ORIGINAL ARTICLE

High-Performance Liquid Chromatography Assessment of Propofol Stability in Pharmaceuticals

Ganesh B. Shevalkar[1a,b], Hemant U. Chikhale[2*], Kishori N. Ahire[1a], Sreya Kosanam[3], Laxmikant B. Borse[4]

[1a]Department of Pharmaceutics, Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra, India, [1b]Department of Pharmaceutics, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India, [2]Department of Pharmaceutical Chemistry, Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra, India, [3]Department of Pharmacy Practice, Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra, India, [4]Department of Pharmacology, Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra, India

Abstract

Purpose: The objective of the current research was to provide a convenient, reliable, selective, and stability-indicating high-performance liquid chromatographic (HPLC) approach for the assessment of propofol in pharmaceutical dosage forms. **Materials and Methods:** A forced degradation study was executed on propofol. The separation was performed on Thermo Scientific, ODS-2 (250 mm × 4.6 mm, 5 µm) column using acetonitrile: water (70:30 v/v) as mobile phase at a flow rate of 1 mL/min. The injection volume and wavelength of the detection were 20 µL and 272 nm, respectively. **Results:** Forced degradation study shows sufficient degradation in alkaline conditions. Whereas, in oxidizing and acidic conditions, less degradation was observed. No degradation was observed in thermal and photolytic conditions. A retention time was observed at 6.633 min. The calibration curve for propofol was found to be linear (R^2 = 0.999) in the concentration from 5 to 30 µg/mL with a limit of detection and limit of quantification of 0.29 µg/mL and 0.89 µg/mL, respectively. The percent relative standard deviation value for all the analytical validation parameters such as specificity, linearity, and precision was found to be <2%. The recovery was found in the range of 98.01–04.72%. **Conclusion:** The method developed using reversed-phase HPLC was found suitable for the determination of propofol in pharmaceutical formulation.

Key words: Forced degradation, high-performance liquid chromatography, propofol, stability, validation

INTRODUCTION

Propofol, an anesthetic and sedative medication, belongs to the alkylphenol derivative class, specifically 2,6-diisopropyl-phenol [Figure 1].[1,2] It predominantly attaches to red blood cells and blood serum proteins, demonstrating a high protein binding rate of approximately 98%. This binding to proteins can vary under different conditions, such as changes in protein levels (e.g., hypo- or hyperalbuminemia), body temperature, pH, or volume, particularly in intensive care settings. It is essential to note that only the unbound form of the drug is responsible for its pharmacodynamic effects. Thus, even minor shifts in propofol's protein binding can potentially lead to adverse consequences.[3,4] Related changes in medication action, such as reducing protein binding from 98% to 96%, would increase the unbound active propofol concentration.[5] As a result, determining free drug concentration is advised to be utilized whenever possible in pharmacokinetic-pharmacodynamics investigations.[6]

Following the International Conference on Harmonization (ICH) guidelines on "stability testing of novel drug substances and products," stress testing was deemed necessary to elucidate the inherent stability properties of the active

Address for correspondence:
Hemant U. Chikhale, Assistant Professor, Department of Pharmaceutical Chemistry, Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra- 422213, India. Contact No. +91-9405211956.
E-mail: hemantch558@gmail.com

Received: 03-02-2024
Revised: 26-05-2024
Accepted: 08-06-2024

ingredient.[7] A robust stability-indicating approach effectively separates the drug from its degradation products. A thorough literature review revealed various high-performance liquid chromatography (HPLC) techniques for propofol estimation in different formulations.[6,8-11] However, the reported methods have limitations such as extended runtime, high cost, and a higher proportion of the organic solvent in the mobile phase.[8] Consequently, devising an analytical strategy to detect propofol in the presence of its degradation products poses a challenge for pharmaceutical scientists.[8] As the novel system, that is, nanostructured lipid carriers (NLC) have been developed for propofol to eliminate limitations like free propofol concentration. Hence, it becomes essential to investigate the stability of propofol in the NLC formulation under diverse environmental conditions such as acidic, alkaline, hydrolytic, oxidative, light, and heat were deemed crucial.[12,13] Consequently, an effort was made to develop a simple, rapid, accurate, precise, specific, affordable, sensitive, and stability-indicating HPLC method for propofol determination in NLC formulations. The developed method underwent validation in accordance with ICH guidelines.

MATERIALS AND METHODS

Materials

Propofol was received as a gift sample from Neon Laboratories Ltd., Mumbai, India. HPLC grade acetonitrile was obtained from Merck Ltd, Mumbai, India. High-purity water was arranged using a Milli-Q water purification system. All other reagents and solvents used in this study were of analytical grade.

Instrumentation

Chromatographic separation was performed on an HPLC system equipped with Jasco PU2080 plus (intelligent HPLC) pump with photodiode array (PDA) detector and autosampler unit. Thermo-Scientific, ODS-2 (250 mm × 4.6 mm, 5 µm) column was used as a stationary phase. The isocratic elution was carried out at a 1 mL/min flow rate using mobile phase composition, acetonitrile: water (70:30, v: v). The injection volume was 20 µL, and detection was carried out at 272 nm using a PDA detector. All the weighing procedures were carried out using an electronic analytical balance (A&D Company Ltd., India). The chromatographic conditions are mentioned in Table 1.

Preparation of standard and test solution

The standard solution was prepared by dissolving 20 mg propofol in 20 mL acetonitrile in a volumetric flask to give the primary stock of 1000 µg/mL. Then, 1 mL of this stock solution was further diluted with the mobile phase to give 100 µg/mL of the standard solution. From this standard solution, dilutions were prepared in the 5–30 µg/mL concentration range.

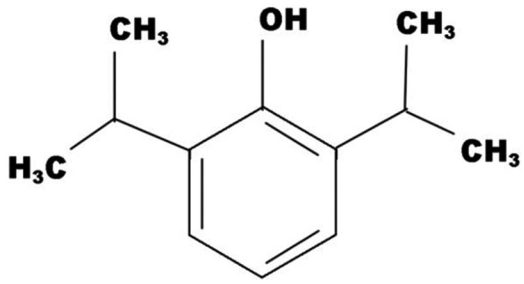

Figure 1: Structure of propofol

Table 1: HPLC instruments and chromatographic conditions

Instrumentation	
HPLC unit	Jasco (Japan)
Pump	Jasco PU 2080 plus intelligent HPLC pump
Detector	Jasco MD 2015 plus (photodiode array detector)
Integrator	Compass C software
Chromatographic conditions	
Column	Thermo-scientific, ODS-2, 250 mm×4.6 mm, 5 µm
Mobile phase	acetonitrile: Water (70:30, v:v)
Flow rate	1 mL/min
Detection	272 nm
Injection volume	20 µL

HPLC: High-performance liquid chromatography

Mobile phase optimization

The mobile phase was optimized based on the trial-and-error method. The trials were conducted using different solvent compositions such as methanol: water, methanol: acetate buffer, acetonitrile: water, and acetonitrile: acetate buffer in variable ratios with and without pH adjustment.[9] The solvent mixtures were filtered through a membrane filter (0.45 µm) and degassed before use. After performing sufficient trials, the mobile phase composition of acetonitrile: water (70:30, v: v) was found suitable for efficient separation of propofol in the column and fulfil all the system suitability criteria. Hence, acetonitrile: Water (70:30, v: v) was selected as a mobile phase for further study.

Experimental (validation of the method)

The HPLC method for propofol was validated as per the ICH Q2 (R1) quality guidelines for specificity, linearity, the limit of detection (LOD) and limit of quantification (LOQ), precision, accuracy, solution-state stability, robustness, assay, and forced degradation study.

Specificity

A placebo of propofol NLC (excluding the drug) was utilized for the specificity study. A portion of the placebo (propofol equivalent to 10 mg) was precisely weighed and transferred to a volumetric flask of 10 mL capacity. 5 mL of acetonitrile was added and sonicated for 10 min. The volume was made up to 10 mL using a mobile phase. The resultant solution was centrifuged (Research Compufuge, Remi PR-24 Centrifuge) for 10 min at 10,000 rpm. The supernatant was diluted with the mobile phase, like the assay procedure, and subjected to HPLC analysis. The standard and test samples (propofol NLC) with a propofol concentration of 100 µg/mL were also subjected to HPLC analysis. The method is considered specific if the placebo does not display any peak at the retention time of the drug.

Linearity

The ability of an analytical procedure (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample, is termed as linearity. The primary stock solution (1000 µg/mL) was suitably diluted using the mobile phase to get six concentrations in the concentration range of 5–30 µg/mL. These individual solutions were then analyzed using HPLC. The peak areas were considered, and a graph was contrived against their respective concentrations. A calibration curve was created, and the linearity range was determined. The coefficient of correlation, y-intercept, and slope of the regression line were computed from the plot. The above experiment was performed in triplicate. The regression coefficient (R^2) of 0.995 is generally considered evidence of the data's acceptable fit to the regression line.

LOD and LOQ

The LOD and LOQ were calculated from the calibration curve data using analysis of variance (ANOVA) analysis according to ICH Q2 (R1) recommendations. LOD and LOQ were calculated using the formula 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of the Y-intercept of the regression equation (n = 3), and S is the slope of the calibration curve.

Precision

Precision is a degree of agreement among individual test results when the method is applied repetitively to analyze multiple replicates in three different conditions. The current technique determined precision by measuring six replicates of a concentration of 10 µg/mL, standard propofol solution, and the % relative standard deviation (% RSD) <2% accepted.

System precision

Sequential, repetitive six injections (n = 6) of the propofol standard (10 µg/mL) in the mobile phase were subjected to HPLC analysis, and their peak responses were monitored. The % RSD of the peak areas of six replicate injections was computed.

Intraday precision (repeatability)

Sample solutions (10 µg/mL) from six different weights were subjected to HPLC analysis, and peak response areas were monitored. The % RSD of the peak areas of six different injections was computed.

Interday precision (ruggedness)

Sample solutions from six different weighings were subjected to HPLC analysis on 2 different days, and their peak responses were monitored. The % RSD for six peak response values was calculated for 1 day, and 12 values were calculated for both days.

Accuracy as recovery

For testing purpose, three experimental levels of the label claim 80, 100, and 120% (8, 10, and 12 µg/mL) were chosen. The placebo combination of the final formulations was prepared and spiked with an equivalent amount (10 mg) of the drug. The mixtures equivalent to the required levels being analyzed were accurately measured and transferred to 10 mL volumetric flasks. 5 mL of acetonitrile was added, followed by sonication at room temperature for 15 min for drug extraction. The volume was made up to 10 mL with the mobile phase. The resulting solution was centrifuged (Research Compufuge, Remi PR-24 Centrifuge) for 10 min at 10,000 rpm. The supernatant was separated and diluted further for HPLC analysis. Each level was performed in triplicate. The % recovery was computed. The mean recovery at each level should be not <95% and not more than 105%.

Solution state stability

Propofol was subjected to solution state stability by keeping the 100 µg/mL solution in phosphate buffer saline (pH 7.4) at room temperature and 10°C for 24 h. The samples were drawn at the specified interval (12 and 24 h) and examined for drug content using the developed HPLC method. % difference was calculated between the initial results (0 times) and results at respective storage conditions (room temperature and 10°C) and sampling time (12 and 24 h). The absolute difference in the assay should be not more than 5%.

Assay

To determine the propofol content in the NLC formulation, a quantity of propofol-NLCs equivalent to 10 mg of propofol was weighed accurately and transferred to a volumetric flask of 10 mL capacity. For extracting the propofol from NLCs, 5 mL of acetonitrile was added to the flask, followed by sonication at room temperature for 10 min. Then, the volume was made up to 10 mL with the mobile phase. The resulting solution was centrifuged (Research Compufuge, Remi PR-24 Centrifuge) at 10,000 rpm for 10 min. The supernatant was diluted with the mobile phase to give a final concentration of 100 µg/mL and subjected to HPLC analysis. The analysis was performed in triplicate.

Forced degradation studies

Forced degradation of propofol was performed to indicate the stability-indicating properties and specificity of the method. The stress conditions used for the degradation study included acid hydrolysis (2 M HCl), alkaline hydrolysis (2 M NaOH), oxidation (10% v/v H_2O_2), heat (80°C), and light. The chromatographic run time was extended to 30 min to check for late-eluting degradation products for all the stressed samples. The propofol stock solution of 1000 µg/mL was used for this study.[13,14]

Acid degradation

The drug was subjected to forced degradation under acidic conditions using 2 M hydrochloric acid (HCl) solution. 2 mL of stock solution was transferred to a 20 mL volumetric flask and 5 mL of 2 M HCl solution was added to it. The solution was heated at 80°C for 60 min in a water bath. After 60 min, the solution was allowed to cool down to room temperature and neutralized with 2 M sodium hydroxide (NaOH) solution. The volume was made up to 20 mL with mobile phase to achieve around 100 µg/mL concentration of propofol. The solution was then subjected to HPLC analysis.

Base degradation

Similar to the acid degradation, 2 mL of stock solution was transferred to a 20 mL volumetric flask, and 5 mL of 2 M NaOH solution was added to it. The solution was heated at 80°C for 60 min in a water bath. After 60 min, the solution was allowed to cool down to room temperature and neutralized with 2 M HCl solution. The volume was made up to 20 mL with mobile phase to achieve around 100 µg/mL concentration of propofol. The solution was then subjected to HPLC analysis.

Heat degradation

For heat degradation, 2 mL of stock solution was transferred to a 20 mL volumetric flask, and 5 mL of water was added to it. The solution was heated at 80°C for 60 min in a water bath. After 60 min, the solution was allowed to cool down to room temperature, and volume was made up to 20 mL with mobile phase to achieve around 100 µg/mL concentration of propofol. The solution was then subjected to HPLC analysis.

Peroxide degradation

For peroxide degradation, 2 mL of stock solution was transferred to a 20 mL volumetric flask, and 5 mL of 10% v/v hydrogen peroxide (H_2O_2) solution was added to it. The solution was heated at 80°C for 60 min in a water bath. After 60 min, the solution was allowed to cool down to room temperature, and volume was made up to 20 mL with mobile phase to achieve around 100 µg/mL concentration of propofol. The solution was then subjected to HPLC analysis.

Light degradation

For light degradation, 2 mL of stock solution was transferred to a 20 mL volumetric flask, and 5 mL of the mobile phase was added to it. The solution was then subjected to light degradation by exposing it to routine daylight for 24 h. After 24 h, the solution volume was made up to 20 mL with mobile phase to achieve around 100 µg/mL concentration of propofol. The solution was then subjected to HPLC analysis.

RESULTS AND DISCUSSION

Specificity

Specificity is the capacity to assess the analyte unequivocally in the presence of components other than active constituents that may be likely to be present. The other components may be impurities, degradants, matrix, etc. Figure 2a shows the standard chromatogram of 100 µg/mL of propofol. Figure 2b shows the chromatogram of the placebo mixture (consisting of all the NLC components except the drug). No peak was observed in the whole chromatogram of the placebo mixture. Figure 2c shows the chromatogram of the propofol in the presence of the placebo, which confirms no change in the chromatogram of the propofol in the presence of a placebo mixture, indicating that the method is specific for the propofol.

Linearity

The calibration curve of propofol in the mobile phase was plotted in the range of 5–30 µg/mL. The method was found to be linear in the selected concentration range with a regression coefficient of 0.9999. The standard plot and corresponding values are depicted in Figure 3 and Table 2, respectively.

LOD and LOQ

LOD and LOQ were found to be 0.29 µg/mL and 0.89 µg/mL, respectively, by ANOVA analysis.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple samples of the same homogeneous sample under prescribed conditions.

System precision

The % RSD of the area of six replicate injections of standard solution (10 µg/mL) was calculated to be 1.75%, which meets the system suitability criterion of % RSD NMT 2% [Table 3].

Intraday precision (repeatability)

Repeatability is a measure of the precision under the same operating conditions over a short interval of time, that is,

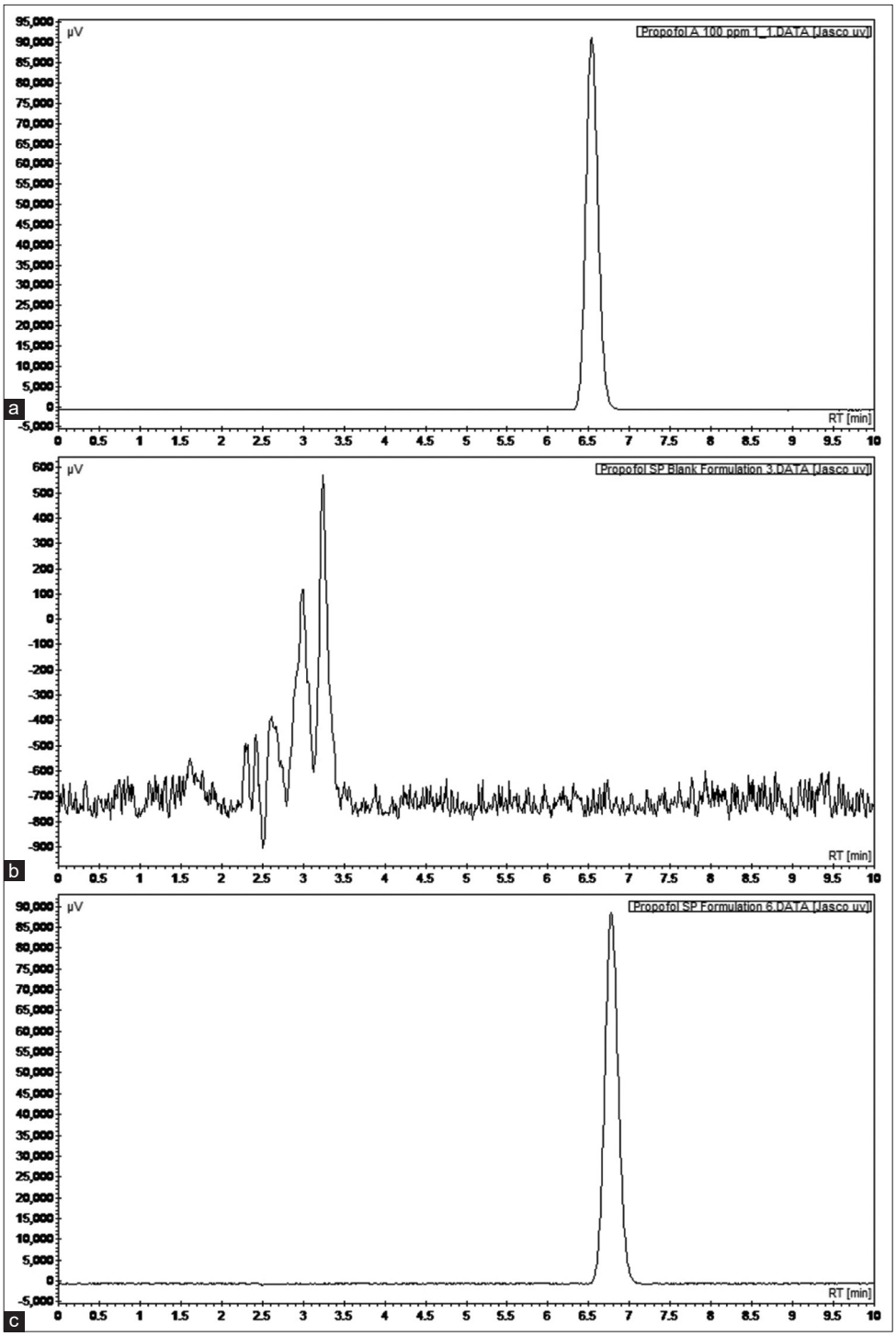

Figure 2: (a) Standard chromatogram of propofol, (b) chromatogram of placebo mixture, (c) chromatogram of propofol and placebo mixture

under normal operating conditions of the analytical method with the same equipment. It is also referred to as intraday precision. Data represented in Table 4 show the variation obtained after injecting six samples of 100 µg/mL from six different weighings. The % RSD of the area from six different weighed injections of standard solution (10 µg/mL) was calculated to be 1.77%, indicating that the method is precise.

Interday precision (ruggedness)

Ruggedness, which expresses within-laboratory variations, was evaluated based on different days. Data obtained from the interday precision assay are given in Table 5. The %RSD for the content of drug of all 12 samples (6 from day 1 and 6 from day 2) was found to be 1.67%, which is below 2%, confirmatory the method is precise.

Table 2: Calibration curve data of propofol in the mobile phase

S. No.	Concentration (µg/mL)	Mean peak areas	Standard deviation *n*=3	% Relative standard deviation
1	5	761.2	9.66	1.26
2	10	1559.13	18.04	1.15
3	15	2397.5	20.71	0.86
4	20	3188.53	12.80	0.40
5	25	4029.46	33.71	0.83
6	30	4805.93	44.22	0.92

Table 3: System precision

Injection number	Retention time (min)	Area
1	6.633	1650.0
2	6.635	1639.5
3	6.641	1649.0
4	6.638	1608.9
5	6.639	1589.7
6	6.632	1589.9
Mean	6.636333	1621.1983
Standard deviation	0.003559	28.4853
% relative standard deviation	0.053629	1.7570

Table 4: Intraday precision (repeatability)

Injection number	Retention time (min)	Area
S1	6.633	1613.3
S2	6.633	1625.8
S3	6.633	1591.0
S4	6.642	1590.7
S5	6.625	1647.6
S6	6.633	1660.0
Mean	6.633167	1621.4
Standard deviation	0.005382	28.7435
% relative standard deviation	0.081139	1.772703

Accuracy

Excellent recovery of propofol was obtained at all the experiment levels (80%, 100%, and 120%) under study [Table 6]. Hence, it can be inferred that the developed method was found to be accurate in the quantitative estimation of propofol using HPLC.

Solution state stability

Results of the solution state stability indicated that the samples were found to be stable for 24 h [Table 7].

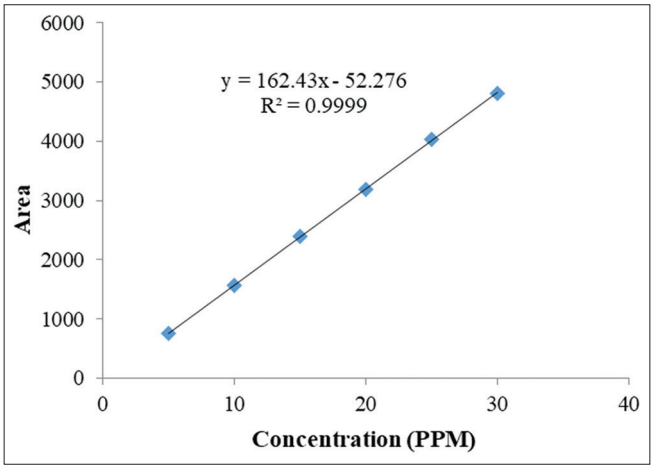

Figure 3: Standard calibration curve of propofol in the mobile phase

Assay

Table 8 shows the assay results of propofol NLC formulation (n = 3). The %RSD of the assay value (1.14) indicated that the method is suitable for determining drug content from NLC formulations of propofol.

Forced degradation studies

Force degradation is carried out to check whether the current method is suitable for identifying the degradation peaks obtained by forcefully degrading the drug under stress conditions.

Acid degradation

Propofol showed degradation when subjected to acid degradation in a solution state in the presence of a concentrated HCl solution (2 M HCl). As shown in Figure 4a, the retention time for an acid-degraded product was found at 2.35 min, which was well separated from the standard propofol peak, having RT at 6.63 min.

Base degradation

Propofol showed degradation when subjected to base degradation in the solution state in the presence of a concentrated sodium hydroxide solution (2 M NaOH). As

Table 5: Interday precision

Sample	Day 1 RT (min)	Day 2 RT (min)	Day 1 Area	Day 2 Area
S1	6.633	6.633	1613.3	1592.5
S2	6.633	6.658	1625.8	1612.7
S3	6.633	6.642	1591.0	1642.8
S4	6.642	6.642	1590.7	1595.6
S5	6.625	6.642	1647.6	1625.6
S6	6.633	6.633	1660.0	1665.8
Mean	6.633167	6.641666667	1621.4	1622.5
Standard deviation	0.005382	0.009136009	28.7435	28.3323
% relative standard deviation	0.081139	0.137555975	1.772703	1.746173
Pooled data for 12 values				
Mean		6.637417	1621.9	
Standard deviation		0.008415	27.2164	
% relative standard deviation		0.12678	1.677962	

Table 6: Accuracy-determination by spiking placebo with drug substance

Level	Sample	Area	Average	Recovery (µg/mL)	% recovery
80% (8 µg/mL)	S1	1266.3	1268.4	7.84 µg/mL	98.01
	S2	1265.1			
	S3	1274.0			
100% (10 µg/mL)	S1	1611.8	1622.2	10.01 µg/mL	100.1
	S2	1633.4			
	S3	1621.4			
120% (12 µg/mL)	S1	2043.8	2035.9	12.56 µg/mL	104.72
	S2	2014.4			
	S3	2049.5			

Table 7: Solution state stability

Time (h)	Storage conditions	Assay (%)
0	Room temperature	99.41139819
12	10°C	99.93950649
	Room temperature	99.63582909
24	10°C	99.39627481
	Room temperature	99.86933403

Table 8: Assay of propofol NLC

Formulation	Assay (Mean±standard deviation)	% relative standard deviation
Propofol NLC	99.34±1.13	1.14

NLC: Nanostructured lipid carriers

shown in Figure 4b, the retention time for the base degraded product was found at 2.47 min, which was well separated from the standard propofol peak having RT at 6.63 min.

Heat degradation

Degradation was not observed when propofol was subjected to heat degradation in solution state for 60 min at 80°C [Figure 4c].

Peroxide degradation

Propofol showed degradation when subjected to oxidative degradation in the solution state in the presence of a concentrated of H_2O_2 (10% v/v H_2O_2). As shown in Figure 4d, the retention time for the oxidative degraded product was found at 7.18 and 10.94 min, which was well separated from the standard propofol peak having RT at 6.63 min. The chromatogram shows an additional peak of peroxide approximately at 2.68 min.

Light degradation

Degradation was not observed when propofol was subjected to light degradation in the solution state for 24 h [Figure 4e]. This study aimed to create a simple,

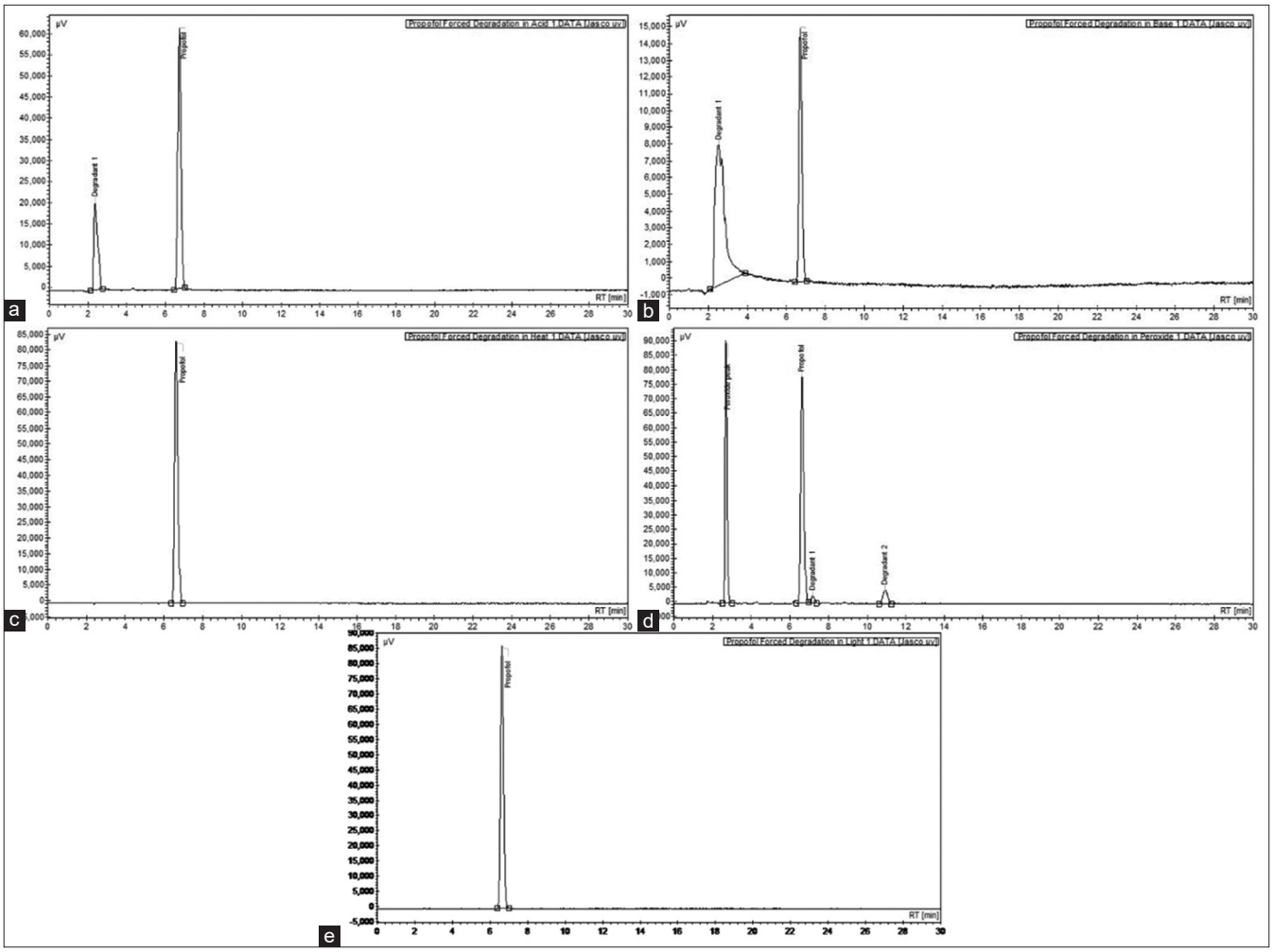

Figure 4: (a) Acid degradation chromatogram of propofol, (b) base degradation chromatogram of propofol, (c) heat degradation chromatogram of propofol, (d) peroxide degradation chromatogram of propofol, and (e) light degradation chromatogram of propofol

rapid, accurate, precise, specific, inexpensive, sensitive, and stability-indicating HPLC technique for determining propofol in bulk and NLC formulations. A thorough literature search revealed several HPLC approaches for quantifying propofol. However, all reported methods have the disadvantage of requiring a long runtime and being less economical with a high proportion of an organic phase. The mobile phase was optimized based on the trial-and-error method in which, different solvent compositions with and without pH adjustment were screened. After performing sufficient trials, the mobile phase composition of acetonitrile: water (70:30, v: v) was found suitable for efficient separation of propofol in the column and fulfilled all the system suitability criteria. Force degradation is performed to see if the current method can identify the degradation peaks obtained by forcefully degrading the drug under stress conditions. In this study, sufficient degradation was observed in alkaline conditions whereas less degradation was observed in oxidizing and acidic conditions. Degradation was not found in thermal and photolytic conditions. All these findings show the potential of the developed method for determining the propofol content in NLCs and bulk formulations.

CONCLUSION

In the presented work of this paper, our investigation demonstrates a sensitive and specific analytical method for determining propofol concentrations in the presence of degradation products described for the first time. The behavior of propofol under various stress conditions was studied and presented. The proposed method exhibited satisfactory linearity (R^2 = 0.9999), reliable accuracy, and adequate repeatability (% RSD = 1.77) for simultaneous quantification of propofol in NLC formulation. Three batches of propofol NLC were assessed by chromatographic analysis. Results showed that this method was reliable and applicable for the quality control of propofol NLC. The information presented herein could be beneficial for quality monitoring of bulk samples and employed to check the quality during stability studies.

REFERENCES

1. Shimizu M, Matsumoto Y, Tatsuno M, Fukuoka M. Glucuronidation of propofol and its analogs by human and rat liver microsomes. Biol Pharm Bull 2003;26:216-9.
2. Darandale SS, Shevalkar GB, Vavia PR. Effect of lipid composition in propofol formulations: Decisive component in reducing the free propofol content and improving pharmacodynamic profiles. AAPS PharmSciTech 2017;18:441-50.
3. Sahinovic MM, Struys MM, Absalom AR. Clinical pharmacokinetics and pharmacodynamics of propofol. Clin Pharmacokinet 2018;57:1539-58.
4. Dinis-Oliveira RJ. Metabolic profiles of propofol and fospropofol: Clinical and forensic interpretative aspects. Biomed Res Int 2018;2018:6852857.
5. Rivera R, Antognini JF. Perioperative drug therapy in elderly patients. Anesthesiology 2009;110:1176-81.
6. Eisenried A, Wehrfritz A, Ihmsen H, Schüttler J, Jeleazcov C. Determination of total and unbound propofol in patients during intensive care sedation by ultrafiltration and LC-MS/MS. J Pharm Biomed Anal 2016;126:148-55.
7. Bajaj S, Singla D, Sakhuja N. Stability testing of pharmaceutical products. J Appl Pharm Sci 2021;2:129-38.
8. Yarbrough J, Harvey R, Cox S. Determination of propofol using high performance liquid chromatography in whole blood with fluorescence detection. J Chromatogr Sci 2012;50:162-6.
9. Puri A, Mehdi B, Panda NB, S Dhawan GD. Estimation of pharmacokinetics of propofol in Indian pateints by HPLC method. J Anal Bioanal Tech 2011;2:120.
10. Theilen HJ, Adam S, Albrecht MD, Ragaller M. Propofol in a medium- and long-chain triglyceride emulsion: Pharmacological characteristics and potential beneficial effects. Anesth Analg 2002;95:923-9.
11. Külling D, Rothenbühler R, Inauen W. Safety of nonanesthetist sedation with propofol for outpatient colonoscopy and esophagogastroduodenoscopy. Endoscopy 2003;35:679-82.
12. Shevalkar G, Pai R, Vavia P. Nanostructured lipid carrier of propofol: A promising alternative to marketed soybean oil-based nanoemulsion. AAPS PharmSciTech 2019;20:201.
13. Shevalkar GB, Mali KD, Sonar VP. Stability indicating high-performance liquid chromatography (HPLC) Method for quantification of ezetimibe: Development and validation. Inventi Rapid Pharm Anal Qual Assur 2021;2021:1-8.
14. Kumar P, Ghosh A, Chaudhary M. Stability indicating method development for simultaneous estimation of ezetimibe and atorvastatin in pharmaceutical formulations by RP-HPLC. Pharm Anal Acta 2012;3:164.

Source of Support: Nil. **Conflicts of Interest:** None declared.