Research Article

Validation of the HS-GC-FID Method for the Determination of Residual Ethanol in Tablets

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ABSTRACT

Current study explains how to make validation of a HS-GC-FID method (based on the Pharmacopeia's method) for the determination of ethanol residue in tablets. A description of the procedure and simple calculation of validation parameters was presented. Specificity of the method was determined. Linearity was observed in the range from 4000 ppm to 6000 ppm of ethanol, Correlation Factor was 0.994. The relative standard deviation for repeatability and intermediate precision was 12.21 % and 3.2 % respectively. The limit of detection was calculated to be 0.887 ppm of ethanol per sample. The total run is 8 minutes.

Keywords: Validation, Residual Solvent-Ethanol, Gas chromatography, Headspace analysis.

INTRODUCTION

The validation is the finding or testing the truth of something, the validation is the procedure in which we ensure that the method is trustworthy[1]. Validation of the analytical procedure is an important issue in the quality control guidance. Validation of the analytical procedure is very important and necessary for screening method, and it is very important part of the analytical method[2-4]. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose[1,5]. Validation is performed in order to show that the result(s) generated by a particular analytical procedure are reliable and accurate[6]. The validation procedure has been developed by time but there are little studies about validation of residual solvent determination especially ethanol. Residual solvents in pharmaceuticals are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products[7].

Determination of residual solvent is necessary because residual solvents can present potential risk to human due to their toxicity. It is also may cause changes in the physiochemical properties of the pharmaceutical product[8-13].Solvents are routinely used in the synthesis and process chemistry of drug substances. These solvents cannot be completely removed by practical manufacturing practices, and the residue should be within the normal limit[14].

European Pharmacopoeia classified the residual solvents in three classes, ethanol was classified in the third class of solvent, It is considered that an amount of this class of 50 mg per day or less (corresponding to 5,000 ppm or 0.5%, when the daily dose does not exceed 10 g) is permitted[7,15].

MATERIALS AND METHODS

Reagents

The following compounds were used during experiments:

Ethanol standard 99.9 from Dikma, Ethanol 96%, N,N-dimethylacetamide diluent, Methanol was used for cleaning laboratory glasses. Distilled water was used to prepare sample and standard solutions.

Instrumentation

Analysis of ethanol was carried out on gas chromatography from Agilent model 7890A equipped with flame ionization detector. Separations were performed on Dikma DM-5 capillary column (30m×0.25mm i.d) with a phase thickness of 0.25 µm film (5% dibenzyl-95% dimethylpolysiloxane). The extraction was performed using Headspace sampler with 20 ml vial.

The initial carrier gas flow was approximately 0.4 ml/min.

Chromatographic conditions are presented in Table 1.

Table 1: Parameters for gas chromatographic analysis (GC-FID procedure)

Carrier gas	Helium 35 cm min^{-1}	
Detector	FID; 250 °C	
Detector gases	Hydrogen 45 cm3 min^{-1}	
	Air 450 cm3 min^{-1}	
Injector	Split/splitless type; 200 °C	
	Split 10:1	
Chromatographic	Dikma DM-5 capillary (5% dibenzyl-95% dimethylpolysiloxane)	
Column	Length	30 m
	Film thickness	0.25 µm
	Internal diameter	0.53 mm
Temperature Program	Initial temperature 50 °C, ramped at 30 °C min−1 to 200 °C , hold 3 min; total analysis time 8 min	

Procedure

Isolation and enrichment of ethanol performed using static headspace technique, while separation performed using gas chromatography with flame ionization detection.

Water with N,N-dimethylacetamide (9:1) was used as dissolving solution. The sample was incubated in headspace vial (5ml in 20 ml vial) at 80 C° for 30 min, 0.5 ml of the vapor phase was injected into the GC-FID system with split mode 10.The oven temperature was programmed from 50 C° to 200 C° at 30 C°/min. 200 C° is held for 3 min.

The total run time is 8 minutes.

Headspace parameters are presented in table 2.

Table 2: Parameters for sample preparation step using headspace technique

Parameter	Value
Thermostat temperature (°C)	80 ± 2
Needle temperature (°C)	85 ± 2
Transfer line temperature (°C)	90 ± 2
Time (min)	30 ± 0.5
Sample amount	5 ml of solution
Injection volume (ml)	0.5
Pressure (kPa)	160 ± 5
Pressurize time (min)	0.5 ± 0.05
Withdraw time (min)	0.2 ± 0.02

Standard solution preparation

Standard solutions were prepared by adding about 10 ml of water to a 100-ml volumetric flask, followed by 10 ml of N,N-dimethylacetamide and an exact amount of ethanol (using a micropipette). Then the flask was filled up with water to the volume of 100 ml. Five milliliters of each standard solution were transferred to a 20-ml headspace vial and analyzed (HS-GC-FID). The results of these experiments were plotted as calibration curves over the concentration range from 4000 ppm to 6000 ppm.

Quantitative analysis

The sample was placed in a 20-ml headspace vial. Then, 5 ml of water with N,N-dimethylacetamide (9:1) were added to the vial. The sample was analyzed using the HS-GC-FID procedure. The chromatographic signal (peak area) and calibration curves of the standard ethanol plotted previously made it possible to estimate the concentration of ethanol. In the sample, once isolation and chromatographic conditions were established, the method validation was performed following ICH recommendations[5].

RESULTS AND DISCUSSION

The first step of the experimental was the validation which includes the following Parameters: selectivity (specificity), linearity, limit of detection (LOD) and limit of quantitation (LOQ), range, repeatability and intermediate precision.

Specificity

The blank sample was injected and analyzed. No significant influence of other compounds on quantification was observed according the basis of the chromatogram obtained. A mixture of methanol and ethanol was prepared and analyzed. On the basis of this chromatogram there has been good resolution between ethanol and methanol.

Linearity

Linearity was verified by analyzing five standard solutions in the range (4000-6000) ppm of ethanol in the vial, four times each. The relationship between the analyte concentration in the sample and the corresponding detector response was calculated using the linear regression method. On the basis of results obtained, linearity was determined for several concentration ranges. The calibration curve equation was

$y=3669351.20x+3972626$andthe correlation factor was 0.994.

Detection limit and quantitation limit

The blank was injected in the same condition, then the noise was determined then detection limit was calculated from the equation

Detection limit= 3*noise according to united state pharmacopeia.

The calculated value was LOD= 0.88 ppm. Then quantitation limit was calculated from the equation

LOQ= 10*noise according to united state pharmacopeia.

The calculated value was LOQ= 2.95 ppm.

Accuracy

Nine samples have been injected for solutions prepared to estimate accuracy. The concentrations used were (80-100-120)% of the standard solution. Three replications for each concentration were evaluated. Then the percentage recovery was calculated. The calculated value was 97.85.

Precision

Nine samples have been injected for solutions prepared to estimate repeatability. The concentrations used were (80-100-120) % of the standard solution. Three replications for each concentration were evaluated. Then the percentage recovery was calculated. The calculated value was 95.87.In the same way this value for intermediate precision was 96.3.

Robustness

The flow rate had been changed in our study (0.4) ml/min increasing and decreasing by 0.1 ml/min to be (0.3-0.4-0.5) in order. Then the five samples of the standard solution had been injected at each flow rate. The relative

standard deviation was calculated, the calculated value was less than 15%

CONCLUSION

In this study, a HS-GC-FID analytical method was developed and validated for the qualitative determination of ethanol in a drug substance. Development was carried out according to requirements of the Eur. Ph. General method[16]. Sample solvent water with n, n dimethylacetamide (9:1) was selected to obtain good recoveries for ethanol. The method was validated within ICH guidelines Q2AR1[5]. Specificity, limits of detection and quantitation, linearity, accuracy, precision (system repeatability, method precision and intermediate precision) and robustness (changes in HS and GC conditions and solutions stability) were determined. Excellent results were obtained within the world wide accepted validation reference values, and particularly taking into account the low concentration levels investigated. This method has been shown to have a high sensitivity since it has a low DL and QL of 0.88 ppm and 2.95 ppm for ethanol. It has also been demonstrated that this method can be readily used to determine ethanol in solid dosage forms. The developed method can be successfully applied for routine determination of ethanol in real samples. Therefore, this method may also work for other residual solvent analysis. This method has a much shorter sample equilibration time and shorter run time comparing with the previously published methods.

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