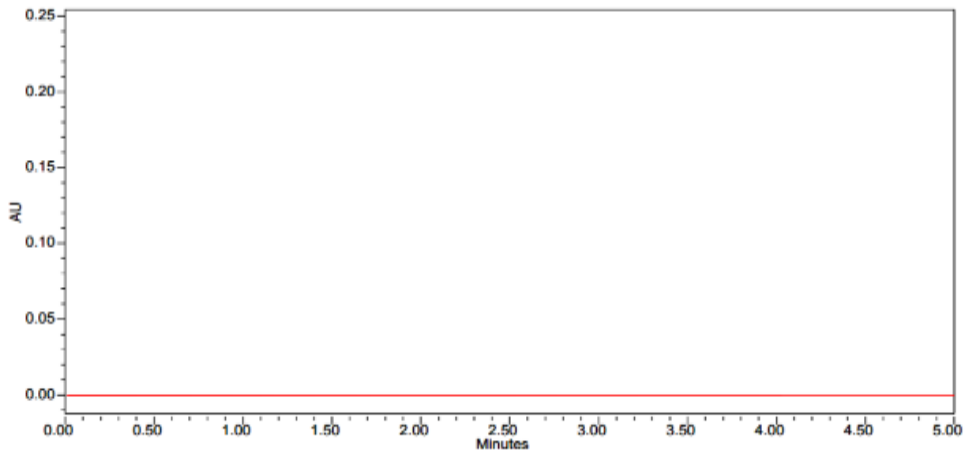

Fig. 6: Chromatogram for Blank Solution.

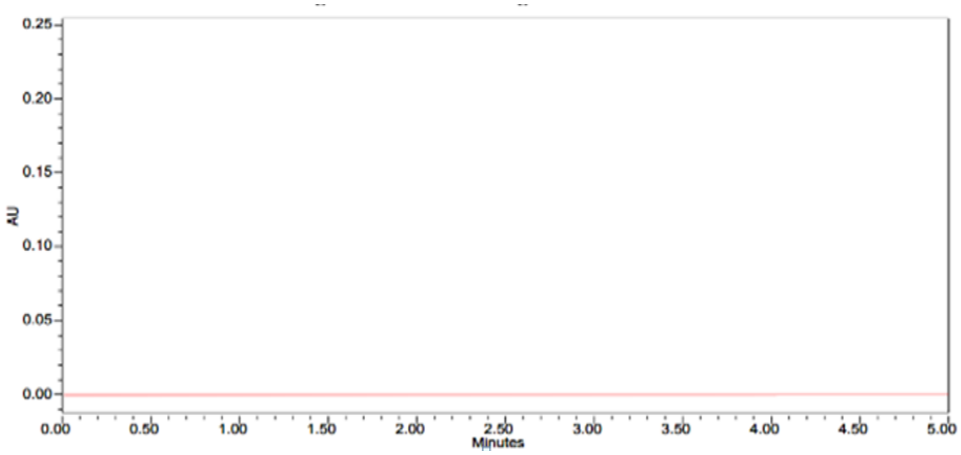

Fig. 7: Chromatogram for Placebo.

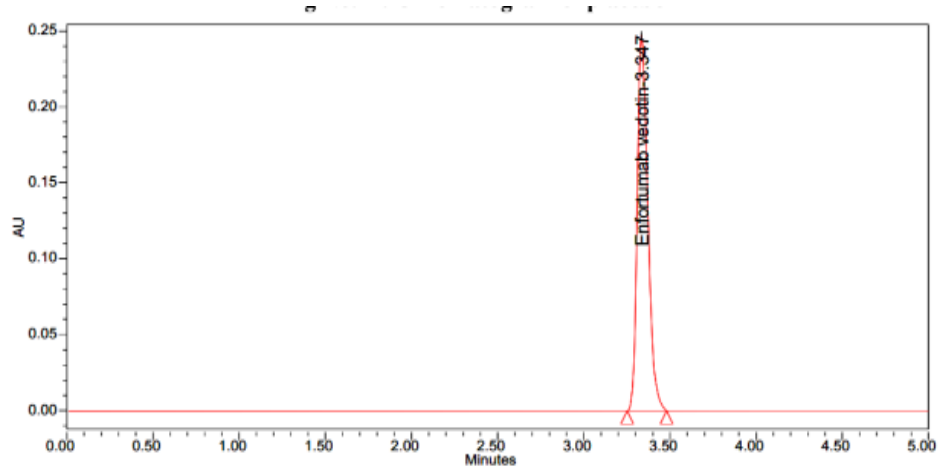

Fig. 8: Optimized Chromatogram for Enfortumab Vedotin Sample.

Observation: Retention time of Enfortumab vedotin was 3.347 min. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Method Robustness

Influence of small changes in chromatographic conditions15 such as change in flow rate 1ml ±(2nm)±0.1ml/min), Wavelength of detection 245nm (&5%) studied to determine±organic phase content in mobile phase 60 (the robustness16-18of the method are also in favour of (Table 9, % RSD 25) the developed RP-HPLC method for the analysis of Enfortumab Vedotin.[31-33]

Table 9: Results of Method Robustness Test.

Parameter	Enfortumab Vedotin					
	Condition	**Retention time (min)**	**Peak area**	**Tailing**	**Plate count**	**% RSD**
Flow rate Change (mL/min)	Less flow (0.9ml)	3.518	2548626	1.02	12721	0.65
	Actual (1ml)	3.347	2748271	0.96	12874	0.21
	More flow (1.1ml)	3.208	2862472	0.91	12954	0.40
Organic Phase change	Less org. (36:64)	3.632	2408547	0.99	12660	0.15
	Actual (40:60)	3.346	2747520	0.92	12855	0.21
	More org. (44:56)	3.127	2948556	0.87	13098	0.31

LOD & LOQ

The detection limit (LOD) and quantization limit20 (LOQ) may be expressed as:

L.O.D. = 3.3(SD/S)

L.O.Q. = 10(SD/S)

Where, SD = Standard deviation of the response S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.30 & 1.00 µg/ml respectively.

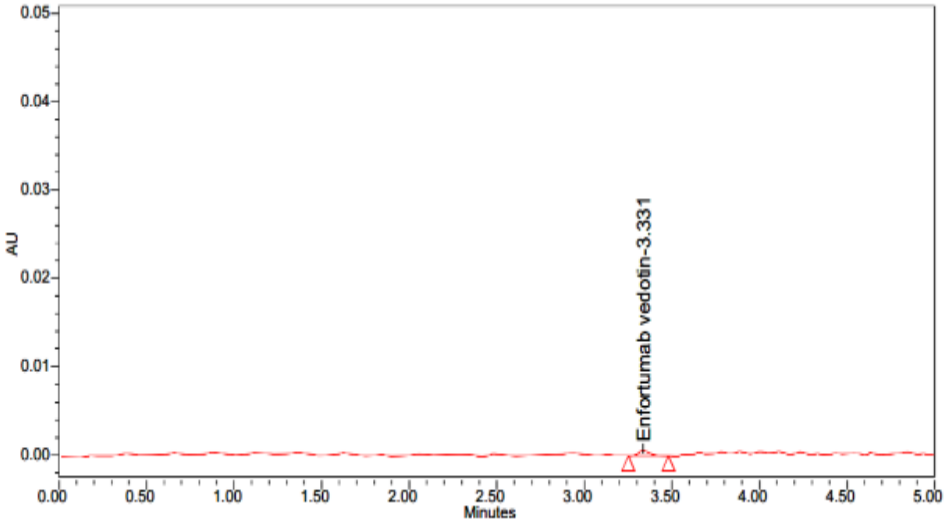

Fig. 9: Chromatogram for LOD.

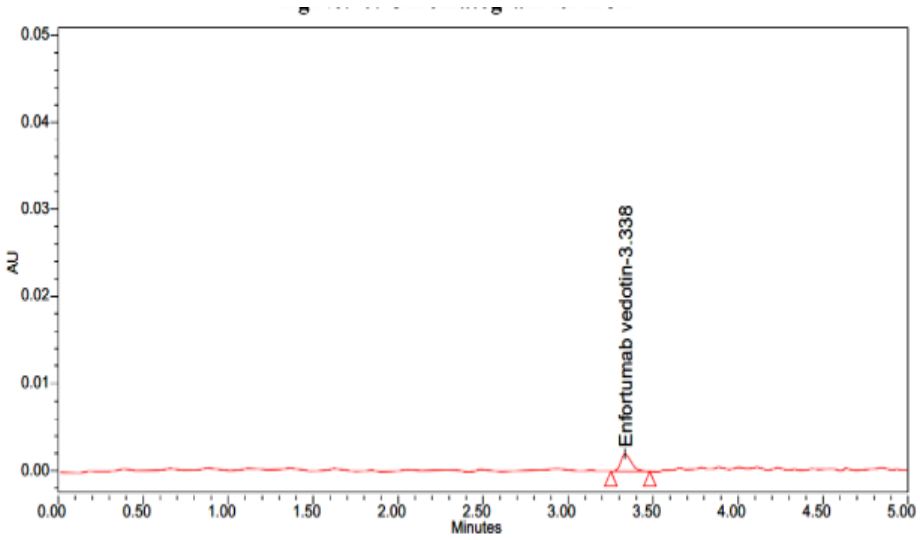

Fig. 10: Chromatogram for LOQ.

Estimation of Enfortumab vedotin in Pharmaceutical Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 10 mg of drug were transferred to 10 ml volumetric flask, and 8 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 10 ml with same solvent. Then 1ml of the above solution was diluted to 10 ml with HPLC grade ACH and Perchloric acid. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system. The solution prepared was injected in five replicates into the HPLC system22 and the observations were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-10.

ASSAY %

$$Assay=AT/AS\times WS/DS\times DT/WT\times P/100\times AW/LC\times 100$$

Where: AT = Peak Area of Enfortumab vedotin obtained with test preparation AS = Peak Area of Enfortumab vedotin obtained with standard preparation WS = Weight of working standard taken in mg WT = Weight of sample taken in mg DS = Dilution of Standard solution DT = Dilution of sample solution P = Percentage purity of working standard Results obtained are tabulated below:

Table 10: Assay of Enfortumab vedotin.

Brand	Area	Sample wt. (mg)	Label amount (mg)	Std. Purity	Amt. found (µg/ml)	% Assay
PADCEV	2737955	10	9.96	99.9	9.96	99.6

OBSERVATION

The % Purity of Enfortumab vedotin 10mg Injection containing Enfortumab vedotin was found to be 99.6%.

Stability Studies

The results of the strain studies indicated the specificity of the tactic that has been developed. Enfortumab vedotin was stable in all stress conditions 25-27 except thermal stress condition. The result of forced degradation studies are given in the following table-11.

Table 11: Results of Forced Degradation Studies of Enfortumab vedotin.

Degradation Condition	Enfortumab Vedotin				
	Area	% Assay	% Degradation	Purity Angle	Purity threshold
Control	2747135	100	0	0.428	7.684
Acid	2397265	87.2	12.8	0.451	7.656
Alkali	2386838	86.8	13.2	0.454	7.629
Peroxide	2288215	83.3	16.7	0.436	7.616
Reduction	2704295	98.4	1.6	0.447	7.608
Thermal	2428413	88.4	11.6	0.413	7.633
Photolytic	2684724	97.7	2.3	0.429	7.667
Hydrolysis	2617236	95.2	4.8	0.452	7.682

IV. CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 271nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Kromasil C18 (150x4.6mm, 3.5µm) particle size because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is ACN+ 0.1% Perchloric acid (40:60) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 5min because analyze gave peak around 3.347 min and also to reduce the total run time. The percent recovery was found to be 99-101 % was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 25-150 µg/ml of the Enfortumab vedotin target concentration. 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 Enfortumab Vedotin.

V. REFERENCES

1. https://go.drugbank.com/drugs/DB01254
2. https://pubchem.ncbi.nlm.nih.gov/compound/Enfortumab
3. https://en.wikipedia.org/wiki/Enfortumab
4. R. Snyder, J. Kirkland, L. Glajch, Practical HPLC Method Development, john Wiley and sons International publication, II Edn., 2011.
5. S. Ashutoshkar, Pharmaceutical Drug Analysis 2nd Edn, New Age International Private Limited Publishers, 2005; 452-474.
6. H. Beckett and J.B. Stenlake, Practical Pharmaceutical Chemistry, 4th End. C.B.S. Publishers and Distributors', New Delhi, 1-9: 157-167.
7. H.H. Williard, L.L. Merit, F.A. Dean, F.A. Settle, Instrumental Methods Of Analysis, 6th Edn, C.B.S. Publishers and Distributors, New Delhi., 430-440, 495-504, 529-545.
8. B.K. Sharma, Instrumental Methods of Chemical Analysis. GOEL Publishing House, Meerut, 286-300.
9. Instant notes on analytical chemistry by D.Kealey and P.J.Haines, © BIOS Scientific Publishers Limited, UK, 2002; 6-7.
10. Gurdeep R. Chatwal, Sham K. Anand, Instrumental methods of Chemical Analysis,5th edition, Himalaya Publishing House(Mumbai), 2005; 2.566.
11. M. E. Swartz, Journal of liquid chromatography, 2005; 28(7/8): 1253-1263.
12. Journal of Chromatography .B, Analytical Technologies in the Biomedical and life Sciences, March 1, 2008; 863(2): 258-265. Published on Jan 18 2008.
13. J. M. Green, a practical guide to analytical method validation, anal. Chem. News & features, 1 May 1996; 305a–309a.
14. P. A. Winslow and r. F. Meyer, defining a master plan for the validation of analytical methods, j. Validation technology, 1997; 361–367.
15. Aoac peer-verified methods program, manual on policies and procedures, Arlington, Va., USA

(1998). 16. R. Patil: J of Chromatographia, 2008; 67: 575.
16. Baht and Leena: J of Liq. Chrom., 2007; 30: 309.
17. H.H. Williard, L.L. Merit, F.A. Dean and F.A. Settle, Instrumental methods of analysis, 7th edition, C.B.S. Publishers, New Delhi, 2002.
18. GN Menon, LB White, Department of Analytical Research, Abbott Laboratories, (pub med-index for MEDLINE).
19. Food and Drug Administration (FDA), "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation;" Federal Register (Notices), 2000; 65(169): 52776–52777.
20. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4): 22-23.
21. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc., 2006; 88-92.
22. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva., 1996; 11. (CPMP/ICH/281/95).
23. Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4): 17-25.
24. A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj *et al. International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015.
25. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.
26. Lalit V Sonawane* Bioanalytical Method Validation and Its Pharmaceutical Application- A Review Pharmaceutica Analytical Acta, 2014, 5: 3. Center for Drug Evaluation and Research (CDER) Reviewer Guidance.
27. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
28. International Conference on Harmonization, Harmonized Tripartite Guideline. Validation of Analytical Procedures. Text and Methodology, November 2005; Q2(R1).
29. International Conference on Harmonization (ICH). Validation of Analytical Methods: Definitions and Terminology. ICH Q2A., 1994.
30. Panchumarthy Ravi Sankar * and Saranu Anusha, Development and Validation of Rp-Hplc Method for the Determination of Nivolumab in Tablet Dosage Form, International Journal of Pharmaceutical Sciences and Research, IJPSR, 2019; 10(10): 4531-4537.
31. Bandi Ramachandra & N.V.S. Naidu*, Validation of Rp-Hplc Method for Estimation of Nivolumab in Bulk and its Pharmaceutical Dosage Forms, International Journal of Pharmacy and Biological Sciences, IJPBS, JAN-MAR. 2014; 4(1): 61-68.
32. Y. S. R. V. S. Jogarao, Ponnuri Bharath, V. Siva Ramakrishna, D. Ramachandran, Development & Validation of RP-HPLC Method for Quantitative Estimation of Nivolumab and its Impurities in Pharmaceutical Dosage Form, Journal of Pharmaceutical Research International, 34(25A): 52-65, DOI: 10.9734/jpri/2022/v34i25A35950.
33. K.S. Nataraj, P. Sivalingachari, S. Sai Naveen, K. Alekhya, Method Development and Validation for the Estimation of Nivolumab Monohydrate Tablets by RP-HPLC, Asian J. Research Chem., September 2013; 6(9): 859-862.