Research Article

Gas Chromatography Method of Cleaning Validation Process for 2-Propanol Residue Determination in Pharmaceutical Manufacturing Equipment

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ABSTRACT

Cleaning validation is an integral operation of good manufacturing practice in pharmaceutical industry. The aim of this study was to validate simple analytical method for detection of 2-propanol residue in equipment, which is likely contaminated with 2-propanol, usually used in the production area. The gas chromatography with flame ionization detection (GC-FID) method was validated on a GC system using DB-FFAP capillary column at the flow rate of 4.9 mL/min. The calibration curve was linear over concentration range from 2.8µg/mL to 110.7µg/mL with a correlation coefficient equal to 0.99981. The detection limit (LOD) and quantitation limit (LOQ) were 1.1µg/mL and 2.8µg/mL, respectively. The simplicity of gas chromatography method makes it useful for routine analysis of 2-propanol residue and is an alternative to corresponding methods.

Keywords: Cleaning validation, GC-FID, 2-propanol.

INTRODUCTION

The cleaning validation processes in the pharmaceutical industry have been regulated by the implementation of the Good Manufacturing Practice (GMP) rules. [1] The purpose of all cleaning validation analysis is to determine, if the contamination of residues is below certain acceptance limits. The acceptance limits of contaminants and residues are determined in International Conference on Harmonisation (ICH) guidelines on their specific toxicity in tested impurities. [2-3] All potential cross-contaminations are related to the determined residues presence. The acceptance limits for residues are not currently advised by regulatory agencies. The acceptance limits should be defined based on logical criteria, such as a risk of residues contamination, as well the quality and the safety of final products. The maximum acceptance limit of ingredient residue is based on mathematical formula and kept at general limit of 10µg/mL. Some acceptance limits have been proposed in published studies. [3-4] The analytical method should be selective for known substance to monitor effectiveness of the cleaning process. The analytical method must be high sensitivity method to determined low residue concentrations. The analytical method for the determination of residue concentrations in the cleaning validation can be specific or non-specific. The specific and non-specific methods are for example high performance liquid chromatography (HPLC), gas chromatography (GC), ion chromatography (IC). [5-12]

The manufacturing equipment used in the pharmaceutical production is usually cleaned with 2-propanol. Isopropanol (or 2-propanol) is defined as a substance with the limit concentration of about 5000µg/mL according to European Pharmacopeia (Ph.Eur.) and United States Pharmacopeia (USP) as a residual solvent Class III. Ph.Eur. methods can be applied for the determination of 2-propanol residue with the limit of 5000µg/mL. Ph.Eur. methods are time-consuming analysis (average time period of 60 minutes).

MATERIALS AND METHODS

Chemicals of high purity level and analytical grade were used. As diluents, filtrated purified water was applied. 2-propanol from Merck was used as a standard.

Gas chromatography conditions

Analysis was performed using gas chromatograph (Agilent Technologies 6890N) equipped with electronic pressure control (EPC), a split/splitless injector and FID detector. Data were acquired and processed using Empower 2 software.

Method: 30 m long DB-FFAP column, 0.320 mm in inner diameter and 1.0µm in film thickness (manufactured by J&W Scientific, USA) was used. Injection port was heated up to 200°C while the temperature of detector was 280°C. Helium was allowed to flow at a velocity rate of 4.9 mL/min. Hydrogen gas and air supply to the detector was

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30 mL/min and 300 mL/min, respectively. The sample was introduced on to the column in a split mode with split ratio, 1.0:1. The column temperature was kept at 35°C for 5 min followed by an increase in the temperature at a rate of 20°C/min to 150°C. The 150°C temperature was held up to 1 min. Volume of injected sample was 1.0µL.

Standard stock solution: About 50.0 mg of 2-propanol was accurately weighed and transferred to a 50.0 mL of volumetric flask and diluted to volume with water, then the solution was mixed.

Standards solution: Appropriate dilutions were prepared in water to obtain the calibration solution from 2.8µg/mL to 11.1µg/mL.

Blank: Purified water.

RESULTS

Specificity: Specificity has been established by injections of standard solution. No peaks were observed in injections of blank (water). Chromatograms of blank (water) and standard 2-propanol are presented in Figure 1.

System precision: System precision has been demonstrated by six replicated injections of standard solution at the 11.1µg/mL concentration. The standard solution is prepared at the working concentration and analyzed as per method. The system precision of the proposed method is expressed in the term of % R.S.D. of data. Table 1 displays the system precision for standard solution.

Linearity: The method has been shown to be linear by a plot of five points in the range LOQ – 110.7µg/mL, with double determination at each level. The linearity curve is drawn by plotting concentration vs. peak area response. Correlation coefficient for 2-propanol was found to be more than 0.99. The calibration curve values of slope, intercept and correlation coefficient are presented in Figure 2 and indicate good linearity.

Accuracy: For accuracy studies, some known amount of 2-propanol standards were spiked into placebo at about 50%, 100%, and 150% of limit 10µg/mL in triplicate. The average recoveries were calculated from 90.2% for 150% of 10µg/mL limits to 93.7% for limits 50% of 10µg/mL limits. The requirements for accuracy studies were 90.0% - 110.0%. So, it may be concluded that the method is accurate.

Limit of detection (LOD) and limit of quantitation (LOQ): The limit of detection of an analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessary, quantified as an exact value. To define the limit of detection, the analyst must determine the minimum concentration of an analyte, which could be observed in a sample. The limit of quantitation is the lowest amount of an analyte in a sample, which can be quantitatively determined with precision and accuracy. LOD and LOQ have been established by six injections at LOD level and six injections by LOQ level. The % R.S.D. was found to be less than 10.0%. The limit of detection and limit of quantitation are demonstrated in Table 2.

Robustness: Robustness has been established by analyzing sample in triplicate as per proposed method and by changing inlet temperature +10%.The percentage of R.S.D was calculated for 2-propanol solvent, all value are listed in Table 3.The difference in relation to the reference value [%] was calculated and was 98.7%. It may be concluded that the method is robust.

System suitability: System suitability has been demonstrated during validation study by analyzed six replicated 2-propanol standard solutions at concentration about 11.1µg/mL. %R.S.D. for retention times for six injections of standard solution at concentration 11.1µg/mL was found 0.12, Tailing factor for 2-propanol peak was found to be 1.29, Theoretical plates for 2-propanol peak was found to be 7032. All data are presented in Table 4.
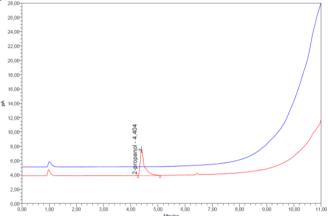

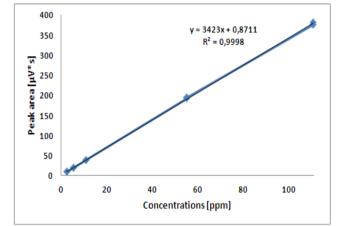**Fig. 1: Chromatograms of blank (water) and standard 2-propanol (concentration about 11.1µg/mL)**

Fig. 2: Linearity curve of 2-propanol

Table 1: The system precision for standard solution 11.1µg/mL

Sample number	2-Propanol (area)
1	38.4
2	38.3
3	37.2
4	38.3
5	38.4
6	38.7
Mean	38.2
Standard deviation	0.5
% R.S.D.	1.4

Table 2: Limit of detection and Limit of quantitation

	LOD [µg/mL]	LOQ [µg/mL]
2-propanol	1.1 µg/mL	2.8 µg/mL
	% R.S.D. (n=6) =9.1	% R.S.D. (n=6) =1.0

Table 3: Data after robustness analysis

2-Propanol, concentration about 11.1 µg/mL	
No. of sample	After changing inlet temperature +10%[area]
1	37.7
2	37.5
3	37.2
Mean	37.7
% R.S.D.	0.7

Table 4: System suitability for determination of 2-Propanol in pharmaceutical area method

Sample number	Retention time [min]	Tailing factor (valley to valley integration)	No. of theoretical plates
1	4.40	1.26	6218
2	4.39	1.21	6969
3	4.39	1.43	7404
4	4.40	1.28	5792
5	4.39	1.31	7389
6	4.39	1.22	7032
Mean	4.39	1.29	6801
Standard deviation	0.005	0.08	656
% R.S.D.	0.12	6.2	9.64

DISCUSSION

The gas chromatography with flame ionization detection method was validated for residual determination of 2-propanol in manufacturing process of pharmaceuticals. The prepared standards procedure makes it rapid and uncomplicated method. Standard time of analysis is about 60 minutes. Our modified method is much shorter and takes about 11 minutes, that makes the examination of cleaning samples less manpower and time-consuming. Our cleaning validation method is more competitive, compared to model methods of determining 2-propanol residue in the pharmaceutical industry equipment. This modification allows to determine the limit of quantitation about 2.8μg/mL. The method was validated with respect to specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, and can be used for analysis of 2-propanol samples obtained from the cleaning validation.

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