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TRABECTEDIN AS AN ANTICANCER AGENT AND RECENTLY DEVELOPED ANALYTICAL TECHNIQUES IN TRABECTEDIN-A SHORT REVIEW

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ABSTRACT

Cancer is a condition in which the unwanted cells spilt up excessively and penetrate neighboring tissues. By the blood and lymph system cancer cells can transmit to other parts of the body. If left untreated cancer may leads to death. If diagnosed early and treated it is curable. Trabectedin is an anticancer agent used for the treatment of cancer. This review focuses on Trabectedin as an anticancer agent and the recently developed analytical techniques in Trabectedin. In this review the analytical techniques in Trabectedin include RP-HPLC, LC-MS method. In RP-HPLC method was developed according to ICH guidelines, for quantifying Trabectedin drug substance and the method is validated. The drugs were injected into a Zorbax SB, C18 (150x4.6mm); 3.5m column kept at room temperature, and the effluent was measured at 215nm. The mobile phase was composed of phosphate buffer (pH 3.0) and acetonitrile in a 70:30 V/V ratio. The flow rate was kept constant at 0.8 ml/min. Trabectedin's calibration curve was linear from 50 to 175 g/ml (r2 for Trabectedin = 1). For the determination of Trabectedin in bulk and pharmaceutical dosage forms, the proposed method was adequate, sensitive, reproducible, accurate, and precise.In LC-MS method was validated in accordance with US Food and Drug Administration guidelines. Linearity, LLOQ, intra- and inter-day accuracy and precision, recovery, matrix effect, selectivity, and stability were among the parameters studied.

Keywords: Trabectedin, RP-HPLC, Anticancer, LC-MS, Validation

INTRODUCTION:

Trabectedin (Yondelin) also named as ET-743 belongs to the category of anti-tumour drug, obtained from marine tunicate Ecteinascidia turbinata. In adults, after the non-success of the current remedy of anthracyclines and ifosfamide trabectedin has become accepted within the European Union in July 2007 for the remedy of soft-tissue sarcomas (STS). In a pre-clinical investigation indicated chemotherapeutic action of invitro and invivo in ovarian, breast, prostate, renal, cancer and non-small cellular lung cancer. Trabectedin is efficient for the treatment of ovarian cancer **[1]**. Soft tissue sarcomas appears from mesenchymal tissues belongs to the category of tumor. Recent therapies include for soft tissue sarcomas include Trabectedin, eribulin, pazapanib other than traditional chemotherapeutic sellers. There are different chemotherapy medications are used, but Trabectedin is specific as it has the ability to persist for many cycles due to loss of increasing lethality **[2]**. Various research offers the mode of action of Trabectedin is greater complicated than usual standard methods of chemotherapeutic substances. Abnormal structure of the compound permits the drug to associate with DNA via covalent binding at the N2 guanine at the minor DNA groove, additionally to protrude stick out of the DNA helix as a consequence being available to bind numerous DNA-binding molecules which includes transcriptional elements and DNA restore proteins **[3]**. It is recommended by European guidelines as an effective second-line treatment for patients with advanced soft tissue sarcomas (STS) and is undoubtedly an appealing treatment option for leiomyosarcoma patients. Trabectedin had identified pleotropic mechanisms of action, which in addition to inducing direct growth. It also has anti-inflammatory and immunomodulatory properties that cause malignant cells to be arrested and killed as a result of inhibiting factors that promote tumour growth, angiogenesis, and metastasis as well as metastasis. The standard trabectedin dose for STS treatment is 1.5 mg/m2. Every three weeks, as an intravenous infusion lasting 24 hours (dosage reductions are permitted) based on the patient's tolerability .The most likely trabectedin side effects are nausea and vomiting, myelosuppression, fatigue, and transaminases, all of which are serious conditions. There is no evidence that trabectedin treatment causes alopecia or other cardiovascular side effects **[4]**. The molecular formula of Trabectedin is $C_{39}H_{43}N_3O_{11}S$ and

the molecular weight is 761.8.Trabectedin belongs to the class of drugs of Antineoplastic and Alkylating agents **[5]**.

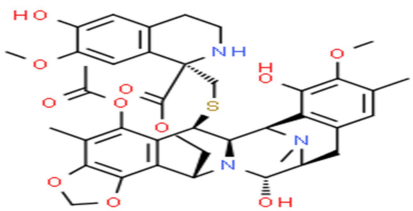

Figure 1: Structure of Trabectedin [5]

This compound's distinct properties make it a valuable tool for deciphering complex mechanisms involving gene transcription regulation and DNA repair **[6]** Trabectedin (Yondelis) is effective against soft tissue sarcoma, Liposarcoma and leiomyosarcoma **[7]**.

NEED OF ANALYTICAL METHOD VALIDATION:

Analytical method validation: Laboratory research is used to validate an analytical approach, ensuring that the procedure's execution attributes meet the requirements for the proposed scientific application. Validation is required for any new or modified procedure to ensure that it is capable of producing predictable and dependable results when used by different administrators using comparable instrumentation in the same or completely different laboratories. The validation of analytical method is used to confirm that the analytical procedure used for a specific test meets the intended requirements. Guidelines from the USP, ICH, and the FDA, among others, can serve as a framework for pharmaceutical method validations. The method validation results can be used to assess its quality and reliability **[8]**.

For the determination of trabectedin the few analytical methods included in literature review includes RP-HPLC **[9]**, LC-MS **[10-12]** stating that the method is selective, accurate, sensitive, and precise.

MATERIALS AND METHODS:

ANALYTICAL METHODS IN ANALYZING TRABECTEDIN SAMPLE

RP-HPLC METHOD:

Reagents and instrumental conditions:

A Zorbax SB, C18, (150x4.6mm) 3.5µm column was used in the study with instrumentation used is UV Spectrophotometer; software used for the study is EMPOWER-2.Mobile phase used is Acetonitrile. HPLC grade Acetonitrile, HPLC grade water, Potassium dihydrogen phosphate, 1-Octane sulphonic acid sodium salt, O-Phosphoric acid, Triethyl amine, Methanol were the reagents used **[9]**.

Procedure for Sample preparation:

The two vials were taken arbitrarily and the capsize of the vials were detached. The rubber septa of the vials were punctured with

a needle, to ensure that all the gases were released. Twenty ml of the diluent was injected into the sample vialed drugs. Transfer the resultant solution into 100 ml ambered volumetric flask, ensuring that the there is no single drop of solution is wasted. Three times the bottles were washed by using 5ml of the diluent. The diluted solution was transferred into the same volumetric flask and diluted with diluent and mix (0.02 mg/ml).

The diluents were separately injected as blank standard preparation and responses for the analytes were recorded **[9]**.

LC-MS Methods:

The earliest LC-MS/MS method for trabectedin quantification required a complex plasma solid-phase extraction (SPE) process and 500 L of sample. It had a lower limit of quantification (LLOQ) of 0.01 ng/mL **[11]**.

Standard and QC Solution preparations:

Two stock solutions of trabectedin and d3-trabectedin in DMSO at 100 g/mL each were prepared. The trabectedin stock solutions were diluted into two series of working solutions with nominal concentrations of 100, 10, and 1 ng/mL in acetonitrile–0.1% formic acid in water (70:30, v/v). These solutions were used to make plasma calibrators and quality control samples. A working solution of d3-trabectedin (internal standard) was prepared at 100 ng/mL in acetonitrile–0.1 percent formic acid in water (70:30, v/v) and stored at 30°C. For each batch of analysis and during the validation study, eight-point calibration curves were established. Working solutions of trabectedinin drug-free plasma were diluted to final concentrations of (A) 2.5 ng/mL, (B) 1.0 ng/mL, (C) 0.5 ng/mL, (D) 0.25 ng/mL, (E) 0.1 ng/mL, (F) 0.05 ng/mL, (G) 0.025 ng/mL, and (H) 0.01 ng/mL. In drug-free plasma, three QC samples were prepared at high (0.80 ng/mL, QC-H), medium (0.16 ng/mL, QC-M), and low (0.04 ng/mL, QC-L) concentrations. Each analysis batch included a double blank (plasma without the internal standard) and a blank (plasma with the internal standard) **[11]**.

RESULTS AND DISCUSSION:

Method Validation:

Validation is the examination and provision of objective evidence that the specific requirements for a specific intended use are met. A method of assessing method performance and demonstrating that it meets a specific requirement. In other words, it understands what your method is capable of delivering, especially at low concentrations **[10]**.

Validation Parameters:

RP-HPLC METHOD

Linearity:

Trabectedin was tested for its linearity by me asuring the absorbance at different concentrations of 50-175 g/ml **[9]**. **Table 1** shows the Linearity data of Trabectedin.

Accuracy:

In order to determine the accuracy of the intended method, recovery studies were conducted by studying the measured concentration and the added concentration of the drug. Each sample was injected three times, and the percentage recoveries of the drugs were then determined. The average percentage recoveries of the trabectedin drug substance were found to be 99.51% **[9]**. **Table 2** shows the accuracy data of Trabectedin.

Precision:

Precision is one of the critical factors which determine the safety of an analytical approach. The precision of the evolved technique was examined and become discovered to be satisfactory. System precision and method precision were carried out **[9]**. **Table 3 and 4** shows the system precision and method precision data for trabectedin.

Assay

The assay of various Trabectedin injectable formulations on the market was performed by injecting a sample of equivalent weight into an HPLC system and performing recovery experiments.

The Assay (percentage purity of Trabectedin sample) was found to be 99.98 % **Figure 1 [9]**.

Method Validation:

LC-MS METHOD:

The method was validated in accordance with the guidelines of the United States Food and Drug Administration Linearity, LLOQ, intra- and inter-day accuracy and precision, recovery, matrix effect, selectivity, and stability were among the parameters investigated **[11]**.

Linearity and LLOQ

The correlation coefficient for calibration curves in the range of 0.01–2.5 ng/mL (interpolated with a 1/x weighting factor) was 0.9902, indicating that the method is linear. Back-calculated concentrations yielded a RE ranging from -13.31 percent to 10.68 percent, while the RSD ranged from 0.04 percent to 13.83 percent when tested over three independent runs. These results show a high level of accuracy and precision. The LLOQ was assigned to the calibrator concentration with the lowest RE and RSD of 20%. The method maintained this level of accuracy and precision at 0.01 ng/mL trabectedin (RE 7.80 percent; RSD 13.83 percent) under the current experimental

conditions. The MRM chromatograms of plasma blank and a plasma sample containing trabectedin at the LLOQ are shown in **Figure 2 [11]**.

Intra and inter day accuracy and precision:

For the QC samples, the intra-day accuracy (RE) ranged from -8.52 percent to 1.19 percent, while the precision (RSD) ranged from 3.95 percent to 12.35 percent. The inter-day accuracy and precision for the QC samples were -6.78 percent to -1.92 percent and 6.57 percent to 10.74 percent, respectively, for the three days of testing. The validation criteria are met by these analyses **[11]**. **Table 5**, **s**hows the data for trabectedin in plasma for Intra and Inter day accuracy and precision.

Recovery and matrix effect:

To maximize the overall recovery of trabectedin from the plasma matrix, several solvent extraction trials were performed. Acetonitrile proved to be the most effective solvent, particularly when containing 1% formic acid, with a mean overall recovery of 54.4 percent (SD = 3.8 percent) . The matrix effect was investigated by comparing the trabectedin MS signals from protein-free plasma samples containing the drug at QC concentrations and drug solutions **[11]**.

Selectivity:

The absence of interfering peaks close to the trabectedin retention time in the MRM chromatograms of six different plasma samples alone (double blanks) and spiked with trabectedin at the LLOQ was revealed by analysis of six different plasma samples alone (double blanks) and spiked with trabectedin at the LLOQ **[11]**.

Stability:

For 4 hours, QC samples were found to be stable at both 4 degree Celsius and room temperature, indicating that the sample preparation procedure can also be performed at room temperature within 4 hours of collection .Furthermore, freeze-thaw cycles had no discernible effect on trabectedin stability, and plasma extracts kept at 4 degree Celsius in the auto-sampler demonstrated good stability up to 24 hours with an accuracy of the QC concentrations less than 8.63 percent. Finally, the long-term stability of QCs at 30 degrees Celsius over 3 months was marked by a slow degradation has not exceeded 15%. Overall, these findings are consistent with those reported in previous studies **[11]**. **Table 6** shows the Stability data for Trabectedin.

Table 1: Trabectedin-Linearity data

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

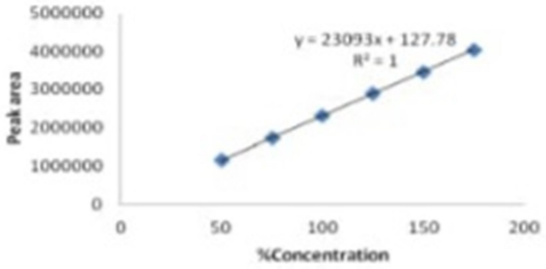

Figure 2: Calibration curve of trabectedin

Table 2: Trabectedin-Accuracy data

Recovery level	Accuracy Trabectedin					Average %Recovery
	Amount taken (mg/ml)	Area	Average area	Amount recovered (mg/ml)	%Recover	
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				

Table 3: Trabectedin System precision data

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	

% RSD of system precision was found to be 0.14

Table 4: Trabectedin method precision data

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	

% RSD of method precision was found to be 0.13

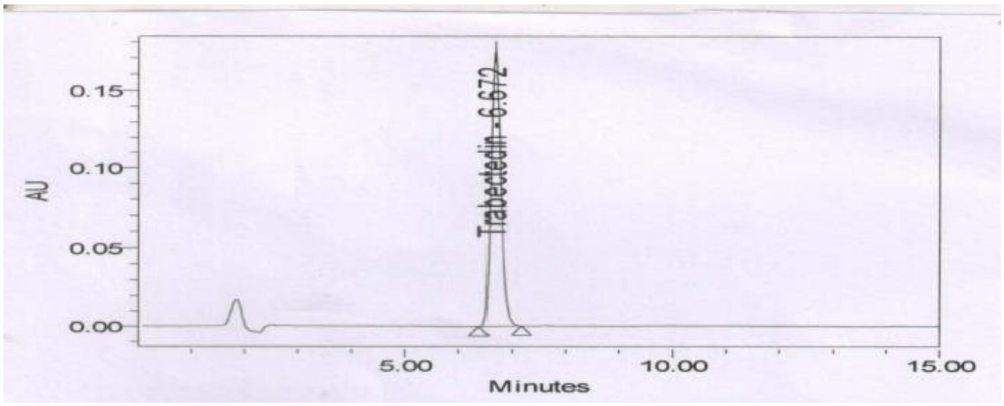

Figure 1: Chromatogram of Trabectedin sample

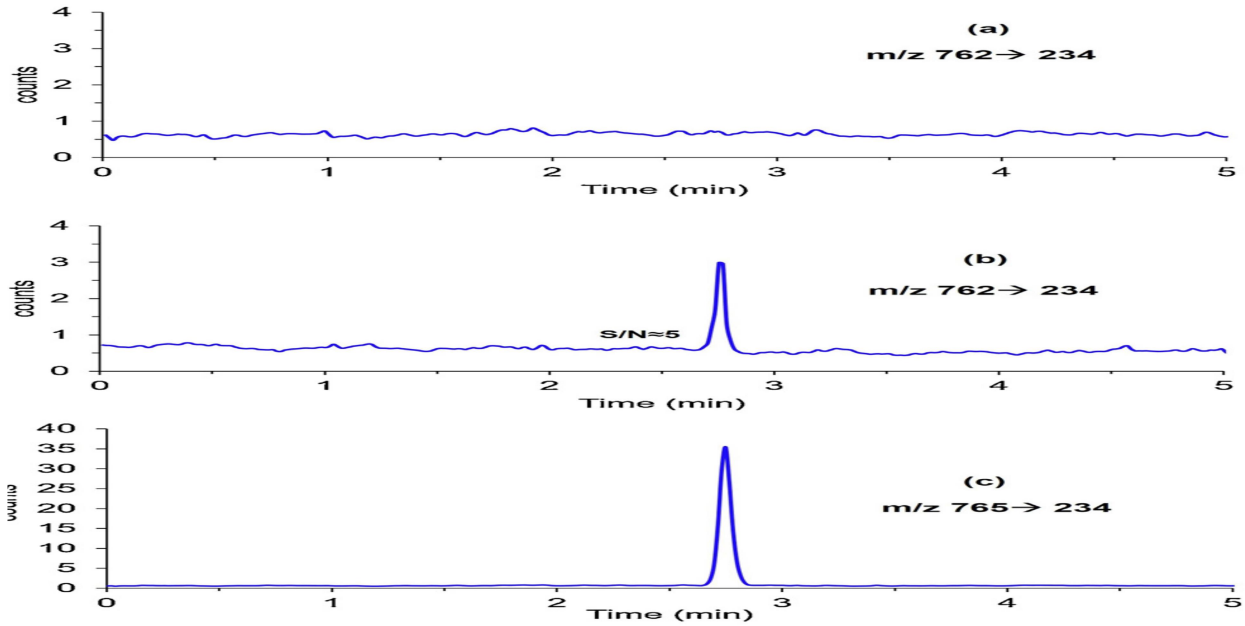

Figure 2: Multiple reaction monitoring (MRM) chromatograms: (a) plasma blank; (b) plasma with trabectedin added at the LLOQ (0.01 ng/mL); and (c) plasma supple-mented with d3-trabectedin (internal standard)

Table 5: Trabectedin in Plasma for Intra and Inter day accuracy and precision

Sample concentration	Intraday		Interday	
	Day1(n-12)	Day2(n-6)	Day3(n-6)	Days1-3(n-24)
LLOQ,0.01ng/mL				
RSD%	13.83	9.30	11.22	12.49
RE%	-0.20	-7.16	7.80	0.72
QC-L,0.04ng/mL				
RSD%	12.35	395	11.66	10.74
RE%	-3.21	-6.24	1.19	-2.87
QC-M,0.16ng/mL				
RSD%	6.26	7.97	6.76	6.57
RE%	-1.50	-1.37	-1.32	-1.92
QC-M,0.16ng/mL				
RSD%	4.75	630	10.61	7.00
RE%	-7.75	-3.10	-8.52	-6.78

Table 6: Trabectedin-Stability data

Conditions	Nominal concentration (ng/mL)	RE (%)
Short-term stability:4˚c,4h	0.04	10.72
	0.16	-0.44
	0.80	-4.47
Short –term stability :room temperature ,4h	0.04	10.39
	0.16	1.59
	0.80	9.60
Plasma extract stability:4 ˚c,24h	0.04	2.16
	0.16	8.43

Mohammad Shahin, K. Ayesha begum *et al* **[7]** has done a study on the YondelisTM (ET-743, trabectedin) in human plasma: a simple and sensitive liquid chromatographic quantitative analysis using column switching and tandem mass spectrometric detection.The authors describe the development of a simple and sensitive assay for the quantitative analysis of the marine anticancer agent Yondelis (ET-743, trabectedin) in human plasma using liquid chromatography with column switching and tandem mass spectrometric (MS/MS) detection. Following methanol protein precipitation, diluted extracts were injected onto a small LC column (10 3.0 mm i.d.) for on-line concentration and sample clean-up. Following that, the analyte and the deuterated internal standard were back-flushed onto an analytical column for separation and detection in an API 2000 triple-quadrupole mass spectrometer. Using 100 l of plasma, the lower limit of quantitation was 0.05 ng mL1, with a linear dynamic range of up to 2.5 ng mL1. The method was validated using the most recent FDA guidelines for bioanalytical method validation. The time required for off-line sample preparation has been reduced by tenfold when compared to an existing LC/MS/MS method for ET-743 in human plasma that used a labor-intensive solid-phase extraction **[12]**.

Mohammad Shahin, K. Ayesha begum *et al* **[7]** has done a study on the Ecteinascidin 743, a new potent marine-derived anticancer drug, was analysed in human plasma using high-performance liquid chromatography in conjunction with solid-phase extraction. A reversed-phase high-performance liquid chromatographic method for quantifying the novel anticancer drug Ecteinascidin 743 in human plasma has been developed and validated. The plasma samples were pretreated using solid-phase extraction (SPE) on cyano columns. To account for variation in injection volumes, propyl-p-hydroxybenzoate was added after the sample pretreatment. The separation was carried out on a Zorbax SB-C18 column (754.6 mm I.D., particle size 3.5 m) with the mobile phase acetonitrile–25 mM phosphate buffer, pH 5.0 (70:30, v/v). The flow rate was set to 1.0 ml/min, and the eluent was measured at 210 nm. The assay's accuracies and precisions are within 15% for all quality control samples and within 20% for the lower limit of quantitation, which was 1.0 ng/ml using 500 ng/ml. The overall recovery of the Ecteinascidin 743 sample pretreatment procedure was 87.05.9 percent. For at least two months, the drug was found to be stable in human plasma at 30°C. Ecteinascidin 743 was stable in human plasma for up to 5 hours at room temperature **[12]**.

CONCLUSION:

The method includes in this review for the quantification of Trabectedin includes RP-HPLC, LC-MS, and methods indicating that it is selective, sensitive, and precise. All the developed methods follow the guidelines of ICH. LC-MS methods for the quantification of trabectedin are used in the bio analytical methods for clinical study.RP-HPLC method developed in this review indicates that the assay of Trabectedin is 99.98% proving the drug sample is pure and can be used for the routine analysis of quality control, and in the formulation.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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