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RESEARCH ARTICLE

Quantitative estimation of ethanol content in eribulin mesylate injection using headspace gas chromatographic with flame ionization detector [HS-GC-FID]

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Abstract

A specific HS-GC method has been developed, optimized, and validated for the quantitative estimation of ethanol content in eribulin mesylate injection. Chromatographic separation was achieved on DB-624 column (30 m x 0.32 mm, 1.8 µm), consisting of 6% cyanopropyl and 94% polydimethylsiloxane as stationary phase and passing nitrogen carrier gas. The performance of the method was assessed by evaluating the specificity, linearity, precision, and accuracy of experiments. The correlation coefficient value of the linearity experiment was 0.9999. The average recoveries for the accuracy were in the range of 98.7 to 102.4%. The results proved that the method is suitable for the quantitative estimation of ethanol content in eribulin mesylate injection.

Keywords: Ethanol content, HS-GC, Eribulin mesylate injection, ICH guidelines, Validation.

Introduction

Eribulin mesylate is assigned chemically as (2R,3R,3aS, 7R,8aS,9S,10aR,11S,12R,13aR, 13bS, 15S,18S,21S,24S,26R, 28R,29aS)-2-[(2S)-3-Amino-2-hydroxypropyl]-3-methoxy-26-methyl-20,27-dimethylidenehexacosahydro-11, 15:18,21:24,28-triepoxy-7,9-ethano-12,15-methano-9H,15H-furo[3,2-i] furo [2′,3′:5,6] pyrano [4,3- b][1,4] dioxacyclopentacosin-5(4H)-one monomethanesulfonate (salt). It is a white powder and freely soluble in water, methanol, ethanol, 1-octanol, benzyl alcohol, dimethyl sulfoxide, N-methylpyrrolidone, dichloromethane and ethyl acetate, soluble in acetone, sparingly soluble in acetonitrile, practically insoluble in tert-butyl methyl ether, n-heptane and n-pentane. Eribulin mesylate was freely soluble at pH 3-7, soluble at pH 9 and slightly soluble at pH 11. Eribulin is a simplified synthetic analogue of halichondrin B, a natural polyether macrolide produced in marine sponges (Bai *et al*., 1991; Budrow *et al*., 2001; Palme *et al*., 2004). Eribulin suppresses microtubule growth without affecting tubulin depolymerisation resulting in sequestration of microtubules into non functional aggregates. This leads to an irreversible mitotic block and thereby cell cycle arrest in the G2-M phase and apoptosis (Jordan *et al*., 2005; Cheng *et al*., 2004; Salvato *et al*., 2011). Probably due to its unique mechanism of action, different to the one of vinca alkaloids or paclitaxel, eribulin has shown an antitumor activity in paclitaxel-resistant ovarian cancer cell lines (Kuznetsov *et al* 2007). Eribulin mesylate used for monotherapy treatment of locally advanced or metastatic breast cancer with tumour progression after treatment with at least two chemotherapy regimens containing an anthracycline and a taxane (EPAR 2011). Molecular formula is $C_{40}H_{59}NO_{11}$ and molecular weight 826.090 g/mol. The chemical structure of eribulin mesylate shown in Figure 1.

The literature survey reveals that several analytical methods have been used to determine the amount of ethanol in various samples. The chemical structure of ethanol is shown in Figure 2. Among these gas chromatography (Chun *et al*., 2016; De Martinis *et al*., 2004; Cornelia *et al*.,

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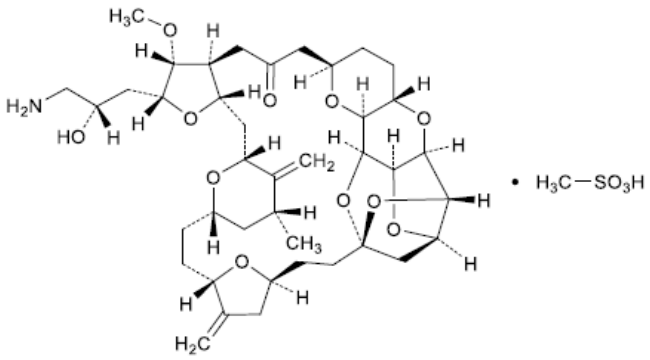

Figure 1: Chemical structure of eribulin mesylate

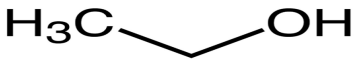

Figure 2: Chemical structure of ethanol

2009; Emrah *et al*., 2010; Hong-tao *et al*., 2014; Kunsman *et al*., 1996; Dustin *et al*., 2011; Dorman *et al*., 2013; Parczewski *et al*., 2002) high-performance liquid chromatography (Ihara *et al*., 2002; Betz *et al*., 1987). However, no analytical method was reported for the quantitative estimation of ethanol content in eribulin mesylate injection.

Hence the author was aimed towards the development of a sensitive GC-HS method was reported for the quantitative estimation of ethanol content in eribulin mesylate injection and the method was validated for specificity, linearity, accuracy, and precision experiments according to ICH guidelines (ICH 2005).

Materials and Methods

Ethanol was obtained from Tianjin Kemiou Chemical Reagents Development Center (Tianin City, China), dimethyl sulfoxide (GC-grade) was obtained from Sigma-Aldrich and pure samples of the drug substance and eribulin mesylate finished dosage form (Injections) for research obtained from local market (Ebunat) manufactured by Natco Pharma.

Instruments and Equipment

Head space GC analysis was conducted using an Agilent GC-HSS 7890B series equipped with 7697A Headspace Sampler. DB-624 column (30 x 0.32 mm, 1.8 µm; J&W Scientific Inc.) was used for analysis. A Mettler Toledo AT261 semi-micro balance was also used for sample preparation.

Preparation of Solutions

Preparation of diluent

Dimethyl sulfoxide used as diluent.

Preparation of standard solution

Accurately weighed and transferred 25.24 mg of ethanol standard into a 25 mL volumetric flask containing about 10 mL of diluent and make up to volume with diluent.

Further transferred 5.0 mL of the above solution into a 50 mL volumetric flask containing about 25 mL of diluent and make up to volume with diluent.

Preparation of placebo solution

Pipette 2 mL placebo solution into the 20 mL headspace vial, cover the vial with a polytetrafluoroethylene film, silastic seal, and then pressurize the aluminium cap with a crimper, seal, and mixed well.

Preparation of sample solution

Pipette 2 mL sample solution into the 20 mL headspace vial, cover the vial with a polytetrafluoroethylene film, silastic seal, and then pressurize the aluminium cap with a crimper, seal, and mixed well.

Instrumentation

Agilent J&W DB-624, (30 m x 0.32 mm, 1.8 µm) column consists of 6% cyanopropyl, 94% polydimethyl siloxane material as a stationary phase. High purity nitrogen gas was used as the carrier gas with the column flow 0.6 mL/min. The initial column oven temperature of 40°C was maintained for 5 minutes and then increased to 140°C at the rate of 20°C/min, followed by holding at 240°C for 12 minutes at the rate of 20°C/min. The run time was 22 minutes. The injection volume was 1.0 mL with a split ratio of 10:1. The injector temperature was 200°C, detector temperature was 250°C.

Chromatographic and Headspace Parameters Conditions

For head space GC, vial equilibration temperature 90°C, loop temperature 95°C, transfer-line temperature 100°C, equilibration time 10 minutes, pressurization time 0.2 minute and injection time of sample 1.0 minute. GC cycle time 30 minutes. The flame ionization detector (FID) detector was used split ratio 10:1, injection port temperature 200°C; detector temperature 250°C; temperature program was set to 40°C for hold time 5 minutes, then raised to 140°C with rate 20°C per minute hold time zero minutes and then maintained at 240°C for with rate 20°C per minute hold time 12 minutes and carrier gas was used nitrogen.

Method Development (ICH 2022)

The objective of the general method is quantitative estimation of ethanol content in eribulin mesylate injection.

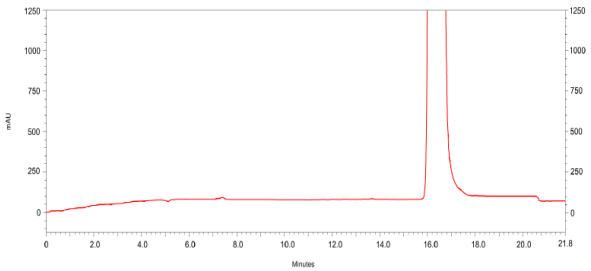

Figure 3: Typical chromatogram of blank

The capillary GC column DB-624 column has reported as suitable for the analysis of a wide range of common ICH residual solvents including ethanol in pharmaceutical products and thus was selected for methods development. The temperature program, split ratio, head space oven temperature, and other head space and GC parameters were investigated and optimized using ethanol standard solution.

Method Validation Results

Specificity and system suitability

The method specificity was validated for potential interference from blank, placebo, standard and sample solution as shown in Figures 3-6. There are no detectable peaks in the chromatograms of blank, placebo. The retention time of ethanol in the chromatogram of sample solution matches well with that from 100 ppm ethanol standard solution (Table 1).

System precision

System precision was demonstrated by preparing standard solution as per method and chromatographed the same into GC system in six replicated injections of standard solution. The peak areas of analyte were recorded for these standard injections. The system precision was evaluated by computing the %relative standard deviation for the peak area of these standard injections. The results of the system precision study are tabulated below in Table 2.

The relative standard deviation of six replicate standard solution results found to be within the specification limit, i.e. 0.39%.

Method precision

Method precision was demonstrated by prepare six samples of eribulin mesylate injection 0.5 mg/mL as per optimized method and injected in to the chromatographic system. The precision of the method was evaluated by calculate the individual assay, mean %assay and %relative standard deviation for each set of samples. The results of the method precision study are tabulated below in Table 3.

Overall and individual % Assay are complies as per test method specification. The relative standard deviation of six assay preparations is 1.19%.

Linearity and range

The linearity of ethanol was evaluated from 50.48 to 151.44 ppm (five levels with duplicate preparations at each level. The peak areas were plotted against the corresponding concentrations and the linear regression was performed. The calibration curve of analytical method was assessed by plotting concentration versus peak area and represented graphically. The observations are tabulated below in Table 4.

Table 1: Specificity and system suitability results

S. No.	*Name*	*Retention time (min)*	*Theoretical plates*	*Tailing factor*
1	Blank	ND	NA	NA
2	Placebo solution	ND	NA	NA
3	Standard solution	6.27	11456	1.3
4	Sample solution	6.29	NA	NA

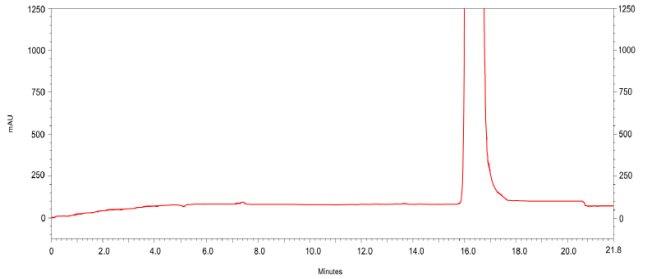

Figure 4: Typical chromatogram of placebo

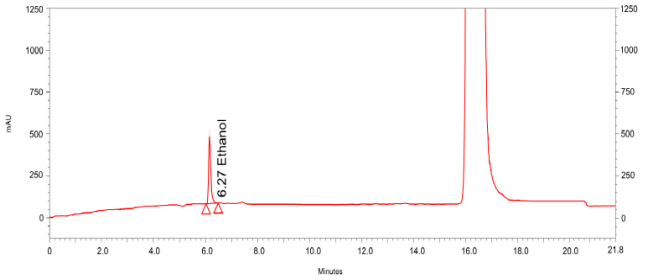

Figure 5: Typical chromatogram of standard

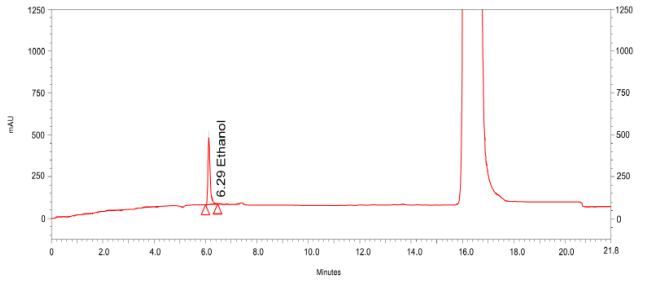

Figure 6: Typical chromatogram of sample

Table 2: System precision results

S. No.	*No. of injections*	*Area response*
1	Inj-1	1852617
2	Inj-2	1852564
3	Inj-3	1867743
4	Inj-4	1849991
5	Inj-5	1867823
6	Inj-6	1859954
	Average	1858449
	SD	7265.20
	%RSD	0.39

Table 3: Method precision results

S. No.	*No. of preparations*	*%Assay*
1	Preparation 1	105.2
2	Preparation 2	104.3
3	Preparation 3	101.9
4	Preparation 4	103.7
5	Preparation 5	104.6
6	Preparation 6	105.8
	Average	104.3
	SD	1.2420
	%RSD	1.19

Table 4: Linearity studies for ethanol

S. No	*Linearity level*	*Concentration (ppm)*	*Area response*
1	Linearity at 50%	50.48	929499
2	Linearity at 75%	75.72	1413945
3	Linearity at 100%	100.96	1859537
4	Linearity at 125%	126.20	2331085
5	Linearity at 150%	151.44	2789695
Correlation coefficient (r^2)			0.9999
Intercept			9739.4000
Slope			18373.7401
100% Y-intercept			0.52

Table 5: Recovery studies for ethanol by proposed method

%Level	*(µg) Recovered*	*(µg) Added*	*% Recovery*	*Mean %Recovery*
Accuracy at 50%-1	50.12	50.38	99.5	
Accuracy at 50%-2	50.36	50.55	99.6	99.7
Accuracy at 50%-3	50.35	50.41	99.9	
Accuracy at 100%-1	99.19	100.48	98.7	
Accuracy at 100%-2	99.51	100.74	98.8	98.8
Accuracy at 100%-3	99.64	100.65	99.0	
Accuracy at 150%-1	152.65	150.34	101.5	
Accuracy at 150%-2	153.85	150.29	102.4	101.7
Accuracy at 150%-3	151.99	150.27	101.1	

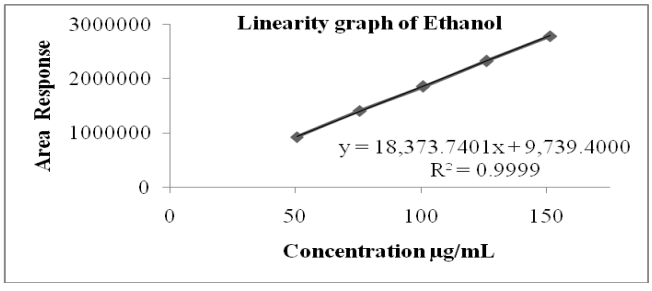

Figure 7: Calibration curve for ethanol

Table 6: Results for solution stability of standard solution

Time interval	*Similarity factor*
Initial	NA
12 hrs	1.01
24 hrs	1.02

Table 7: Results for solution stability of sample solution

Time interval	*%Assay*	*%Assay difference*
Initial	105.2	NA
12 hrs	104.5	0.7
24 hrs	103.9	1.3

The correlation coefficient (r^2) was found to be 0.9999. Therefore the GC method was found to be linear standard curve were calculated and given in Figure 7 to demonstrate the linearity of the proposed method. From the data obtained which is given in Table 4 the method was found to be linear within the proposed range.

Accuracy

The accuracy of the test method was demonstrated by preparing recovery samples of eribulin mesylate at 50 to 150% of the target concentration level. The recovery samples were prepared in triplicate preparations on ethanol standard spiked to placebo, analyzed as per the proposed method for each concentration level (Table 5). The samples were chromatographed and the percentage recovery of each sample was calculated for the amount added.

Solution stability

The stability of standard and sample solutions were prepared in duplicate and stored at ambient laboratory condition (25 ± 5°C) respectively (Tables 6 and 7).

Therefore, the standard solution, sample solutions were stable for 24 hours at room temperature condition.

Discussion

A simple, economic, accurate and precise HS-GC method was successfully developed. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of selectivity, precision, linearity, accuracy, and solution stability.

For selectivity the chromatograms were recorded for blank, placebo, standard and sample solutions of eribulin. Selectivity studies reveal that the peak is well separated from each other. Therefore the method is selective for the quantification of ethanol in eribulin mesylate injection.

For system precision studies six replicate injections were performed. %RSD was determined from the peak areas of ethanol. The acceptance limit should be not more than 2 and the results were found to be 0.39% within the acceptance limits.

For method precision studies six samples preparations were performed. %RSD was determined from the %Assay of ethanol. The acceptance limit should be not more than 2 and the results were found to be 1.19% within the acceptance limits.

The linearity results for ethanol in the specified concentration range 50 to 200% (50.48 – 151.44 ppm) are found satisfactory, with a correlation coefficient greater than 0.99. Calibration curve was plotted and correlation co-efficient for ethanol found to be 0.9999, respectively.

The accuracy studies were shown as % recovery for Ethanol at 50 to 150% of the target concentration level. The limit of % recovered shown is in the range of 98 and 102% and the results obtained were found to be within the limits. Hence, the method was found to be accurate.

The solution stability of the standard solution, sample solutions were stable for 24 hours at room temperature condition.

Conclusion

A rapid, sensitive, and selective HS-GC method for quantification of ethanol in eribulin mesylate injection reported in this study. The developed method was validated for various parameters as per ICH guidelines like specificity, system suitability, precision, linearity, accuracy and solution stability. The results obtained were within the acceptance criteria. So, it can be concluded that the developed method is simple, precise, cost-effective, eco-friendly, safe and can be successfully employed for the quantitative estimation of ethanol content in eribulin mesylate injection.

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