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Research Article

Development and Validation of a Gas Chromatographic Method for Determination of Residual Solvents in Propofol API

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ABSTRACT

Objective: To develop and validate a simple, sensitive, and reliable Headspace Gas Chromatography method coupled with Flame Ionization Detection (HS-GC-FID) for the determination of residual solvents in Propofol Active Pharmaceutical Ingredient (API) in accordance with International Council for Harmonisation (ICH) guidelines.

Methods: Chromatographic separation was achieved using a DB-624 column with nitrogen as the carrier gas under optimized temperature programming conditions. N-Methyl-2-pyrrolidone (NMP) was used as the diluent for the preparation of standard and sample solutions. The residual solvents analyzed in the study were isopropyl alcohol (IPA) and cyclohexane. The developed method was validated as per ICH Q2(R2) guidelines for specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness.

Results: The developed method demonstrated good specificity with well-resolved peaks and no interference at the retention times of IPA and cyclohexane. Excellent linearity was observed over the studied concentration range with correlation coefficients (R^2) of 0.9904 and 0.9908 for IPA and cyclohexane, respectively. Precision studies showed low %RSD values, indicating good repeatability of the method. Accuracy studies demonstrated acceptable recovery results within the specified limits. The obtained LOD and LOQ values confirmed adequate sensitivity for trace-level analysis of residual solvents. Robustness studies indicated that minor deliberate variations in chromatographic conditions did not significantly affect the analytical performance of the method.

Conclusion: The validated HS-GC-FID method was found to be simple, precise, accurate, robust, and suitable for routine quality control analysis of residual solvents in Propofol API, ensuring compliance with pharmaceutical safety and regulatory requirements.

Keyword: Propofol, Residual Solvent, Headspace Gas Chromatography, ICH Guideline.

INTRODUCTION

The objective of analytical method development is to develop new methods for estimating chemical compounds using analytical instruments, including phytoconstituents, active pharmaceutical ingredients, biological samples, volatile components of crude aerial parts of plants, and the chemical composition of plant products and formulations. Because they provide precise answers quickly, take little effort, and use fewer chemicals during analysis, analytical instruments have become far more important than chemical methods for estimating any substance.[1] Organic volatile impurities known as residual solvents are utilized or created throughout various stages of the synthesis or production of pharmaceutical drug compounds, intermediates, excipients, or medicinal products. [2] The creation of active pharmaceutical ingredients (API) under GMP (good manufacturing practice) conditions requires sufficient control over the quality of the various components utilized in the synthesis. Therefore, organic residual solvents must be controlled and their purity evaluated before every GMP synthesis.[3] The use of Class I solvents, which include five residual solvents, should be avoided since they are known or suspected to be environmental risks and human carcinogens. It is necessary to identify and measure class I solvents. Class II solvents, which comprise 29 residual solvents, can induce other irreversible toxicity such teratogenicity or neurotoxicity, or they are non-genotoxic animal

carcinogens.[4] These solvents should be used sparingly. There are certain restrictions for class II solvents. There is no requirement for a health-based exposure limit for class III solvents, which include 26 residual solvents with little hazardous potential to humans. PDEs in class 3 solvents are at least 50 mg daily. Lastly, Class 4 solvents are those for which there is insufficient toxicological information. In order to comply with regulations and guarantee patient safety, the process of determining residual solvents becomes essential for quality control of therapeutic substances and drug products.[5] [6] The amount of residual solvents deemed safe for human use and in pharmaceutically finished goods is recommended and limited by the worldwide conference on harmonization. Guidelines and daily exposure limits for numerous solvents have been published by the ICH. These solvents have been divided into three groups based on how poisonous they are. [7] Organic solvents are frequently used in the manufacture of excipients, pharmacological ingredients, and drug product formulation. Their toxicity, impact on the drug substance's crystal quality, and potentially disagreeable taste or odour make them undesirable in the finished product. Organic volatile pollutants or residual solvents are common terms used to describe these small levels of organic solvents. Finding residual solvents in medication components, excipients, or pharmaceutical formulations is one of the most difficult and demanding analytical tasks in the pharmaceutical industry. [8]

Propofol is a sedative and anesthetic drug that is a member of the 2,6-diisopropyl-phenol class of alkylphenol derivatives [Figure 1] [9,10]. mainly sticks to red blood cells. and blood serum proteins, showing a high rate of over 98% protein binding. This binding to proteins can be impacted by changes in body temperature, pH, volume, or protein levels (such as hypo- or hyperalbuminemia), particularly in intensive care settings. It is crucial to remember that the drug's pharmacodynamic effects are solely caused by its unbound form. Therefore, even slight changes in the protein binding of propofol may have negative effects. [11,12] Related changes in pharmacological activity, such as a drop in protein binding from 98% to 96%, would increase the concentration of unbound active propofol.Therefore, it is advised to use free drug concentration determination whenever possible in pharmacokinetic-pharmacodynamics research [13][14].

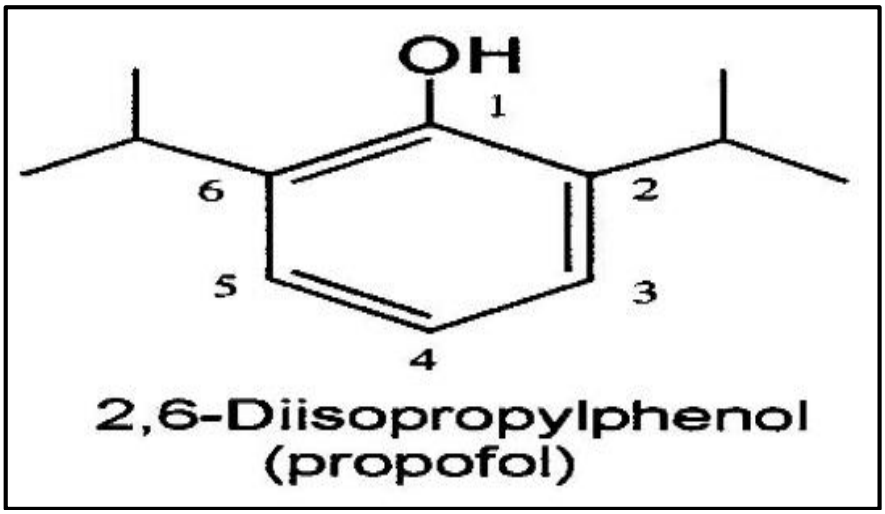

Figure 1. Structure of Propofol

MATERIALS AND METHODS

Material

Used chemicals were obtained from following supplier:

Cyclohexane, Iso Propyl Alcohol, N-Methyl-2-Pyrrolidone - Laxachem organics Pvt. Ltd. Also, API Propofol was obtained from Laxachem organic Pvt. Ltd.

Instrumentation

The sample was loaded using a Headspace sampler (Agilent Technologies 7697A HS) and a Gas Chromatograph (Agilent Technologies 8890 GC system) with a flame ionization detector. A micropipette (100–1000 µL) and an analytical balance (Shimadzu AUW 220D) were utilized.

Chromatographic condition

Chromatography condition: A DB-624 column with a length of 30 m, an internal diameter of 0.53 mm, and a film thickness of 3.0 micrometers was utilized for the gas chromatographic examination. As a carrier gas, nitrogen was employed. The flow rate was maintained at 5 mL/min. The split ratio was 10:01. The oven temperature was first held at 60 degrees Celsius for one minute, then increased at a rate of 20 degrees Celsius per minute to 250 degrees Celsius for nine minutes. The detector temperature was 280 degrees Celsius

PREPARATION OF SOLUTIONS

Preparation of Stock Solution

Ready using a calibrated analytical scale, 0.6250 g of isopropyl alcohol and 0.4850 g of cyclohexane were precisely weighed to create the stock solution of residual solvents. A 25 mL volumetric flask that was dry and clean was used to properly transfer the weighed amounts. To guarantee that both analytes were completely dissolved, the flask was filled with about 10 mL of an appropriate diluent and gently swirled. If necessary,
the solution was allowed to reach room temperature before being diluted with the same diluent until no visible particles were left. To make sure the solution was uniform, the flask was inverted multiple times. This stock solution served as the primary standard solution for further dilutions in method development and validation studies.

Preparation of Standard Solution

Using a calibrated pipette, 1.0 mL of the previously made stock solution was transferred into a 50 mL volumetric flask to create the working standard solution. To guarantee that the analytes were distributed evenly, around 20 milliliters of diluent were added, and the mixture was thoroughly mixed. After that, the volume was adjusted with diluent and blended once more using repeated inversion. This standard solution was used for system suitability testing, calibration, and routine analysis during the validation process.

Preparation of Sample Solution

The sample solution was prepared by accurately weighing approximately 0.5 g of propofol API and transferring it into a clean, dry headspace vial. Care was taken to minimize sample exposure to ambient conditions to prevent loss of volatile components. To the vial, 5 mL of diluent was added using a calibrated pipette. The vial was immediately sealed with a suitable septum and crimp cap to prevent evaporation of residual solvents. The contents were mixed, if necessary, to ensure uniform distribution of analytes within the matrix. This preparation was used for the determination of native residual solvent content in the sample.

Preparation of Spiked Sample Solution

The spiked sample solution was prepared to evaluate method performance characteristics such as accuracy and precision. Approximately 0.5 g of propofol sample was accurately weighed and transferred into a headspace vial. To this, 5 mL of the prepared standard solution containing known concentrations of isopropyl alcohol and cyclohexane was added. The vial was immediately sealed to prevent loss of volatile components and ensure integrity of the sample. The contents were allowed to equilibrate, ensuring proper interaction between the sample matrix and the added analytes. This spiked preparation was used for recovery studies and method validation experiments.

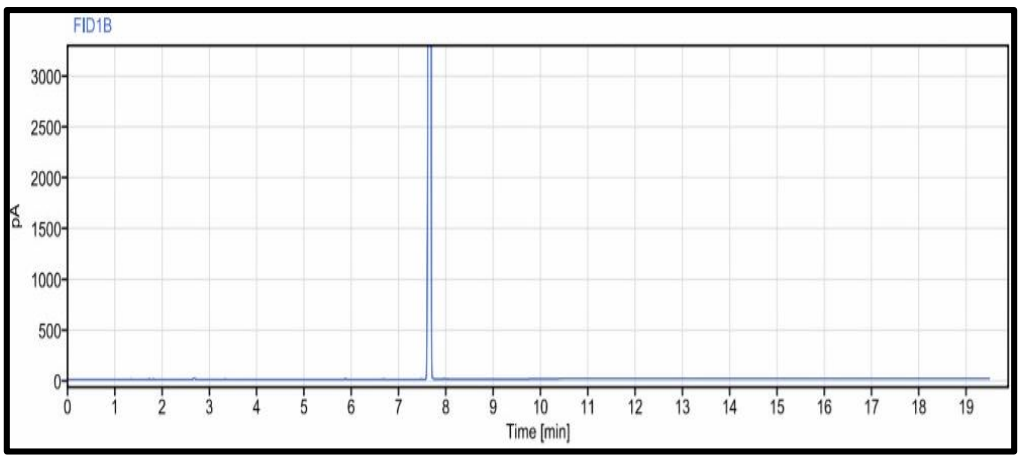

Figure 2. Chromatogram of Blank

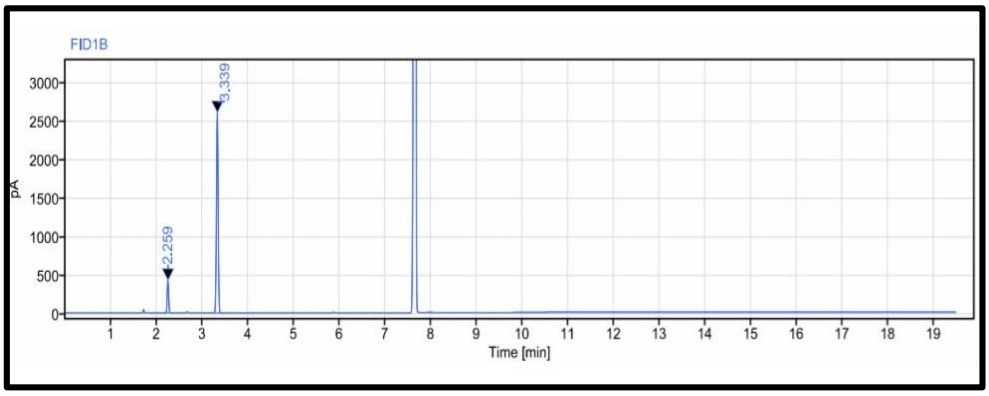

Figure 3. Chromatogram of Standard.

Validation of Methods

The technique was evaluated based on the ICH harmonized tripartite guideline's specificity, limit of detection and limit of quantitation, linearity, accuracy, repeatability, ruggedness, system adaptability, and method precision of residual solvents (1997, 2002).

RESULT AND DISCUSSION

Specificity

IPA, cyclohexane, and benzene were added to the Propofol API sample separately. Each sample was then chromatographed to look for any interference between the leftover solvent peaks. A resolution standard including isopropyl alcohol, cyclohexane, and benzene was used to assess the new method's specificity. The results showed that the retention times were, in order, 2.26, 3.34, and 3.49 minutes. Every peak had a clear resolution and no interference. The method's specificity was confirmed by the benzene peak's obvious separation from nearby peaks.

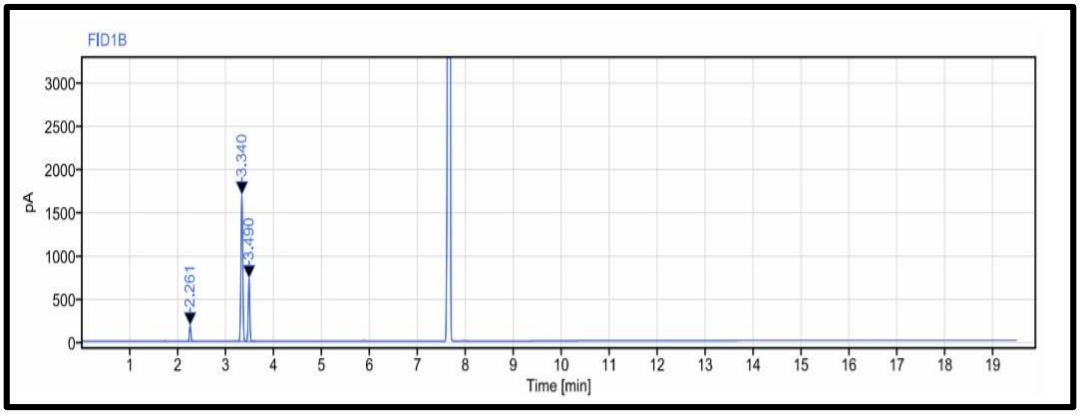

Figure 4. Chromatogram of Specificity

Accuracy

By injecting a known quantity of residual solvent at accuracy levels at LOQ, 100%, and 150% of standard stock solution, accuracy the degree to which measured values and their actual value are close was assessed(n=3). Table 1 lists the percentage recovery of residual solvents. The approach is accurate for determining residual solvents in Propofol API because these values are substantially within the specified limits.

Table 1: Accuracy Data of IPA and Cyclohexane

Accuracy Level	Cyclohexane (%)	Iso Propyl Alcohol (%)
LOQ	89.9	108
100%	90.2	100.2
150%	109.8	116.9

Limit of Detection & Limit of Quantification:

The signal to noise ratio approach was used to establish the limits of detection and

quantitation (LODs and LOQs) of residual solvents in Propofol API. The S/N ratios for both IPA and Cyclohexane were found to be well above the acceptance criteria (≥3 for LOD and ≥10 for LOQ), indicating good sensitivity of the method. The %RSD at LOQ level was within 15% for both analytes, confirming acceptable precision. Hence, the developed method is sensitive and precise for the analysis of residual solvents are given in below table 2.

Table 2: LOD & LOQ

Parameter	Cyclohexane	IPA
LOD	72.63	11.80
LOQ	232.21	34.38
LOQ Precision % RSD	4.56	7.64

Precision

System precision, method precision, and intermediate precision were used to assess precision. Six injections(n=6) of standard solution were used to evaluate the system's precision, and it was discovered that the peak area's %RSD fell within acceptable bounds. Six spiking sample preparations(n=6) were analyzed to establish method precision, and the %RSD values were within acceptable bounds, suggesting satisfactory repeatability. The findings of evaluating intermediate precision on several days and by various analysts(n=6) were found to be consistent with %RSD within acceptable bounds. Overall, the technique showed good precision

Table 3: System Precision

Parameters	IPA	Cyclohexane	Acceptance Criteria
Mean RT	2.257	3.338	-
SD	0.001	0.001	
% RSD	0.00	0.00	NMT 1.0
Mean Area	825.46	7660.44	-
SD	48.33	364.81	-
% RSD Area	5.86	4.76	NMT 15.0

Table 4: Method Precision (Repeatability)

Parameters	IPA	Cyclohexane	Acceptance Criteria
Mean Area	825.46	7660.44	-
SD	48.33	364.81	-
% RSD	5.86	5.76	NMT 15.0

Table 5: Intermediate Precision

Parameters	IPA	Cyclohexane	Acceptance Criteria
Mean RT	2.257	3.338	-
SD(Area)	0.001	0.001	-
% RSD	0.04	0.03	NMT 1.0
Mean Area	837.18	7756.22	-
SD	44.45	418.42	-
% RSD Area	5.30	5.39	NMT 15.0

Robustness

The robustness of the developed method was assessed by introducing small deliberate variations in detector temperature (258–262°C), flow rate (4.9–5.1 mL/min), and injector temperature (218–222°C). The effects were evaluated for IPA and cyclohexane in terms of mean peak area, retention time, and %RSD. For IPA, the %RSD of peak area ranged from 4.9% to 10.2% and %RSD of retention time was ≤0.44%, while for cyclohexane, %RSD of peak area ranged from 3.3% to 8.0% with no significant variation in retention time (%RSD = 0.00%). All values met the acceptance criteria (NMT 15% for area and NMT 1.0% for RT), confirming the robustness of the method.

Table 6: Robustness IPA

Parameter	Condition	Mean area	%RSD area	Mean RT(min)	%RSD RT
Detector temp	258◦C	828.512	10.2	2.258	0.00
Detector temp	262◦C	755.827	7.4	2.258	0.00
Flow rate	4.9ml/min	826.478	7.8	2.286	0.44
Flow rate	5.1ml/min	860.033	4.9	2.226	0.00
Injector temperature	218◦C	689.910	6.0	2.260	0.00
Injector temperature	222◦C	682.787	5.0	2.259	0.00

Table 7: Robustness Cyclohexane

Parameter	Condition	Mean area	%RSD area	Mean RT(min)	%RSD RT
Detector temp	258◦C	7770.438	8.0	3.339	0.00
Detector temp	262◦C	7419.538	3.3	3.370	0.00
Flow rate	4.9ml/min	7689.527	6.2	3.377	0.00
Flow rate	5.1ml/min	7857.775	5.7	3.307	0.00
Injector Temperature	218◦C	5972.330	3.9	3.340	0.00
Injector Temperature	222◦C	6118.125	4.1	3.339	0.00

Linearity

Linearity was evaluated using five Concentration levels (n=5) for IPA and Cyclohexane. The proposed HS-GC technique was used to analyse standard solutions of IPA and cyclohexane across a specified concentration range in order to assess linearity. Plotting concentration against peak area was used to create calibration curves, and linear regression analysis was carried out. With a correlation coefficient (R^2) of 0.9904 for IPA and 0.9908 for cyclohexane, the approach demonstrated exceptional linearity.

Peak area grew proportionately with concentration in both situations, demonstrating a robust linear connection. The obtained results verify that the method is linear, dependable, and appropriate for accurate quantification over the study range by adhering to analytical validation guidelines.

Table 8: Linearity Data for IPA

Linearity (PPM)	Peak area
149.22	245.76
248.70	375.73
397.92	580.21
497.92	750.25
596.88	958.9
$r^2 = 0.9904$	

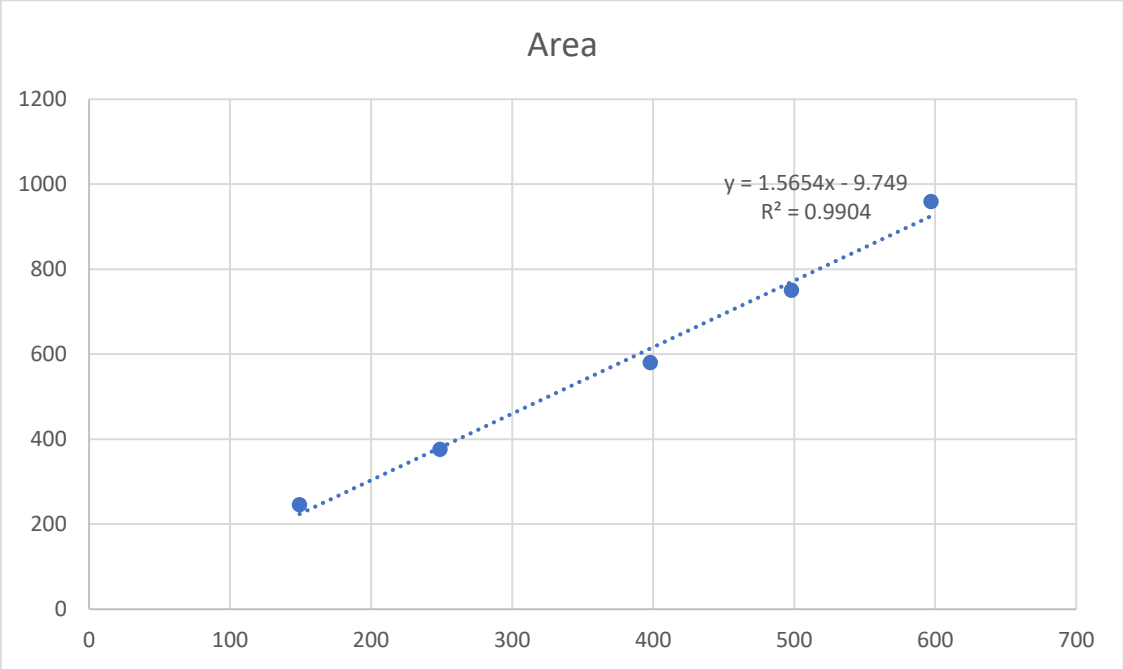

Figure 5. Calibration Curve of IPA

Table 9: Linearity Data for Cyclohexane

Linearity (PPM)	**Peak area**
116.73	2152.34
194.56	3357.33
311.30	5412.4
389.13	6747.75
466.95	8810.02
r^2 = 0.9908	

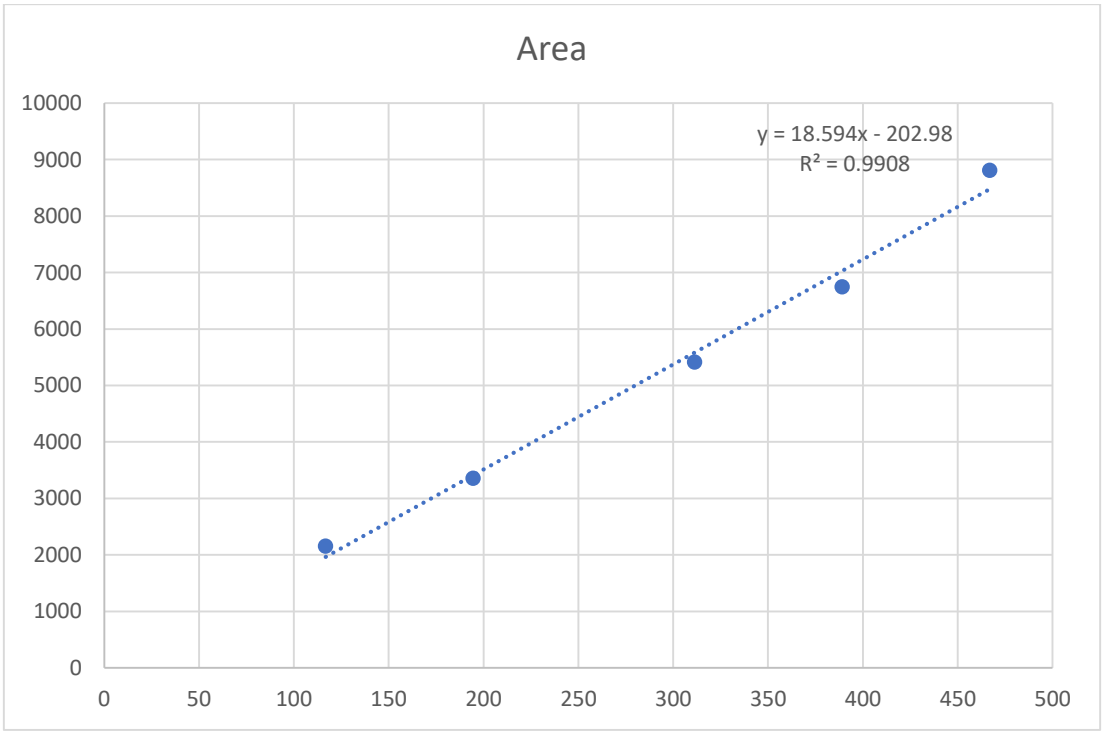

Figure 6. Calibration Curve of Cyclohexane

DISCUSSION

The developed HS-GC-FID method showed good specificity with well-resolved peaks of IPA, cyclohexane, and benzene at retention periods of 2.26, 3.34, and 3.49 minutes, respectively, without interference, the devised HS-GC-FID method demonstrated good specificity. The method's accuracy was confirmed by accuracy experiments, which revealed recovery values of 89.9–109.8% for cyclohexane and 100.2–116.9% for IPA. With LOQ precision %RSD < 15%, the LOD and LOQ values were determined to be 72.63 ppm and 232.21 ppm for cyclohexane and 11.80 ppm and 34.38 ppm for IPA, respectively. Precision studies demonstrated strong repeatability and reproducibility, with %RSD values falling within acceptable bounds. Additionally, robustness experiments showed that minor modifications to the chromatographic settings had little effect on the outcomes. With correlation coefficients (R^2) of 0.9904 for IPA and 0.9908 for cyclohexane, the technique demonstrated outstanding linearity. Overall, it was discovered that the devised method was robust, sensitive, accurate, exact, specific, and appropriate for routine measurement of residual solvents in Propofol API.

CONCLUSION

A simple, rapid, accurate, and reliable headspace gas chromatographic (HS-GC) method was successfully developed and validated for the determination of residual solvents, namely cyclohexane and isopropyl alcohol, in Propofol API. The developed method demonstrated excellent specificity, with well-resolved peaks and no interference from other components. All validation parameters, including accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness, were found to be within the acceptable limits as per ICH guidelines.

The recovery studies confirmed the accuracy and reliability of the method within the specified range. Precision studies, including system precision, method precision, and intermediate precision, showed low %RSD values, indicating good reproducibility and consistency of the analytical method. The method also exhibited adequate sensitivity based on satisfactory LOD and LOQ values. Furthermore, robustness studies revealed that small deliberate variations in chromatographic conditions did not significantly affect the analytical performance of the method.

Therefore, the proposed HS-GC method can be effectively employed for the routine analysis of residual solvents in Propofol API. The validated method is suitable for quality control applications and supports regulatory compliance while ensuring the quality, safety, and efficacy of pharmaceutical products.

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