Analytical method Validation for the determination of Residual solvents Analysis in Trabectedin drug substance by Head space Gas chromatography (HSGC)

B.Prathima,[1] M.Vijey Aanandhi,[*] N.K. Srinivasa Rajagopalachari

[1]Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical sciences,

Vels Institute of Science Technology and Advanced studies (VISTAS), Pallavaram,

Chennai, Tamilnadu, India.

[*]Corresponding author

Name: Dr.M.Vijey Aanandhi, Department of Pharmaceutical Chemistry and Analysis,School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced studies(VISTAS), Pallavaram,Chennai, Tamilnadu, India.

Email:hodpchemistry@velsuniv.ac.in

Abstract:

The aim of the work is focused on the Validation for the determination of Residual solvents analysis in Trabectedin drug substance by Head space gas chromatography (HSGC).Trabectedin is an oncology class of drug used for the treatment of cancer. In Pharmaceutical industry the analysis of Residual solvents is an important factor to be considered as the toxicity of many of these solvents may cause possible risk to human health. Hence the limits of these residual solvents must be noted and followed by pharmaceutical industries which was set and stated by ICH. The Chromatographic separation was performed using GC with Flame Ionization detector (FID) using Helium as “Carrier Gas” with CP Select -624 (60m X0.32mm, 1.8µm film thickness) column and Split ratio 10:1 with N-Methyl-2-pyrrolidone as diluent

Results: All the Solvents (Methanol, Ethanol, Hexane, Dichloromethane, Isopropyl ether, Ethyl acetate, Acetonitrile, Tetrahydrofuran, Acetone, Isopropyl Alcohol) mentioned under ICH Q3C (4) were eluted correspondingly at the individual retention time and the method has been validated as per ICH Q2R1 Guideline.

Introduction:

FDA(Food and Drug Administration) accepted Trabectedin(Yondelin) is the first marine-derived anti-neoplastic drug used to manage and cure liposarcoma(a rare type of cancer that begins in fat cells) , leiomyosarcoma(a malignant cancer tumor of smooth muscle cells that can arise almost anywhere in the body, but is most common in uterus, abdomen, or pelvis) that are unable to clear through surgical procedure or that have been spread from one part of the body to another which is termed as Metastasized. Trabectedin possess combined anti-cancer effectiveness when joined

with the chemotherapy. (1) The approved dose of Trabectedin is 1.5mg/m^2 which is given intravenously over 24 hours every 21 days. In a Phase 1 study for children 3 hour infusion reveled that the approved dose of Trabectedin for children was 1.1mg/m^2 . Trabectedin at an elevated lethal dose are more danger to the patients suffering from hepatic dysfunction. (2)

For the analysis of residual solvents Flame ionization detector (FID) is the most commonly used detector in the pharmaceutical industry due to its high separation efficiency and sensitivity for volatile organic compounds.(3)

Keywords: FDA-Food and Drug Administration Act, ppm- Parts per million, FID-Flame Ionization detector, HSGC- Head Space coupled with Gas Chromatography, mL/min-Milli Liter per Minutes, °C-degree Celsius, mg-Milligram, LOD-Limit of Detection, LOQ-Limit of Quantification, S/N-Signal to Noise, RSD-Relative standard deviation

Methodology:

Instrument and Analytical conditions:

Instrumentation: Agilent Gas chromatography 7890 GC System with G1888 Head space sampler with FID detector.

Mobile phase: Helium as Carrier Gas

Diluent: N-Methy-2-Pyrrolidone

Head space condition:

Column: CP Select -624 (60m X0.32mm, 1.8µm film thickness) column , Oven Temperature: 85°C, Loop Temperature: 110 °C, Transfer line temperature: 120°C, GC Cycle time: 55 minutes, Equilibration time: 30 minutes, Pressurization time: 0.20 times, Loop fill time: 0.20 min, Loop Equilibration time: 0.05 min, Injection time:1.0 min, vial size: 20 mL, shake mode : Low

Chromatographic conditions:

Oven Temperature (T1): 40 °C, Time(t1):27 min, Rate(1): 20 °C per min, Oven Temperature(T2): 240 °C, Time(t2): 8 min, Injector Temperature: 200 ° C, Detector temperature: 270 °C, Gas Flow: 2.0 mL/min, spit ratio:10:1, Hydrogen flow: 40 mL/min, Zero air flow: 400 mL/min, Run time: 45 minutes

Software used: Empower-3.

Method Validation Parameters/ Performance Characteristics:

Specificity: Prepared individually each standard solvent solution, Trabectedin sample solution and standard solvents spiked at specification level of Trabectedin sample solution. These solutions were analyzed to verify the retention time of standard solvents. No blank interference was observed .Refer Table-1 for Specificity results.

Table-1

Peak name	Retention time		Results obtained from sample solution (in ppm
	Standard solution	Spike sample solution	
Methanol	5.99	5.99	46
Ethanol	8.09	8.10	22
Isopropyl alcohol	10.10	10.10	21
Acetone	9.59	9.60	19
Acetonitrile	11.08	11.09	Not detected
Dichloromethane	11.40	11.40	Not detected
Hexane(Fr-2,Fr-3,Fr-4&Fr-6)	10,83,11,93,13,21&16.77	10,83,11,94,13.22&16.78	5
Diisopropyl ether	14.48	14.48	Not detected
Ethyl acetate	18.93	18.94	Not detected
Tetrahydrofuran	20.16	20.16	Not detected

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ was established for each standard solvents by S/N ratio method. Refer Table-2 for LOD & LOQ results. Acceptance Criteria: LOD: S/N ratio ≥ 3, LOQ: S/N ratio ≥ 10, Precision at LOQ (%RSD): %RSD of peak area of each standard solvent in LOQ solution should be not more than 15.0%.

Table-2

Standard solvent	LOD concentration in ppm w.r.t sample	Average S/N Ratio	LOQ Concentration in ppm w.r.t sample	S/N Ratio	Precision at LOQ(%RSD)
Methanol	19.87	4.90	49.68	10.78	3.78%
Ethanol	20.90	3.50	64.31	11.57	5.03%
Isopropyl alcohol	17.79	4.37	69.56	12.08	5.53%
Acetone	4.84	5.30	17.74	11.62	1.96%
Acetonitrile	14.43	4.91	55.10	14.27	3.43%
Dichloromethane	40.57	4.19	109.54	11.14	3.38%
Hexanes	1.81	4.56	6.04	13.60	2.93%

Diisopropyl ether	2.07	4.39	9.56	13.25	3.60%
Ethyl acetate	16.13	4.17	45.16	11.80	2.89%
Tetrahydrofuran	10.52	4.07	28.04	10.96	3.90%

Linearity and Range: Linearity was demonstrated by analyzing solutions containing each standard solvent ranging from LOQ to 150% concentration (i.e. LOQ level, 40%, 80%,100%,120% & 150%). Table 3 summarized the results for Linearity.

Table-3

Name of the standard solvent	***Correlation coefficient (Acceptance Criteria: NLT 0.995)***	***Regression equation***	***Slope***	***Intercept***	***%Intercept (Acceptance criteria: ± 5%)***
Methanol	1.000	0.999	0.02150	0.29222	0.5
Ethanol	1.000	0.999	0.02194	0.88514	0.4
Isopropyl alcohol	1.000	0.9999	0.02226	0.63837	0.6
Acetone	1.000	1.0000	0.07623	1.23500	0.3
Acetonitrile	1.000	0.9991	0.02468	0.50429	5
Dichloromethane	1.000	0.9996	0.01394	0.09981	1
Hexanes	1.000	0.9999	0.47004	-1.12615	-0.8
Diisopropyl ether	1.000	1.0000	0.22646	-1.01231	-0.1
Ethyl acetate	1.000	1.0000	0.04902	0.78780	0.3
Tetrahydrofuran	1.000	1.0000	0.08286	-0.04233	-0.1

Accuracy: Accuracy of the method was determined by Analyzing Trabectedin sample solutions spiked at each standard solvent at 50%, 100% and 150% of the working strength. The Percentage recoveries were tabulated under Table-4.

Table-4

Name of the standard solvent	***Average Recovery at 50 % level***	***Average Recovery at 100 % level***	***Average Recovery at 150 % level***	***Acceptance criteria***
Methanol	97.6	95.8	96.5	% Recovery should be between 80 % to 120%
Ethanol	100.0	97.6	98.1	
Isopropyl alcohol	101.4	98.6	99.0	
Acetone	108.8	104.6	104.9	
Acetonitrile	106.0	98.6	96.9	
Dichloromethane	101.0	98.6	98.9	
Hexanes	103.6	102.4	102.0	
Diisopropyl ether	102.3	100.8	101.1	
Ethyl acetate	102.1	99.3	99.5	

Tetrahydrofuran	100.9	99.1	99.2	

System Precision: Demonstrated by showing that the %RSD obtained for the standard solvents in the range from 0.16% to 0.61%.

Method Precision: Precision of the method was analyzed using Trabectedin sample solution spiked with each standard solvent of working concentration. Results was tabulated under Table-5.

Table-5: Method Precision

Name of the solvent	Average content of residual solvents in ppm from Spiked sample solution	%RSD obtained
Dichloromethane	621 ppm	1.42%
Ethanol	9914 ppm	1.23%
Diisopropyl ether	4988 ppm	1.78%
Ethyl acetate	5068 ppm	1.32%
n-Hexane	293 ppm	2.00%
Acetonitrile	406 ppm	1.56%
Methanol	2896 ppm	1.21%
Tetrahydrofuran	759 ppm	1.28%
Acetone	4957 ppm	1.36%
Isopropyl alcohol	5036 ppm	1.26%

Intermediate precision: Verified through variation of analyst, different day, column and instrument. All the set acceptance criteria were meeting satisfactory.

Robustness: Robustness was performed with one parameter changed while keeping the other two unchanged from actual parameter. Refer Table-6 for Robustness parameters .

Table-6: Robustness parameters

Parameter	Actual	Low	High
Carrier gas (Helium) gas	2.0 mL/min	1.8 mL/min	2.2 mL/min
Column oven temperature	40°C	38°C	42°C
Headspace oven temperature	85°C	83°C	87°C
Vial Equilibration time	30 min	28 min	32 min

Verified through variation of carrier gas flow, column oven temperature , head space oven temperature and vial equilibration time . All the set acceptance criteria were meeting satisfactory.

RESULTS AND DISCUSSION:

Analytical Method Validation Parameters for the determination of residual solvents in Trabectedin drug substance were meeting the acceptance criteria for all the parameters successfully for individual standard solvents . No blank interference was observed for individual standard solvents.

Typical chromatograms for specificity, linearity, accuracy, precision , robustness were tabulated from Figure-1 to 5.

Figure-1: Typical chromatogram for specificity

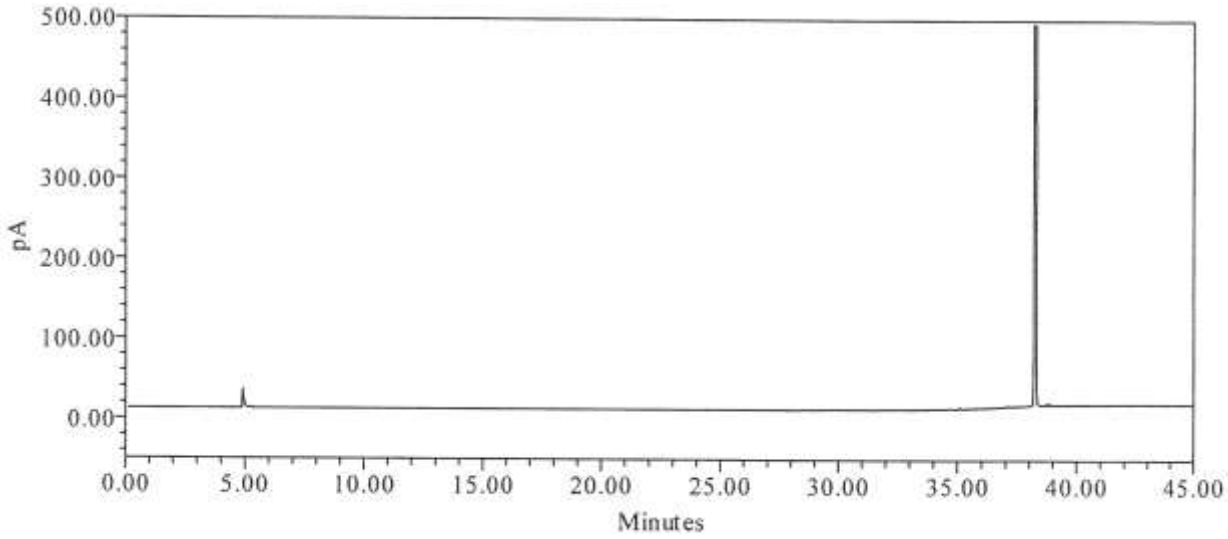


Figure-2: Typical chromatogram for Linearity

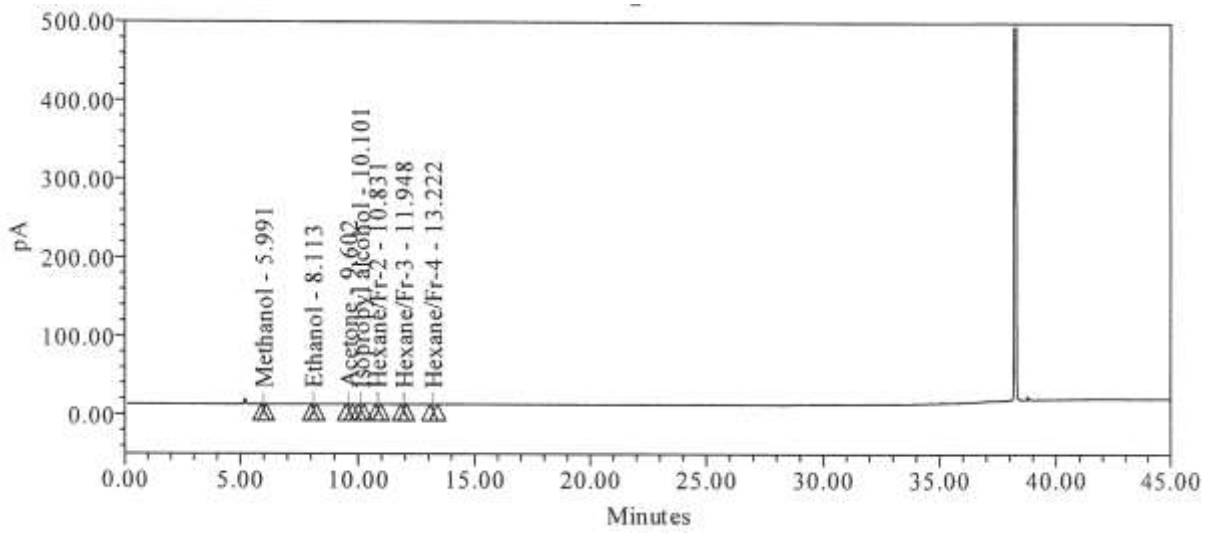


Figure-3: Typical chromatogram for Accuracy

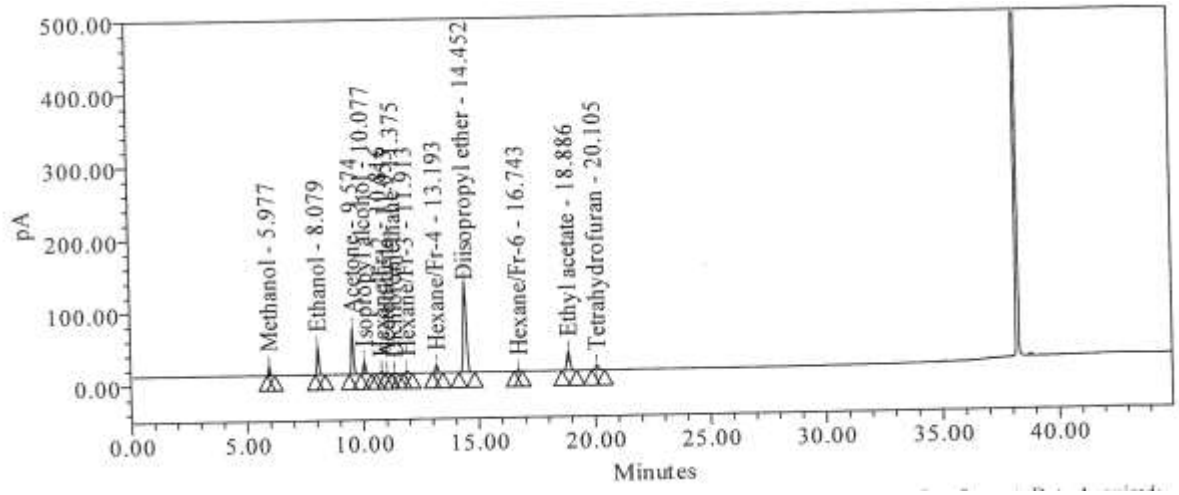


Figure-4: Typical chromatogram for Precision

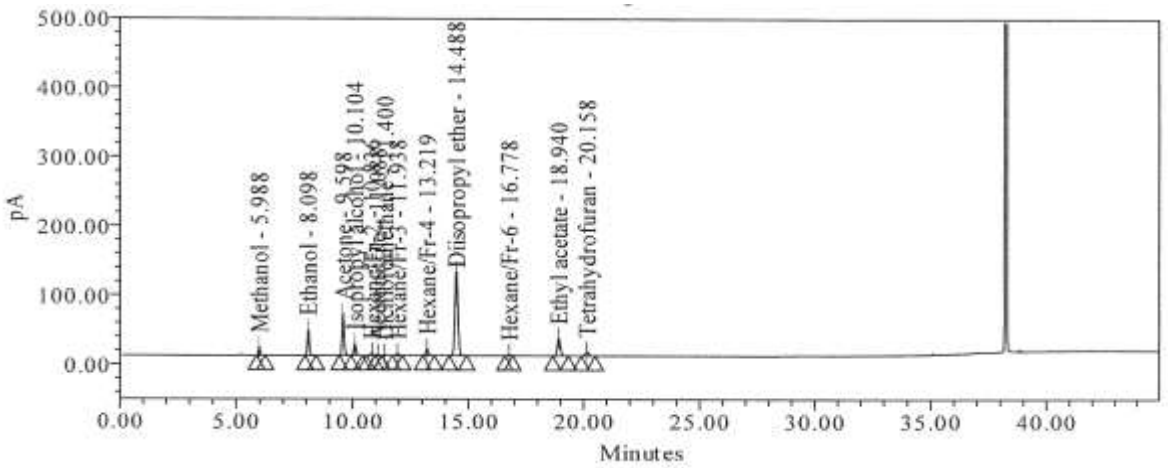
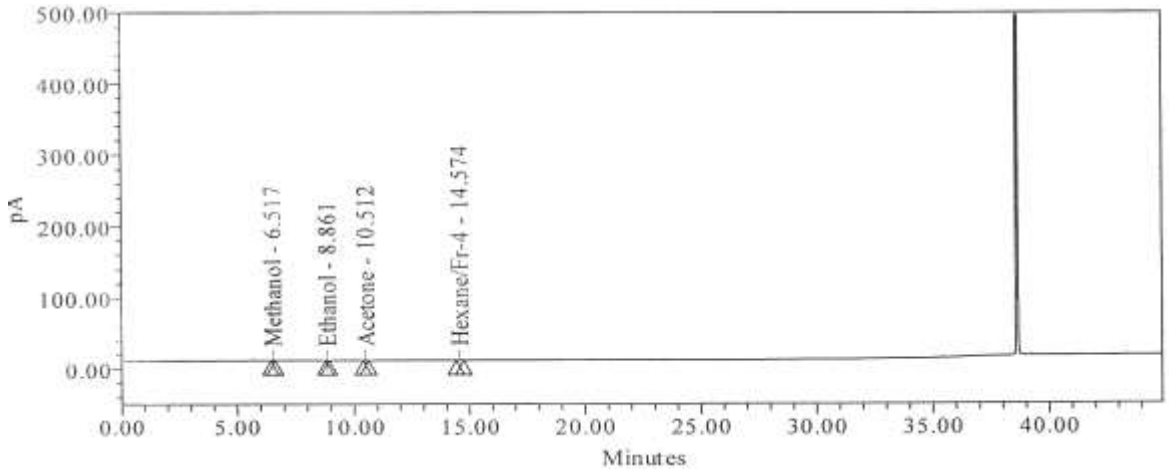


Figure-5: Typical chromatogram for Robustness



Conclusion:

The developed HSGC Method for the determination of residual solvents in Trabectedin drug substance by Head Space Gas Chromatography equipped with Flame Ionization detector has been validated successfully as per ICHQ2R1 (5) guidelines for its intended purpose. The validated method shall be used for routine analysis for the determination of residual solvents by HSGC method in Trabectedin drug substance.

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