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GC-FID ASSESEMENT OF RESIDUAL SOLVENTS PRESENT IN ADDITIVE-POLYSORBATE-80

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ABSTRACT

Residual solvents in pharmaceuticals are defined here as organic volatile chemicals that are used or produced in the manufacture of drug substance or excipients or in the preparation of drug products. Polysorbate-80 is a non-ionic surfactant and emulsifier mostly used in foods and cosmetics. The organic solvents such as isopropyl alcohol, 1, 4-dioxane, ethylene glycol and acetic acid are frequently used in manufacturing of polysorbate-80. Even after such manufacturing process of polysorbate-80, some solvents still remain in small quantities, which are potential toxic to humans. All these points signify that the need for some efforts for quantification of residual solvents present in polysorbate-80. The method utilized the Agilent 7700 with FID (DB-wax, 30m x 0.53mm, 1µ) capillary column, nitrogen as carrier gas with a flow rate of 1.8 mL /min. The critical experimental parameters such as oven temperature, zero air, make up flow, injection volume; split ratio and selection of diluent were studied and optimized. The retention time for residual solvents individually and in spiked standard solution was determined as 6.674, 9.477, 16.615 and 18.540 min for isopropyl alcohol, 1, 4-dioxane, acetic acid and ethylene glycol respectively. The proposed method was also applied for the quantification of residual solvents present in marketed polysorbate-80. The developed method was statistically validated as per standard guidelines, and results obtained from validation proved that the proposed method was scientifically sound. The proposed method was fruitfully applied for the quantification of residual solvents (which are involved in the manufacturing process) present in polysorbate-80.

Keywords: Polysorbate-80, GC, FID, residual solvents

INTRODUCTION

The manufacturing process/ synthetic process of pharmaceutical products/ excipients mostly occurred in the presence of distinct organic volatile solvents, which are not desirable in the final product, because of its toxicity, no therapeutic effect and interference with the quality of the pharmaceutical substance while storage of them. These organic solvents still remain in pharmaceutical products even after various manufacturing process (or) techniques used in at the end. This type of solvents, generally called as residual solvents. According to standard guidelines, quantification of residual solvents in pharmaceutical substances is obligatory for their release testing. Now a days, due to increasing demand analytical techniques, which are helpful to control the minimum quantity of organic solvents in final pharmaceutical products excipients by identifying /quantifying them. Polysorbate-80 is a non-ionic surfactant used as excipient, solubilizer and emulsifier mostly used in foods, cosmetics and pharmaceutical products. It is chemically known as polyoxyethyle-20-mono-oleate, appear as amber coloured viscous liquid and freely miscible with water. The residual solvents such as isopropyl alcohol, 1,4-dioxane, ethylene glycol and acetic acid are frequently used in manufacturing of polysorbate-80. Sometimes these solvents still remain in polysorbate-80, even after various manufacturing /synthetic process[1-2].

Extensive literature review revealed that few analytical methods are available for quantification of residual solvents present in some pharmaceutical drug substances and excipients by gas chromatography

(most useful technique to quantify residual solvents) but there is no analytical method available for quantification of residual solvents present in polysorbate-80 [3-15].

Keeping these points into consideration, we undertaken the present investigation with the objective to develop and validate the simple gas chromatographic method for quantification of residual solvents present in polysorbate-80.

EXPERIMENTAL

Materials

Acetic acid, 1,4 –Dioxane , dimethyl sulphoxide , iso-propyl alcohol and ethylene glycol (AR grade) were purchased from Merck chemicals Co (Mumbai). HPLC grade methanol and N-methyl pyrollidine were procured from SD fine chemicals Ltd (Mumbai). Polysorbate-80 (AR grade) were obtained from Hi-media, Mumbai.

Instrumentation and operating gas chromatographic conditions

Gas chromatograph Azilent technologies 7700 equipped with a flame ionization detection, standard oven for temperature ramping, and split/splitless injection port. The analytes of interest were separated on a DB-Wax capillary column (30m x 0.53mm, 1.0µm film thickness) with nitrogen as carrier gas. An analytical balance (AVW 220D from Sartorious) and ultra-sonicator (RK-106 from Bandeliasonorex) were used for this investigation purpose.

Preparation of Standard stock solution

Accurately weigh and transfer 1000 mg of IPA, 1000 mg of acetic acid, 124 mg of ethylene glycol and 76 mg of 1, 4-dioxane into 100 mL volumetric flask containing 50 mL of diluent, dissolve and dilute to volume with diluent.

Preparation of standard solution

Accurately transfer 1.0 mL of standard stock solution into 10 mL volumetric flask containing 5 mL of diluent, dissolve and diluted upto the mark with diluent.

Preparation of Sample solution (assay of commercially available polysorbate-80)

The proposed method was evaluated by the assay of commercially available polysorbate-80 (Hi-media) for quantification of residual solvents present in it. The results obtained for residual solvents were compared with the corresponding specification limits of standard guidelines. Accurately weigh and transfer 2.0 g of sample into 10 mL volumetric flask containing 2 mL of diluent, dissolved and diluted upto the mark with diluent.

Procedure for calibration plot

Calibration plot for the contemplated method was determined by injecting series of standard solutions of residual solvents prepared over the concentration range of LOQ to 150%. Six replicates were performed at each level and subjected to the contemplated method, then contrived the graph of concentration (at X-axis) versus average peak area of solvent (at Y-axis). Evaluate the squared coefficient (r2), correlation coefficient (r), slope and Y-intercept.

Method validation

The method was validated according to the ICH guidelines and all validation parameters useful to evaluate the overall performance of qualitative analytical method were investigated including specificity, linearity, precision, accuracy, robustness, limit of detection and limit of quantification[16].

RESULTS AND DISCUSSION

To release the product into the market, quantification as per the standard guidelines of residual solvents in final pharmaceutical product is mandatory due to this toxicity. It may contain some residual solvents even

after the last part of manufacturing process. These facts stand for the need of some efforts to quantify the residual solvents in polysorbate-80. Hence present inference was undertaken with an objective of developing GC- analytical method for identification of residual solvents present in polysoebate-80. For this investigation purpose, we made several trials and finally optimized the gas chromatographic conditions, which is the most popular technique to quantify the residual solvents in Polysorbate-80. The method utilized the Agilent 7700 with FID (DB-wax, 30m x 0.53mm, 1µ) capillary column, nitrogen as carrier gas with a flow rate of 1.8 mL /min. The critical experimental parameters such as oven temperature, zero air, make up flow, injection volume, split ratio and selection of diluent were studied and optimized. Optimized chromatographic conditions mentioned in Table-1.

Method validation

The method was validated for all the residual solvents under the study undertaken for specificity, linearity, precision, accuracy, LOD & LOQ, robustness and system suitability as per ICH guidelines.

Specificity

Specificity of the method was determined by injecting blank solution under the same experimental conditions and was checked for the peak interference at the retention time of the each solvent in the standard and sample solution. The blank did not show any peaks at the retention time of the each solvent in the standard and sample solution indicating that the method is specific. Specificity chromatograms are shown in Figures-1 to 4.

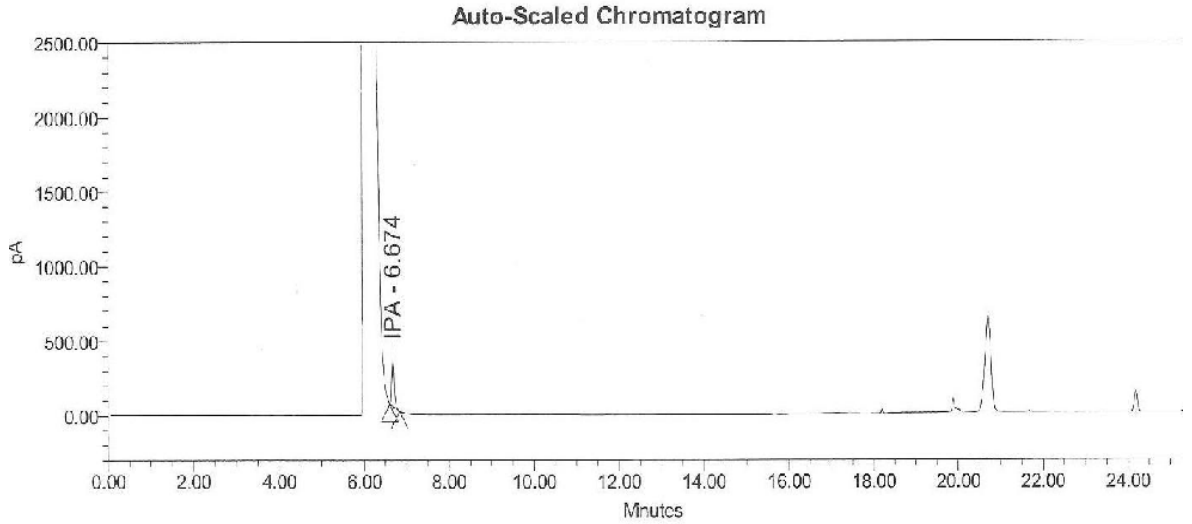

Fig-1: Standard Chromatogram of Isopropyl Alcohol

Table-1: Operating gas chromatographic conditions

Parameters	Condition
Column	DB-WAX,30m x 0.53mm,1.0µm
Column Flow	1.8 mL min^{-1}
Carrier Gas	Nitrogen
Split Ratio	1:5
Injector Temperature	180^0C
Injection Volume	1.0µL
Detector	FID
Detector Temperature	220^oC
Zero Air	300 mL min^{-1}

Hydrogen gas	30 mL min^{-1}
Makeup Flow	25 mL min^{-1}
Diluent	methanol (Gradient Grade)

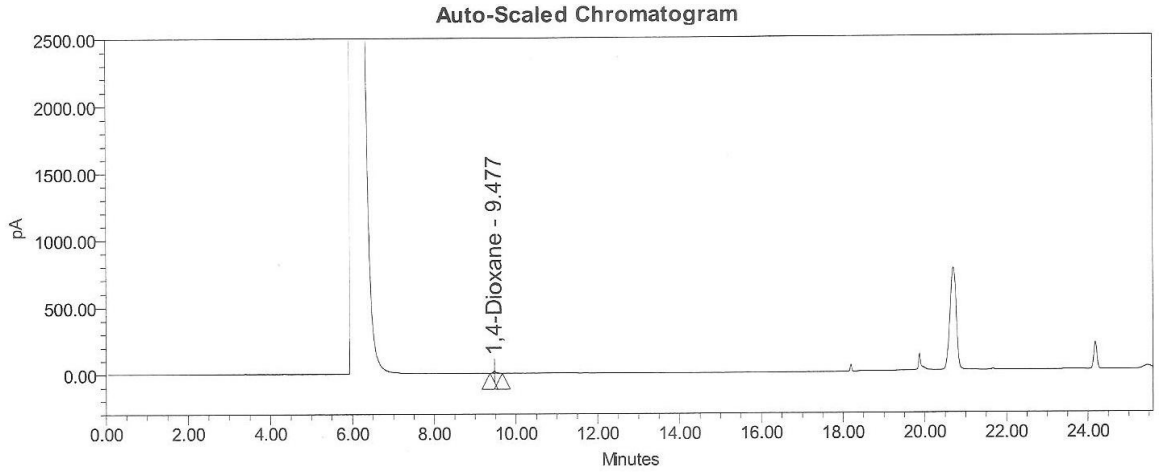

Fig-2: Standard Chromatogram of 1,4-Dioxane

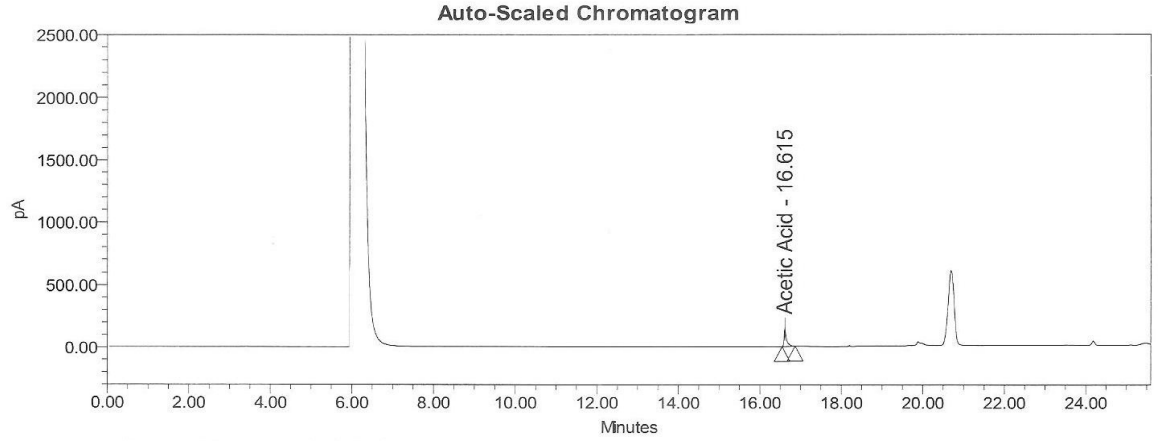

Fig-3: Standard Chromatogram of Acetic acid

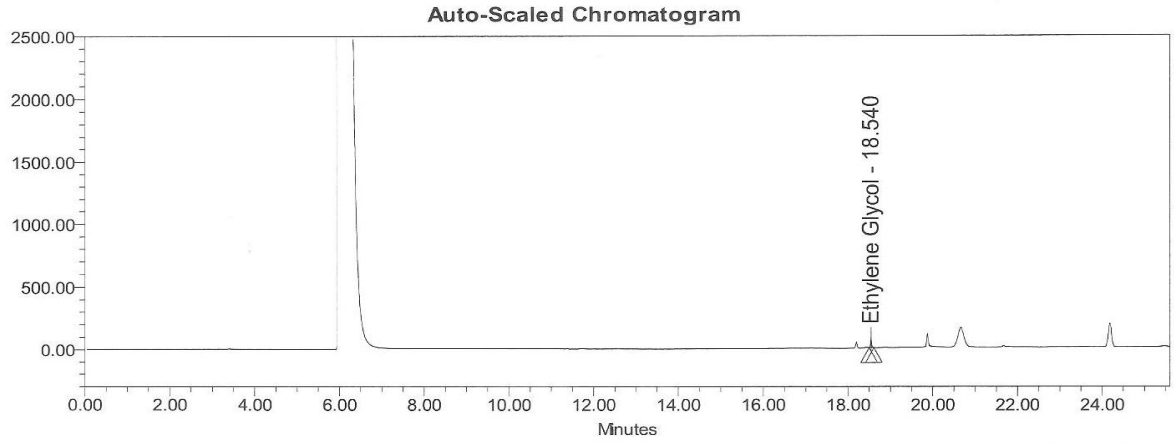

Fig-4: Standard Chromatogram of Ethylene Glycol

Linearity

Linearity of the method was determined over the concentration range of LOQ, 50% 80% 100% 120% and 150%. Linearity data, correlation coefficient (r2), slope and Y-intercept of the method are shown in Table-2. The calibration plot of concentration (at X-axis) versus average peak of solvent (at Y-axis) is shown in Figures-5 and 6.

Table-2: Linearity data about four residual solvents

Level	IPA		1,4-dioxane		Acetic acid		Ethylene glycol	
	Conc. (ppm)	peak area[a]	Conc. (ppm)	peak area[a]	Conc. (ppm)	peak area[a]	Conc. (ppm)	peak area[a]
LOQ	612.5	157.15	6.7	1.66	477.3	42.64	42	12.92
50	2505.2	686.17	193.8	40.22	2497.5	282.08	324.2	73.84
80	4008.3	1148.27	310	65.31	3996	493.12	518.8	120.20
100	5010.4	1572.25	387.6	86.80	4995	670.98	648.5	156.31
120	6012.5	1765.83	465.1	100.61	5994	801.11	778.1	181.27
150	7515.6	2263.82	581.3	124.97	7492.5	1017.48	927.7	226.01
Correlation coefficient		0.9964	0.998		0.997		0.9994	
Slope		0.3079	0.2171		0.1411		0.2313	
Intercept		-51.4458	-0.4382		-47.3631		1.8210	

[a] Mean of 6 determinations

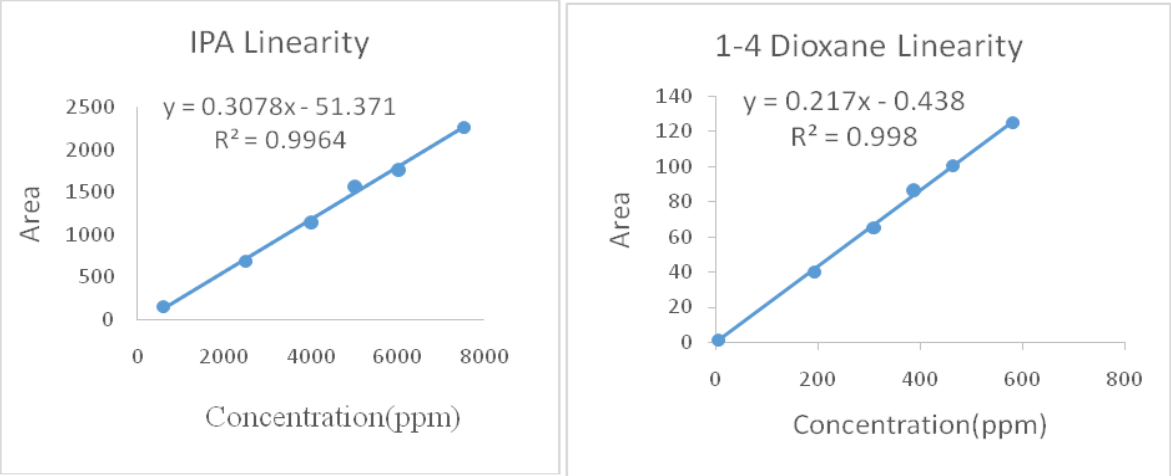

Fig-5: Linearity plot of Isopropyl alcohol and 1,4 Dioxane

Precision

System precision and method precision was determined by injecting six replicate injections of standard solution and sample solution respectively and analyzed as per ICH guidelines. Intermediate precision was carried out to demonstrate the reproducibility of sample results obtained by the analytical method for the variability of instrument, column (serial number/ lot number), analyst and day. Peak responses for each solvent peak were measured and %RSD was calculated. The %RSD for the solvent content of each solvent from the six preparations of system precision solution should be not more than 15.0.The obtained % RSD values were within the acceptable range. The results of this study were summarized in Table-3.

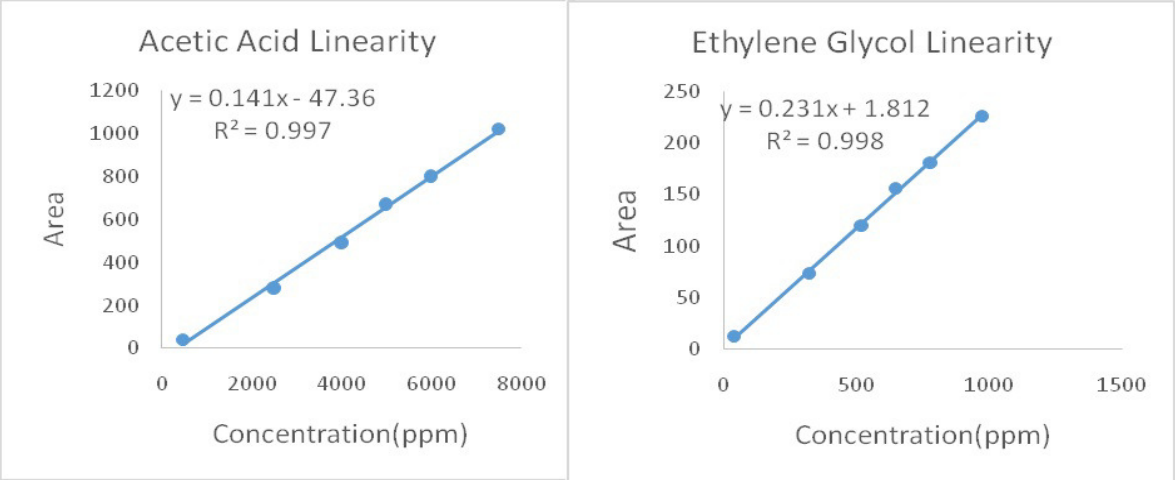

Fig-6: Linearity plot of Acetic acid and Ethylene Glycol

Table-3: Precision data of proposed method

Solvent name	System Precision		Method Precision		Intermediate Precision		
	Mean[a](ppm)	%RSD[b]	Mean[a](ppm)	%RSD[b]	Mean[a] (ppm)		%RSD[b]
					Analyst 1	Analyst 2	
IPA	1358	2.7	5394	6.3	5394	4649	9.3
1,4 dioxane	78	2.3	446	1.3	446	389	7.3
Acetic acid	647	1.9	6523	1.8	6523	6029	4.5
Ethylene glycol	126	3.5	675	1.4	675	645	2.9

[a] Mean of 6 determinations
[b] Relative standard deviation

Accuracy

Accuracy of the method was assured by applying the standard addition technique. The % recovery was calculated. The mean % recovery of each solvent at LOQ level should be not less than 70.0 and not more than 130.0**.** The mean % recovery of each solvent at 50%, 100% and 150% level should be not less than 80.0 and not more than 120.0. The % recovery values for the method are shown in Tables- 4 and 5. The % recovery values obtained were within the limits indicating the method as accurate.

Table-4: Accuracy data for Isopropyl alcohol and 1,4 –dioxane

Spike Level	Isopropyl alcohol		1,4 dioxane	
	Conc. Of solvent found (ppm)	% Mean Recovery[a]	Conc. Of solvent found (ppm)	% Mean Recovery[a]
LOQ	590	90.4	7.0	104.5
50%	2405	95.0	195.8	106.0
100%	4594	93.7	381.9	104.1
150%	7416	98.4	530.8	95.7

Table-5: Accuracy data for Acetic acid and Ethylene glycol

Spike Level	Acetic acid		Ethylene glycol	
	Conc. Of solvent found (ppm)	% Mean Recovery[a]	Conc. Of solvent found (ppm)	% Mean Recovery[a]

LOQ	1065	88.0	84.4	87.0
50%	3183	97.5	356	93.2
100%	5713	99.2	655	91.9
150%	7868	95.6	921	88.9

a mean of 6 determinations

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for the proposed method were determined using calibration standards. LOD and LOQ are calculated by using 3.3 σ /s and 10 σ/s, respectively, where s is the slope of the calibration curve and σ is the standard deviation of y- intercept of the regression equation. LOD and LOQ are shown in Table-6.

Table-6: LOD & LOQ values for residual solvents

Parameter	IPA	1,4 dioxane	Acetic Acid	Ethylene Glycol
LOD (ppm)	202	2.3	157	14
LOQ (ppm)	611	6.8	476	42

Robustness

This study was performed by making small and deliberate variations in the method parameters. The variations in the column flow was done at low column flow (1.7 mL min^{-1}) and high flow (1.9 mL min^{-1}) and the results revealed that the method is robust at flow between 1.7 mL min^{-1} and 1.9 mL min^{-1}) column flow variations. The variations in the column oven temperature were done at low column oven temperature (55° C) and high column temperature (65°C) and the results revealed that the method is robust at column oven temperature between 55°C to 65°C variations. Results for all the variations were found to be within the acceptance criteria i.e. %RSD values were less than 15%. as shown in Table-7.

Table-7: Robustness studies of proposed method

Parameter	% RSD[a]			
	IPA	1,4 dioxane	Acetic Acid	Ethylene Glycol
Effect of Variation Flow (mL min^{-1})				
1.7	3.2	0.6	0.6	0.7
1.8	2.7	2.3	1.9	3.5
1.9	3.1	2.9	3.8	3.6
Effect of Variation in Column oven temperature				
55° C	2.3	2.5	2.6	1.8
60° C	2.7	2.3	1.9	3.5
65° C	6.3	2.6	2.3	0.8

[a] Relative standard deviation (n=6)

System suitability

System suitability was evaluated by injecting six replicates of standard solution into the chromatographic system as per the test method and then measured solvents peak area for all six standard chromatogram and % RSD was calculated. The %RSD for the area of each solvent from the six replicate injections of standard solution should be not more than 15.0. Results are summarized in Table-8. The results obtained reveal that the system meets the required system suitability.

Table-8: System suitability values of proposed method

Parameter	%RSD			
	IPA	1,4 dioxane	Acetic Acid	Ethylene Glycol
Linearity	1.6	1.9	2.3	0.7
System precision	2.7	2.3	1.9	3.5

Method precision	2.7	2.3	1.9	3.5
Intermediate precision	0.6	0.9	3.9	4.3
Accuracy	2.3	2.1	1.0	1.6
LOQ	3.1	1.4	1.7	0.4
Acceptance criteria	Not more than 15.0			

Application of the proposed method (Analysis of commercially available polysorbate-80)

The proposed method was evaluated by the assay of commercially available polysorbate-80 (Hi-media) for quantification of residual solvents present in it. The results obtained for residual solvents were compared with the corresponding specification limits of standard guidelines and reported in Table-9 and Figure-7. This revealed that concentration of residual solvents present in polysorbate-80 in ppm levels which were less than the specified limits.

Table-9: Assay results commercially available polysorbate-80

Solvent name	R_t	Area	Amount found (ppm)
IPA	6.673	1581	4213.8
1,4 dioxane	9.478	91	391
Acetic acid	16.612	839	5296
Ethylene glycol	18.541	137	578

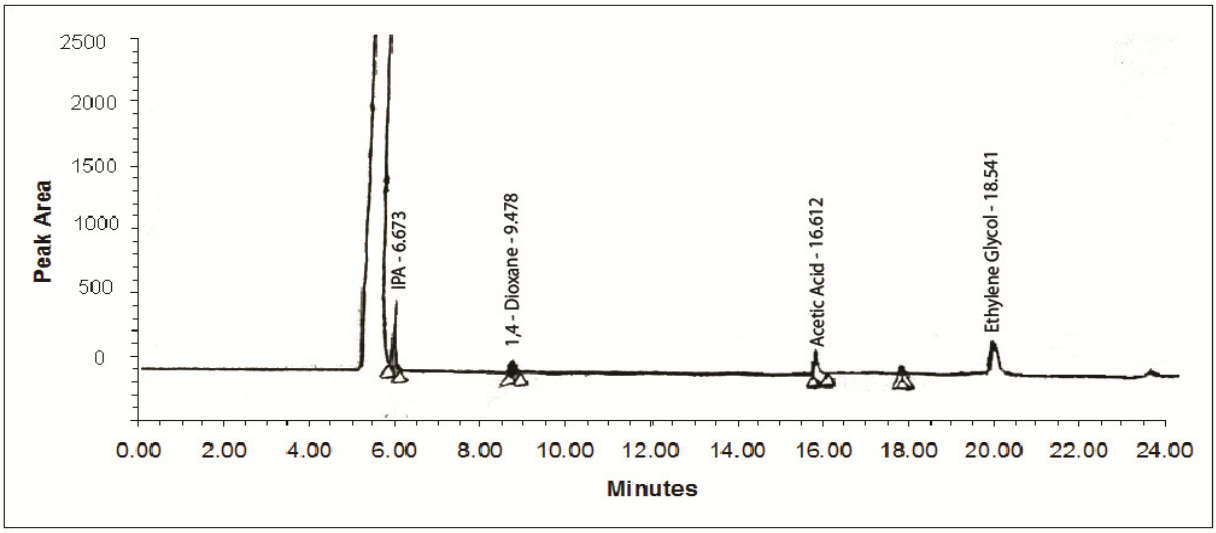

Fig-7: Assay Chromatogram of Polysorbate-80

CONCLUSION

As per standard guidelines, the quantification of residual solvents are mandatory for the release testing of all pharmaceutical products and excipients into the market because of its potential toxicity and may interfere with the quality of the them. Based on this point, we undertaken the present investigation with the objective to develop and validate simple analytical method for simultaneous quantification of residual solvents present in excipient polysorbate-80 by gas chromatography with flame ionization detector (which is powerful tool to quantify the residual solvents). A single, specific and highly selective gas chromatographic method was developed and validated for the quantification of residual solvents present in excipient polysorbate-80 (which are involved in synthetic process of it). The residual solvents 1,4 di-oxane, ethylene glycol, acetic acid, iso propyl alcohol were well separated from each other and quantified by the proposed method. Proposed method was also applied for the quantification of residual solvents in

marketed available polysorbate-80 and they were present in ppm (specification limits) as per standard guidelines. The proposed method was validated as per the standard guidelines, results revealed that the method was scientifically sound. Based on the results, finally we hope that the proposed method effectively applied for the quantification of residual solvents (which are involved in the manufacturing process) present in polysorbate-80.This investigation may helpful to the manufacturers of polysorbate-80 for controlling and minimization of the residual solvents level present in it.

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