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Research Article

GAS CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF RESIDUAL ISOPROPYL ALCOHOL CONTENT IN PHARMACEUTICALS

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ABSTRACT

The analysis of residual solvents in pharmaceuticals such as drugs, excipients etc are important because most of them contain residual solvents in which isopropyl alcohol (IPA) is mainly present. Residual solvents are entrapped within the formulation either during synthesis, extraction of drug substances or during coating of formulations. Many residual solvents generally cannot be removed completely by standard manufacturing process or techniques and are left behind, preferably at low levels in formulations. Pharmaceutical excipients containing IPA were taken for gas chromatographic method development and validation. A simple, sensitive, and precise gas chromatographic method for the analysis of pharmaceutical excipients has been developed, validated and used for the determination of IPA. Nitrogen was used as carrier gas and method development involved optimization of chromatographic conditions such as; selection of column, pH, flow rate, temperature, and concentration. Method performance is determined primarily by the quality of the procedure itself. The two factors that are most important in determining the quality of the method are selective recovery and standardization. Properly developed method was finally utilized for validation. The search for the reliable range of a method and continuous application of this knowledge is called validation. Validation was performed according to the requirement of ICH validation guidelines. During method validation, parameters such as precision, linearity, accuracy, limit of quantification & detection and specificity were evaluated, which remained within acceptable limits. The results obtained from validation proved that the proposed method was scientifically simple and reliable. The proposed GC method was successfully applied for the quantification of IPA present in pharmaceutical excipients.

Keywords: Gas chromatography; Isopropyl alcohol, Method development, validation

1. INTRODUCTION

Residual solvents in pharmaceuticals are volatile organic chemicals that are used in and are produced during the synthesis of drug substances or can be in excipient used in the production of drug formulations [1]. These residual volatiles are remains from processing agents. Many of these volatile organic chemicals generally cannot be completely removed by standard manufacturing processes or techniques and are left behind, preferably at low levels. Residual solvent analysis of bulk drug substance and finished pharmaceutics products is necessary for a number of reasons [2]. High levels of residual organic solvents represent a risk to human health because of their toxicity. Residual organic solvents also play a role in the physicochemical properties of the bulk drug substance. Crystallinity of the bulk drug substance can be affected. Differences in the crystal structure of the bulk drug may lead to changes in dissolution properties and problems with formulation of the finished product. Finally, residual organic solvents can create odor problems and color changes in the finished product and thus can lead to consumer complaints [3]. Often, the main purpose for residual solvent testing is its use as a monitoring check for further drying of bulk pharmaceuticals or as a final check of a finished product.

Isopropyl alcohol (IPA) also known as the 2-propanol as well as iso-propanol, is a colorless liquid and becomes extremely viscous with decreasing temperature. IPA is used as residual solvent in lots of pharmaceutical ingredients, substances and finished products. Extensive literature review shows that only few analytical methods are available for quantification of IPA as residual solvents in pharmaceutical drugs and excipients, but Gas chromatography is most useful and precise technique for determination of such solvents [4-8]. Many alternatives to gas chromatography have been used to determine the level of residual solvent in pharmaceutical products and these procedures are either non-specific or they have high detection limits. The oldest and simplest method for determining the quantity of volatile residue is measuring the weight loss of a sample during heating. Loss on drying (LOD) suffers from the main disadvantage of being nonspecific. It is also found that no specific analytical data is present for estimation of IPA in various pharmaceutical products and drugs. Keeping these points into consideration, aim of present study was to develop and validate a simple, accurate, rapid, precise, sensitive and reliable gas chromatographic method for estimation of isopropyl alcohol content in pharmaceuticals.

2. MATERIALS AND METHODS

2.1. Materials

Isopropyl alcohol (IPA) was procured as gift sample from Sigma Aldrich. N, N-Dimethylformamide and Propionic acid were obtained from CDH Fine Chemicals, India. Milli-Q water was prepared by triple glass distillation and filtered through a nylon 0.22 µm-47 mm membrane filter.

2.2. Instrumentation and operating gas chromatographic operation

The Gas chromatography system [Perkin Elmer-500] was used in this present study and detection was performed by means of flame ionization detector*,* with Headspace sampler (Perkin Elmer, HS-40 XL or equivalent), Column (Fused silica column; 30 m long; 0.32 mm internal diameter), Data handling system (Turbo matrix; Totalchrom Navigator). Analytical balance, Auto pipette 100-1000 µL (Eppendorf), Headspace vials (Perkin Elmer-20 ml) and Headspace septa (20 mm Butyl rubber) were also used.

2.3. Preparation of standard solution of IPA

The standard solution of IPA was prepared by accurately weighing 71.43 mg of IPA and transferred into a 100 mL volumetric flask containing about 25 mL of diluent (N, N-Dimethylformamide and Milli-Q water in 50:50 ratio). Volume was made up with diluent and shaken well. 7 mL of this standard solution was transferred to a headspace vial and vial was sealed immediately. Six vials of standard in the same manner were prepared.

2.4. Preparation of sample solution of IPA

Sample solution was prepared by weighing about 1 g of sample and transferred into a headspace vial. Added 7 mL of diluent and sealed the vial immediately. Manually shaken the vial for about 2 minutes and vortexed for about 5 minutes. Prepared the sample vial in duplicate.

2.5. System suitability

The system is suitable for analysis of IPA, if the relative standard deviation for six replicate injections is not more than 15.0 %.

2.6. Determination of IPA content

Placed the sealed vials of sample solution, in duplicate, in the magazine and the Headspace analyzer was run. The area count of the eluting peaks was noted from the chromatographic report. The retention time of IPA is about 4.4 min. The content of IPA was determined by using following formula:

$$\text{Isopropyl Alcohol } (\mu g/g)) = \frac{AT}{AS} \times \frac{DS}{DT} \times \frac{P}{100} \times 10^3$$

Where,

AT - Area counts of Isopropyl Alcohol peak in the chromatograms of sample solution

AS - Average area counts of Isopropyl Alcohol peak in the chromatograms of standard solution as obtained under system suitability

DS - Dilution factor of standard solution

DT - Dilution factor of sample solution

P - Purity of Isopropyl Alcohol in % GC Area

2.7. Validation of GC Method for analysis of IPA

Validation was done as per ICH guideline. The developed *GC* method was validated with respect to the following parameters such as precision, limit of quantitation (LOQ), limit of detection (LOD), linearity, accuracy and system suitability [9-13].

3. RESULTS AND DISCUSSION

An understanding of the nature of the various residual solvents present in pharmaceuticals is the foremost prerequisite for successful GC method development for analysis of IPA. In addition, successful method development should result in a fast, simple and time efficient method that is capable of being utilized in a manufacturing setting.

The primary goal is column selection and several columns were initially investigated to finalize a method for the separation and quantitation of IPA as residual solvents. Fused silica column; 30 m long; 0.32 mm internal diameter) coated with 6 % cyanopropylphenyl-94 % dimethylpolysiloxane as stationary phase of 1.8 µm film thickness was used for IPA. A linear thermal gradient was chosen to provide elution of the solvents' peak during the isothermal segment of the chromatographic run for better quantification. An initial hold of 8 min at 40°C and finally a linear thermal gradient to 200°C at 30°C/min were used. For the system precision, six replicate injections of standard solution were given in the GC system and data is shown in Table 1, which indicates acceptable level of precision for the analytical system.

Table 1: System Precision of IPA

Standard Area (µV*sec)	
Injections	**IPA**
1	1023835.93
2	1028653.58
3	1026186.70
4	1030962.64
5	1033883.03
6	1042158.23
Mean	**1030946.69**
SD	**6523.799**
% RSD	**0.63**

Table 2: Method Precision of IPA

Sample ID	IPA	
	Area (µV*sec)	**Amount (µg/g)**
MP – 1	49193.82	234
MP – 2	51183.88	244
MP – 3	48029.75	230
MP – 4	53518.71	259
MP – 5	52313.77	251
MP – 6	52772.89	254
Mean		**245**
SD		**11.5**
% RSD		**4.69**

For the method precision, six sample solutions were prepared and analyzed by the proposed method and result is shown in Table 2.

Table 3: Intermediate Precision of IPA-Spiked

IPA (µg/g)		
Sample No	**Set I**	**Set II**
1	234	225
2	244	256
3	230	230
4	259	222
5	251	224
6	254	258
Mean	**245**	**236**
SD	**11.5**	**16.6**
% RSD	**4.69**	**7.03**
Overall Mean	**241**	
Overall SD	**14.5**	
Overall % RSD	**6.02**	

The % RSD values indicate that the method has an acceptable level of precision. For intermediate precision, six samples of pharmaceuticals were prepared and analyzed by the proposed method by different analysts on different columns and on different days and verified method ruggedness. For acceptance, % RSD should not be more than 15.0%.

The limit of quantitation and detection for IPA in pharmaceutical excipient sample were determined by showing precision of six replicate injections of various lower concentration solutions of the solvent. Data is shown in Table 4 and 5.

Table 4: LOD and LOQ of IPA

LOD LOQ Level	**LOD - LOQ - 1**		**LOD - LOQ - 2**		**LOD - LOQ - 3**	
Amount (µg)	**24.97**		**49.94**		**74.91**	
Conc.(µg/g)	**24.97**		**49.94**		**74.91**	
Injection	**Area counts**	**S/N**	**Area counts**	**S/N**	**Area counts**	**S/N**
1	5771.57	5.290	8422.11	9.484	16019.02	14.983
2	4951.64	5.069	8898.42	9.892	15421.14	15.060
3	5125.93	5.222	9789.13	10.596	15463.18	14.669
4	5891.83	5.661	10330.70	10.434	15116.04	14.695
5	5705.77	5.651	9978.28	10.241	15604.21	13.810
6	5678.21	5.540	8478.38	11.199	15887.37	15.109
Mean	**5520.83**	**5.4**	**9316.17**	**10.3**	**15585.16**	**14.7**
SD	**384.553**	**-**	**820.622**	**-**	**329.176**	**-**
% RSD	**6.97**	**-**	**8.81**	**-**	**2.11**	**-**

Table 5: Compiled LOD-LOQ of IPA

Compiled LOD LOQ				
	LOD		LOQ	
Name	Amount (μg)	Conc. (μg/g)	Amount (μg)	Conc. (μg/g)
IPA	24.97	24.97	74.91	74.91

The linearity of response for IPA was determined in the range of LOQ to 120 % of their specification limit. Result is shown in Table 6, indicates that the response is linear over the specified range. An acceptance criterion is correlation coefficient which should not be less than 0.980.

Table 6: Linearity of Response for IPA

Linearity Range (ppm)	Amount (μg)	Mean Area Counts
75	74.51	14248.99
719	719.26	136504.29
1439	1438.51	276859.86
2158	2157.77	425030.32
3596	3596.28	716896.73
5035	5034.79	1007672.51
6078	6077.58	1271986.64
Slope		**207.34**
Intercept		**-15826.90**
Correlation Coefficient		**0.9994**

For accuracy, the sample solution of pharmaceutical excipient was spiked with IPA at LOQ, 50 %, 100 %, and 120 % of specification level in triplicate. The samples were analyzed by the proposed method, the amount of IPA recovered was calculated and result is shown in Table 7. The result indicates that the method has an acceptable level of accuracy. Percentage recovery in the range of 80-120 for IPA is acceptable. Individual and overall % RSD of % recovery should not be more than 15.0.

For system suitability, standard solution was injected on different days during the validation study. Using the system suitability software, IPA peak was calculated and % RSD of area counts of six replicate injections of standard solutions was calculated. Data is shown in Table 8 and % RSD was found to be less than 15%, which indicate that overall system is suitable for analysis of IPA.

Table 7: Accuracy Data of IPA

Level	% Recovery
50% of Specification level -1	97.82
50% of Specification level -2	99.92
50% of Specification level -3	99.22
100% of Specification level -1	100.82
100% of Specification level -2	99.59
100% of Specification level -3	99.70
120% of Specification level -1	104.95
120% of Specification level -2	105.64
120% of Specification level -3	104.21
Overall Mean	**101.32**
Overall SD	**2.843**
Overall %RSD	**2.81**

Table 8: Overall System Suitability of IPA

Experiment Name	% RSD
System precision, Method precision, Accuracy and LOD/LOQ	0.63
Intermediate precision, Linearity, Specificity	0.44

4. CONCLUSION

The rapid and highly selective GC method was developed and validated for the quantification of IPA residual solvent present in pharmaceutical excipients through an understanding of the synthetic process, nature of solvents and nature of stationary phases of columns. This method was very suitable and easy for this analysis. In the validation of developed method for estimation of IPA, all the validation parameters such as; precision, LOQ, LOD, linearity, accuracy and system suitability were properly determined and all parameters were found in the acceptable range. GC method was shown to be specific for determination of IPA as residual solvents was successfully applied, lead to monitor and control the presence of IPA on a manufacturing level. The method was found to be applicable for the routine analysis of the pharmaceuticals in pharmaceutical industry.

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