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Gas Chromatographic Assessment of Residual Solvents Present in Excipient-Benzyl Alcohol

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Abstract

Purpose: This article describes a simple and rapid gas chromatographic method for identification of residual solvents present in benzyl alcohol. Benzyl alcohol is used in foods and pharmaceutical products. The organic solvents such as benzene, chlorobenzene and toluene are frequently used in manufacturing of benzyl alcohol. Even after such manufacturing process, some solvents still remain in small quantities.

Methods: Method for the quantification of residual solvents present in benzyl alcohol was done by gas chromatography with flame ionization detector and utilizes the Agilent 7700 with FID (DB-624, 30 m × 0.53 mm, 3 µ) capillary column, nitrogen as carrier gas with a flow rate of 2.5 ml mn^{-1}. The critical experimental parameters such as oven temperature, zero air, make up flow; injection volume, split ratio and the selection of diluent were studied and optimized.

Results: The retention time of various residual solvents taken individually and in spiked standard solutions were determined as 8.824, 13.467 and 11.461 min for benzene, chlorobenzene and toluene respectively. The proposed method was applied for the quantification of residual solvents present in marketed benzyl alcohol and was statistically validated as per standard guidelines.

Conclusion: The 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 benzyl alcohol.

Keywords: Benzyl alcohol; GC; FID; Residual solvents

Introduction

Organic volatile chemicals may be present in pharmaceutical products (or) excipients, which are involved in the manufacturing/ synthesis process, but they are not desirable in the final product due to their toxicity, non therapeutic effect and may interfere with the quality of the pharmaceutical substance. These organic solvents still remain in pharmaceutical products even after subjecting to various manufacturing process (or) techniques. These types of solvents are called as residual solvents. As per standard guidelines, quantification of residual solvents in pharmaceutical substances is enforced for their release testing. Now days, there is an increase in the stipulate for analytical techniques, which are helpful to control the minimum quantity of organic solvents in final pharmaceutical products/ excipients by identifying /quantifying them. Benzyl alcohol is a colorless liquid with a mild pleasant aromatic odor. It is partially soluble in water and completely miscible in alcohols and diethyl ether. Benzyl alcohol is used in foods and pharmaceutical products it is also a precursor to a variety of esters, used in the soap, perfume and flavor industries. It has been used as a dielectric solvent for the dielectrophoretic reconfiguration of nano wires and as a bacteriostatic preservative at low concentration in intravenous medications, cosmetics and topical drugs. The organic solvents such as benzene, chlorobenzene and toluene are frequently used in manufacturing of benzyl alcohol [1,2]. Even after such manufacturing process of benzyl alcohol, 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 benzyl alcohol. 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 benzyl alcohol.

Extensive literature review revealed that few analytical methods are available for quantification of residual solvents present in some pharmaceutical drug substances and excipients [3-14] by gas chromatography which is the most useful technique to quantify residual solvents. It also accomplished that, there is no analytical method available for the quantification of residual solvents present in benzyl alcohol.

Keeping these points into deliberation, we contemplated a method with an objective of development and validation of the simple gas chromatographic method for the quantification of residual solvents present in benzyl alcohol.

Materials and Methods

Materials

Benzyl alcohol, benzene, chlorobenzene, methanol and toluene (HPLC grade) were purchased from Merck chemicals Co (Mumbai). DMSO (AR grade) was purchased from Merck chemicals Co (Mumbai).

Instrumentation and operating gas chromatographic condition

Gas chromatograph Azilent technologies 7700 equipped with flame ionization detection, standard oven for temperature ramping, and

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split/split less injection port. The analytes of interest were separated on a DB-624 capillary column (30 m × 0.53 mm, 3.0 µm 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.

Methods

Preparation of standard stock solution: Benzene 50 mg, chloro benzene 50 mg and toluene 50 mg were accurately weighed and transferred into 100 mL volumetric flask containing 50 mL of diluent, dissolved and diluted up to the mark with diluent.

Preparation of standard solution: Accurately transferred 1 mL of standard stock solution into 10 mL volumetric flask containing 5 mL of diluent, dissolved and diluted up to the mark with diluent.

Preparation of sample solution (assay of commercially available benzyl alcohol): The proposed method was evaluated by the assay of commercially available Benzyl alcohol (Hi-media) for the quantification of residual solvents present in it. The results obtained were compared with the corresponding specification limits of standard guidelines. Accurately weighed 5.0 g of sample was transferred into 10 mL volumetric flask containing 2 mL of diluent, dissolved and diluted up to the mark with diluent.

Procedure for calibration plot

Condition the column for 2 hours at 200°C column oven temperature before starting the analysis. The linearity of analytical method was established at LOQ to 150% of specification level concentration. A series of standard solutions were prepared of different concentrations at LOQ, 50%, 80%, 100%, 120% and 150% from standard solution to 10 mL with diluent to obtain the required concentration. Inject the blank as diluent once, six replicate injections of standard solution and then inject the sample solution. Inject these solutions into the GC system and record the area of solvent peak. Contrive the graph of concentration (at X-axis) versus average peak of solvent (at Y-axis) and assess the 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 analytical method were investigated including specificity, linearity, precision, accuracy, and robustness, limit of detection and limit of quantification [15].

Results and Discussion

Residual solvents, which may be present in the final pharmaceutical products /excipients, are not desired due to their probable toxicity and their quantification in pharmaceutical products is mandatory as per standard guidelines to release into the market. Benzyl alcohol is used in foods and pharmaceutical products. 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 benzyl alcohol. Hence present inference was undertaken with an objective of developing GC- analytical method for identification of residual solvents present in benzyl alcohol. Several trials were performed to optimize the most suitable chromatographic conditions which are having the ability of quantifying the residual solvents. Optimized chromatographic conditions includes nitrogen as a mobile phase with flow rate of 2.5 ml min^{-1}, DB-wax (300 m × 0.53 mm, 3 µm) as a column and other operating gas chromatographic conditions are specified in Table 1. The retention times with good resolution were observed as 8.824, 13.467 and 11.461 min for benzene, chloro benzene and toluene respectively. The chromatographs are shown in Figure 1.

Method validation

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

The method was validated for all the residual solvents under the study for their specificity, linearity, precision, accuracy, LOD and 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 no peak interference at the retention time of the each solvent in the standard and sample solution, indicating that the method is specific.

Linearity: Linearity of the method was determined over the concentration range of LOQ, 50% 80% 100% 120% and 150%. Correlation coefficient (R^2), slope and Y-intercept were calculated from Linearity data and shown in Table 2.

Limit of detection (LOD) and limit of quantification (LOQ): The LOD and LOQ for the proposed method were determined using calibration standards and calculated using 3.3 σ /s and 10 σ/s formulae respectively, where s is the slope of the calibration curve and σ is the standard deviation of y- intercept of the regression equation. Results are shown in Table 2.

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

Parameters	Condition
Column	DB-624, 30 m × 0.53 mm, 3.0 µm
Column Flow	2.5 ml min^{-1}
Carrier Gas	Nitrogen
Split Ratio	1:2
Injector Temperature	180°C
Injection Volume	2.0 µL
Detector	FID
Detector Temperature	240°C
Zero Air	300 ml min^{-1}
Hydrogen gas	30 ml min^{-1}
Makeup Flow	25 ml min^{-1}
Diluent	DMSO

Table 1: Optimized gas chromatographic conditions.

Parameter	Benzene	Toluene	Chloro benzene
Linearity range (ppm)	0.9-3.2	3.7-162.7	2.5-153.3
Regression equation	Y=4.525x+0.142	Y=46.34x+33.60	Y=3.5661x+3.7082
Correlation coefficient	0.996	0.9993	0.9997
Slope	4.5257	46.34	3.5661
Intercept	0.1424	33.60	3.7082
LOD (µg ml^{-1})	0.3	1.2	0.8
LOQ (µg mL^{-1})	0.9	3.7	2.5

Table 2: Linearity plot details of three residual solvents.

of instrument, column (serial number/ lot number), analyst and day. Peak responses for each solvent peak were measured and %RSD was calculated. The %RSD of each solvent from the six preparations of system precision should not be more than 15.0 and obtained results were within the acceptable range. The data of this study were summarized in Table 3.

Accuracy: Accuracy of the methods 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, at 50%, 100% and 150% level should not be less than 80.0 and not more than 120.0. Results obtained were within the limits indicating the method as accurate and are shown in Table 4.

Robustness: This study was performed by making small and deliberate variations in the method parameters. The variation in the column flow ± 0.1 ml min-1, column oven temperature ± 5°C was done and the results obtained were within the acceptance criteria indicating the method is robust within the specified range. % RSD values were less than 15% as shown in Table 5.

System suitability: System suitability was evaluated by injecting six replicates of standard solution into the chromatographic system as per the test method and solvents peak area was measured.% RSD was calculated and the results revealed that the system meets the required system suitability. Results are summarized in Table 6.

Application of the proposed method (Analysis of commercially available benzyl alcohol): The proposed method was evaluated by the assay of commercially available benzyl alcohol (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 7. This revealed that concentration of residual solvents present in benzyl alcohol in ppm levels which were less than the specified limits.

Conclusion

As per standard guidelines, the quantification of residual solvents is mandatory for all the pharmaceutical products and excipients before their release into the market because of its potential toxicity and may be the interference with the quality. Based on this point, we developed a method with an objective of development and validation of simple analytical method for simultaneous quantification of residual solvents present in excipients- benzyl alcohol by gas chromatography with flame ionization detector (which is powerful tool to quantify the residual solvents). The residual solvents benzene, chlorobenzene and toluene were well separated from each other and quantified by the proposed method. This method was also applied for the quantification of residual solvents in the marketed benzyl alcohol, which were present in ppm specification limits as per standard guidelines. The proposed method was validated as per the standard guidelines and the results revealed that the method was scientifically sound. Finally we conclude that the proposed method can be effectively applied for the quantification of residual solvents present in benzyl alcohol. This investigation may be helpful to the manufacturers for controlling and minimization of the residual solvents.

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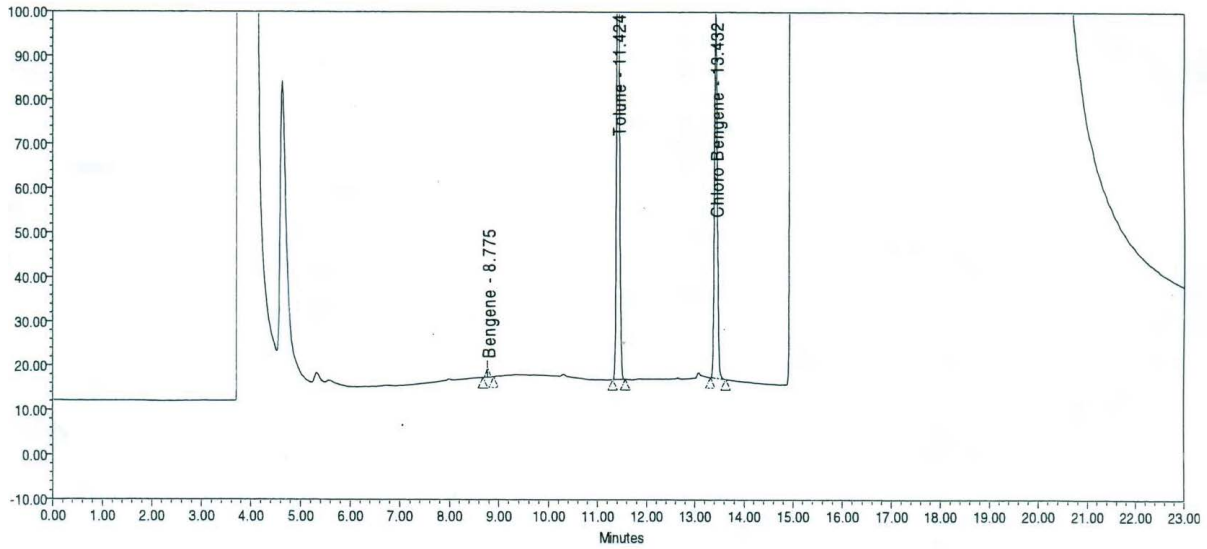

Figure 1: Chromatogram of Benzyl alcohol residual solvents.

Solvent name	System Precision		Method Precision		Intermediate Precision		
	Cocentration Mean[a] (ppm)	%RSD[b]	Concentration Mean[a] (ppm)	%RSD[b]	Mean[a] (ppm)		%RSD[b]
					Analyst 1	Analyst 2	
Benzene	12.55	1.5	2.0	4.9	2.0	2.1	5.9
Toulene	624.04	0.8	103.8	1.5	103.8	102.3	1.4
Chloro benzene	434.02	0.7	96.5	1.5	96.5	101.5	2.9
[a]Mean of 6 determinations							
[b]Relative standard deviation							

Table 3: Precision data of proposed method.

Solvent name	Parameter	Spike levels			
		LOQ	50s%	100%	150%
Benzene	Conc. Of solvent (ppm) Conc. Of spiked solvent (ppm) % Mean Recovery[a]	0.9 0.9 100.0	1.1 1.0 107.0	2.3 2.1 109.5	3.5 3.1 109.6
Toluene	Conc. Of solvent (ppm) Conc. Of spiked solvent (ppm) % Mean Recovery[a]	3.5 5.0 122.1	51.8 55.3 104.4	103.7 107.7 102.8	155.5 166.1 104.8
Chloro benzene	Conc. Of solvent (ppm) Conc. Of spiked solvent (ppm) % Mean Recovery[a]	2.6 2.7 103.8	51.6 52.2 101.6	103.2 103.2 100.0	154.7 159.3 104.0

Mean of 6 determinations

Table 4: Accuracy data of proposed method.

Parameter	% RSD[a]		
	Benzene	Toluene	Chlorobenzene
Effect of Variation Flow			
2.4 ml/min	3.6	0.6	0.7
2.5 ml/min	1.5	0.8	0.7
2.6 ml/min	2.6	1.7	1.7
Effect of Variation in Column oven temperature			
55°C	4.2	2.7	1.9
60°C	1.5	0.8	0.7
65°C	3.2	2.2	1.7
[a]Relative standard deviation (n=6)			

Table 5: Robustness data.

Parameter	Benzene	Toluene	Chloro benzene
% RSD	2.6	1.7	1.9
Acceptance criteria	Not more than 15.0		

Table 6: System suitability.

Solvent name	R_t	Area	Amount found (ppm)
Benzene	8.775	13	2.8
Toluene	11.424	434	134
Chloro benzene	13.432	624	120

Table 7: Assay results of commercially available Benzyl alcohol.

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