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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TRABECTEDIN IN BULK AND PARENTERAL DOSAGE FORM BY RP-HPLC

G. Sirisha*, M. Gayathri Devi, M. Gowri Manoja, G. Sudhakar
Srinivasarao College of Pharmacy, Visakhapatnam.

Abstract:
A new RP-HPLC method for the quantitative determination of Trabectedin was developed and validated as per ICH guidelines. The drugs were injected into Zorbax SB, C18,(150x4.6mm); 3.5µm column maintained at ambient temperature and effluent monitored at 215nm. The mobile phase consisted of phosphate buffer (pH 3.0) and Acetonitrile in the ratio of 70:30 V/V. The flow rate was maintained at 0.8 ml/min. The calibration curve for Trabectedin was linear from 50-175µg/ml (r^2 for Trabectedin = 1). The proposed method was adequate, sensitive, reproducible, accurate and precise for the determination of Trabectedin in bulk and pharmaceutical dosage forms.

Keywords: *Trabectedin, Linearity , Accuracy, Validation.*

Corresponding author:
G.Sirisha,
Srinivasarao College of Pharmacy,
Visakhapatnam
Email id:sirisha.honey1@gmail.com

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INTRODUCTION:

Trabectedin is Indicated for treatment of advanced soft tissue sarcoma in patients refractory to or unsuitable to receive anthracycline or ifosfamide chemotherapy in Europe, Russia and South Korea. It is investigated for use/treatment in cancer/tumors (unspecified), gastric cancer, ovarian cancer, pediatric indications, sarcoma, and solid tumors. It interacts with the minor groove of DNA and alkylates guanine at the N2 position, which bends towards the major groove. In this manner, it is thought that the drug affects various transcription factors involved in cell proliferation, particularly via the transcription-coupled nucleotide excision repair system and blocks the cell cycle at the G2 phase, while cells at the G1 phase are most sensitive to the drug. It also inhibits overexpression of the multidrug resistance-1 gene (MDR-1) coding for the P-glycoprotein that is a major factor responsible for cells developing resistance to cancer drugs. The agent is also thought to interfere with the nucleotide excision repair pathways of cancer cells, suggesting that it could be effective in the treatment of many cancer types including melanoma and sarcoma, as well as lung, breast, ovarian, endometrial and prostate cancers. It is (1'*R*,6*R*,6a*R*,7*R*,13*S*,14*S*,16*R*)-6',8,14-trihydroxy-7',9 dimethoxy-4,10,23-trimethyl-19-oxo-3',4',6,7,12,13,14,16-octahydrospiro[6,16-(epithiopropano-oxymethano)-7,13-imino-6a*H*-1,3-dioxolo[7,8]isoquino[3,2-*b*][3]benzazocine-20,1'(2'*H*)-isoquinolin]-5-yl acetate with $C_{16}H_{14}C_lN_{3O}$ • HCl. It is less soluble in water is low (0.01 mg/mL), but higher in acid (up to 1.1 mg/mL) [1-6]. Various analytical methods have been reported for the estimation of Trabectedin including spectrophotometric methods and HPLC. The suggested HPTLC and HPLC methods for assay of Trabectedin are quite expensive and need complex and sophisticated instrumentation. HPLC is the most widely used technique for the estimation of Trabectedin in human plasma, saliva, cerebrospinal fluid, as well as for studying the drug metabolites in the urine. The present research work describes a HPLC and UV spectrophotometric method for estimation of Trabectedin, in API [7-8]. The present method aims at developing a simple, accurate and precise RP-HPLC method for its estimation in bulk and Pharmaceutical dosage forms.

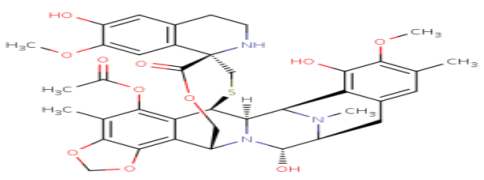


Fig. 1: Chemical structure of Trabectedin

MATERIALS AND METHODS:

Instrumentation:

A Waters module equipped with a UV spectrophotometer for finding out the λmax values of the drug used throughout this study. A Zorbax SB, C18,(150x4.6mm) 3.5µm column was employed for the method development. The chromatographic system was monitored by EMPOWER2 software. Analytes were monitored by UV detection at 215 nm using an isocratic mode with phosphate buffer (pH 3.0): Acetonitrile in the ratio 70:30 was used as mobile phase. The flow rate was set at 0.8 ml/min and effluent was monitored at 215 nm. The temperature and run time were maintained at 25°C and 15 min respectively. Solubility of the compounds was enhanced by sonication on an ultrasonicator (Bandelin Sonorex).

Chemicals and solvents:

The reference sample of Trabectedin was obtained from Natco pharma Ltd , India. HPLC grade water (prepared by using 0.45 Millipore Milli –Q) was procured from Standard Reagents, Hyderabad. HPLC grade Acetonitrile, HPLC grade water, Potassium dihydrogen phosphate, 1-Octane sulphonic acid sodium salt, O-Phosphoric acid, Triethyl amine, Methanol were bought from Merck, Mumbai.

Selection of mobile phase:

The objective of this experiment was to optimize the method for estimation of Trabectedin based on the literature survey. Various mobile phases were tested to select the best possible system. The various mobile phases used included Acetonitrile : water (40:60), phosphate buffer (pH 3.0): methanol (70:30), water: methanol (50:50) , Better peak resolution and adequate retention time were obtained with the ratio of phosphate buffer (pH 3.0):Acetonitrile (70:30).

Preparation of the Buffer:

1.36 g of potassium dihydrogen phosphate, 0.5 g of 1-Octane sulphonic acid sodium salt and 1.0 ml of triethylamine were dissolved in 1000 ml of purified water and the pH was adjusted to 3.0±0.05 with orthophosphoric acid.

Preparation of Mobile Phase:

The mobile phase was prepared by mixing 700 ml of phosphate buffer and 300 ml of Acetonitrile in a 1000 ml clean and dry flask. The mobile phase was then degassed using Ultra-Sonicator to remove dissolved gases and the resultant mobile phase was filtered through a 0.45 µm membrane filter under vacuum. Diluent is mixture of water and methanol in the ratio of 50:50 v/v.

Preparation of the standard solution:
2.0 mg of the trabectedin working standard was accurately weighed and transferred in to 100 ml amber coloured volumetric flask. 20 ml of methanol was added, sonicated to dissolve and diluted to volume with the diluent. 5ml of above solution was transferred in to a 50ml Amber coloured volumetric flask, diluted to volume with the diluent (0.02 mg/ml).

Preparation of the sample solution:
2 vials of the drug samples were selected randomly, flip of the vials were removed,the rubber septa of the vials were pricked with a needle,so that the gas present in the vials was released completely**.** 20 ml of the diluent was injected into each vial. This solution was transferred into a 100 ml amber coloured volumetric flask carefully without any loss of solution. The vials were rinsed thrice with 5 ml of the diluent each time. The solution was transferred into the same volumetric flask and diluted to volume with the diluent and mix (0.02 mg/ml). Separately injected the (20µl) of diluents as blank standard preparation and responses for the analyte peaks were recorded.

VALIDATION:
Prior to validation studies blank solution was injected and chromatogram was noted. Optimized conditions maintained were the drug was eluted with good retention time and peak area which was shown in the fig. 3.

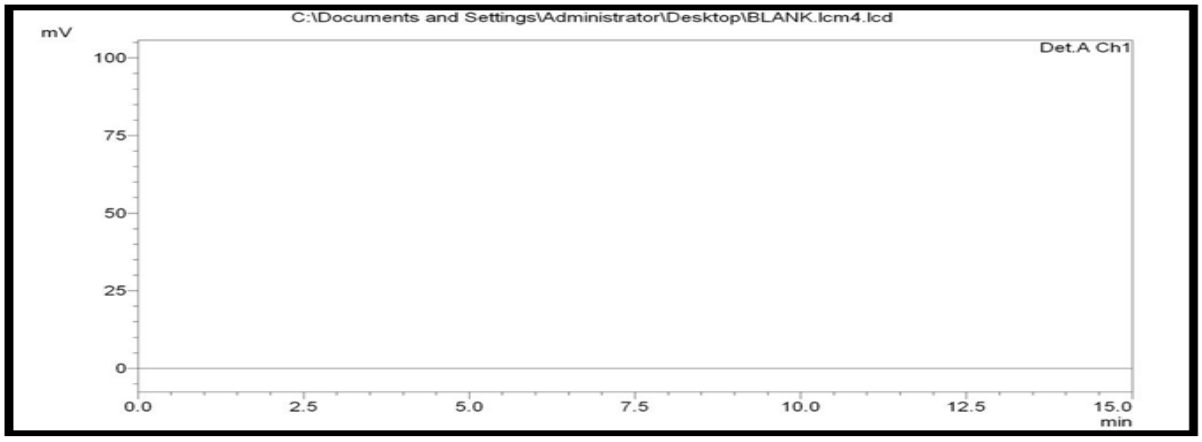


Fig. 2: Blank chromatogram

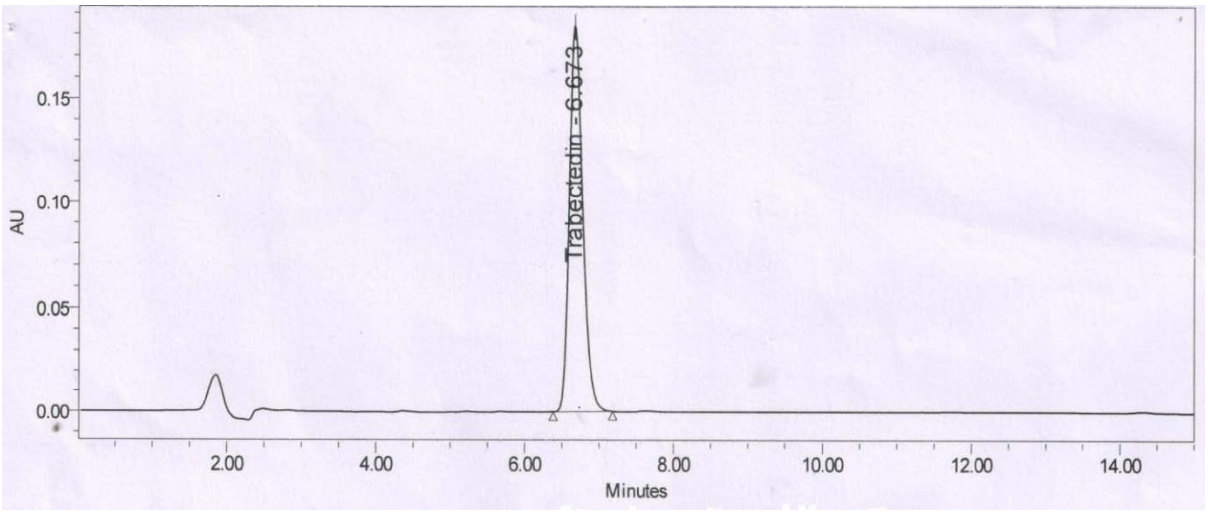


Fig. 3: Optimized Chromatogram of Trabectedin

Linearity:
The linearity of the method was established by determining the absorbance of different concentrations of Trabectedin over a range of 50-175µg/ml respectively. The linearity data was given in table 1.

Table 1: Linearity data of Trabectedin

Actual Concentration (mg/ml)	% Linearity Level	Peak Area
0.01	50	1154007
0.015	75	1732629
0.02	100	2310239
0.025	125	2887601
0.03	150	3462301
0.035	175	4042091

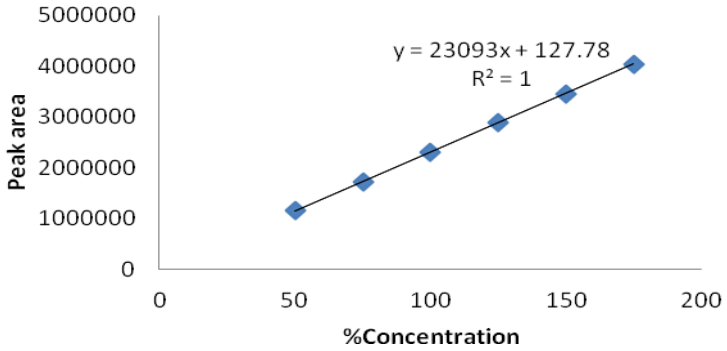


Fig. 4: Calibration curve of Trabectedin

Accuracy:
To determine the accuracy of the proposed method, recovery studies were carried out by analyzing the samples were carried out by analyzing the measured concentration and the added concentration of the drug. Each sample was injected thrice .The percent recoveries of the drugs were estimated. The accuracy data was represented in table 2.

Table 2: Accuracy data of Trabectedin

Recovery level	Accuracy Trabectedin					Average %Recovery
	Amount taken (mg/ml)	Area	Average area	Amount recovered (mg/ml)	% Recovery	
80%	0.016	1733804	1735731	0.00796	99.5	99.51
	0.016	1738538				
	0.016	1734851				
100%	0.02	2166943	2166507.6	0.00999	99.90	
	0.02	2168262				
	0.02	2164318				
120%	0.024	2600321	2606518	0.0119	99.16	
	0.024	2597101				
	0.024	2622132				

Precision:

Precision is one of the important factors which determine the reliability of an analytical method. The precision of the developed method was tested and was found to be suitable. Both system and method precision were performed and are given in table 3,4.

Table 3: System precision data of Trabectedin

Number of Injections	RT	Peak Area
1	6.661	2166730
2	6.665	2173173
3	6.662	2168564
4	6.669	2166943
5	6.672	2164318
6	6.672	2169845
Average	**2168262**	
SD	**3044.77**	
%RSD	**0.14**	

Table 4: Method precision data of Trabectedin

Number of Injections	RT	Peak Area
1	6.665	2173173
2	6.662	2168564
3	6.669	2166943
4	6.672	2164318
5	6.661	2166730
6	6.662	2168564
Average		**2168048.667**
SD		**2955.3065**
% RSD		**0.136**

Robustness:

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like volume of injection, wavelength which may differ but the responses were still within the limits of the assay.

Table 5 : Robustness data of Trabectedin

Drug / Variation Parameter	Variation	Rs	Tailing Factor	Number of Theoretical Plates (Efficiency)
Flow rate (± 0.2)	0.6 ml/min	7.1	1.29	6871
		7.2	1.27	6912
		7.1	1.29	6920
	0.8 ml/min	6.661	1.27	6544
		6.665	1.28	6591
		6.672	1.27	6677
	1 ml/min	6.112	1.19	6622
		6.121	1.20	6598
		6.121	1.23	6598

Table 6: Robustness data of Trabectedin

Drug / Variation Parameter	Variation	Rs	Tailing Factor	Number of Theoretical Plates (Efficiency)
pH (± 0.2)	3.0	6.681	1.29	6389
		6.661	.130	6398
		6.72	1.29	6389
	3.2	6.672	1.27	6449
		6.661	1.26	6554
		6.669	1.28	6458
	3.4	6.689	1.29	6501
		6.681	1.30	6498
		6.672	1.32	6498

Table 7: Robustness data of Trabectedin

Drug / Variation Parameter	Variation	Rs	Tailing Factor	Number of Theoretical Plates (Efficiency)
Organic phase (± 5 %)	35 %	6.532	1.20	6498
		6.538	1.19	6476
		6.539	1.20	6489
	30 %	6.662	1.24	6546
		6.667	1.21	6539
		6.668	1.13	6558
	25 %	6.874	1.27	6449
		6.894	1.29	6459
		6.876	1.30	6466

LOD and LOQ:

Limit of Detection (LOD)

$$LOD = \frac{3.3\sigma}{S}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte

Limit of Quantification (LOQ)

$$LOQ = \frac{10\sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of theS calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The results of LOD and LOQ for the drug are shown in table 8 below.

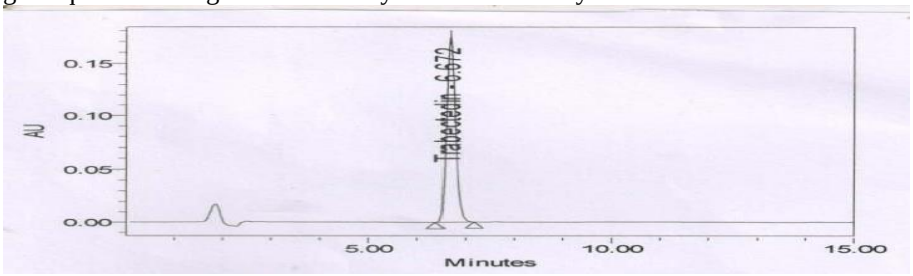
Table 8: Results of LOD and LOQ

	Trabectedin	
S.No.	**Concentration ng/ml**	**Peak Area**
1	4	927.12
2	5	1139
3	6	1301
4	7	1517
5	8	1721
S.D.	**1.58**	**311**
Slope	196.6	

The LOD for this method was found to be 0.016 ng/ml and The LOQ for this method was found to be 0.050ng/ml.

Assay:

Assay of different parenteral formulations of Trabectedin available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out.



The %Assay of Trabectedin working sample was 99.98**%.**

DISCUSSION:

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and economical RP-HPLC method. It was successfully applied for the determination of Trabectedin in pharmaceutical dosage forms without the interferences of other constituents in the formulations. Different mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was done based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Phosphate buffer (3.0 pH) : Acetonitrile (70:30) with 0.8ml/min flow rate was quite robust. The optimum wavelength for detection was 215 nm at which better detector response for drug was obtained. The average retention time of Trabectedin was found to be 6.667 mins .The calibration was linear in concentration range of 50-175 mcg/ml for Trabectedin **.** The low values of % RSD indicate the method was precise and accurate. Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. These results show the accuracy and reproducibility of the assay. The LOD and LOQ values were also in limits. The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. There was no significant difference in the results achieved by the proposed method.

CONCLUSION:

The proposed method for the assay of the Trabectedin in the commercially available dosage formulation was simple, accurate, economical, and rapid. It can be easily adopted for routine quality control for monitoring the assay in the API, in-process samples, and the finished tablet formulation.

REFERENCES:

1.Sharma BK. Instrumental methods of chemical analysis, Introduction to Analytical chemistry: Goel Publishing House Meerut, 2004; 233-238, 23th edition.
2.Michael E, Schartz IS, Krull. Analytical method development and Validation, 2004; 25-46.
3.ICH, Validation of Analytical Procedure. Text and Methodology Q2(B), International conference on Harmonization, IFPMA, Geneva, Switzerland, 2005.
4.Lichter; et al. Worthen LW, ed. "Food-drugs from the sea. Proc: Aug 20–23, 1972". 173. Marine Tech Soc: 117–127.
5. http://www.drugbank.ca/drugs/DB05109
6.http://en.wikipedia.org/wiki/Trabectedin
7.Monique Zangarini, Laura Ceriani, Federica Sala, Elena Marangon, Renz, Bagnati, Maurizio D'Incalci, Federica Grosso, Quantification of Trabectadin in Human plasma; J Pharm Biomedical analysis; 2014;95:107-112.
8.Csaba Gajdos and Anthony Elias ; trabectedin-safety-and-efficacy-in-the-treatment-of-advanced-sarcoma-, Clinical medicine insights:oncology; 2011;5, 35.