Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF PROPOFOL IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT
The objective of the current study was to develop a simple, accurate, precise and rapid RP-HPLC method and subsequently validate as per ICH guidelines for the determination of Propofol using mobile phase acetonitrile and methanol (pH-5.3), in the ratio of 25:75 v/v was considered to be the optimal composition as the solvent. The proposed method involves the measurement of retention time at selected analytical wavelength. 254.0 nm was selected as the analytical wavelength. The retention time of PROP was found to be 3.534 min. The linearity of the proposed method was investigated in the range of 100-500 µg/ml with regression coefficient value of 0.9996. The method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % RSD (Relative Standard Deviation) value indicating high grade of precision of the method.

Keywords: Propofol, RP-HPLC Method, Validation and Relative standard deviation.

INTRODUCTION
Propofol chemically is 2, 6 – Di isopropyl phenyl. It is a short-acting medication that results in a decreased level of consciousness and lack of memory for events. Its uses include the starting and maintenance of general anesthesia, sedation for mechanically ventilated adults, and procedural sedation. It is also used for status epilepticus if other medications have not worked. It is given by injection into a vein. Maximum effect takes about two minutes to occur and it typically lasts five to ten minutes.[2]
Common side effects include an irregular heart rate, low blood pressure, burning sensation at the site of injection, and the stopping of breathing. Other serious side effects may include seizures, infections with improper use, addiction, and propofol infusion syndrome with long-term use. It appears to be safe for using during pregnancy but has not been well studied in this group. However, it is not recommended during cesarean section. Propofol is not a pain medication, so opioids such as morphine may also be used.[3] Propofol is believed to work at least partly via a receptor for GABA

On literature survey it was revealed that several HPLC, Gas chromatography and GC/MS methods have been developed for the estimation of propofol in blood, plasma and urine samples[4,5]. HPLC method for the estimation of the same has been developed by comparison with different extraction methods also[6-9]. Since no method has been found for the estimation of propofol in bulk and pharmaceutical formulation, Here an attempt has been made to develop and validate simple , sensitive and precise RP HPLC method for the estimation of propofol in pure form and in formulation forms.

MATERILA AND METHODS
Instrument used
A Shimadzu class HPLC unit accomplished with SPD-20AD UV-Visible detector, Enable C18 (250*4.6*5) Column (Shimadzu); LC-20 AD Pump; Quantitative HPLC was performed on a isocratic mode with 20 µl injection of sample

loop (manual). The output signal was monitored and integrated using software LAB SOLUTIONS (Shimadzu).

Preparation of Mobile phase

The HPLC grade acetonitrile and methanol was filtered through 0.4μm membrane filter. Mobile phase was prepared by mixing 250 ml of acetonitrile with 750ml of methanol and pH-5.3 was adjusted by ortho-phosphoric acid and sonicated for 15 min.

Standard stock solution

Propofol (100mg) was accurately weighed and transferred in a 100ml volumetric flask. Volume was made up to 100ml with mobile phase to obtain a concentration of 1000μg/ml (Stock-I). From this stock solution-I aliquots of 1.0ml, 2.0ml, 3.0ml, 4.0ml, and 5.0ml were withdrawn and transferred into 10 ml volumetric flasks and made up the volume with mobile phase to obtain a concentrations of 100μg/ml, 200μg/ml, 300μg/ml, 400μg/ml, and 500μg/ml respectively.

Preparation of calibration curves

Appropriate dilutions were prepared separately and 20 μl of each was injected into the HPLC system and their chromatograms were recorded under the same chromatographic conditions as described below. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

Chromatographic condition

The mobile phase containing both acetonitrile and methanol in the ratio of 25:75 was selected as the optimum composition of mobile phase, because it was found that this solvent system was very ideal for the selected drug. The flow rate was set to 1.0 ml/min and UV detection was carried out at 254.0nm. The mobile phase and samples were degassed by sonication for 15 min and filtered through 0.4 μm membrane filter paper. All determinations were performed at constant column temperature (25^{0}C).

Selection of analytical concentration range

Appropriate aliquots were pipetted out from the standard stock solution (solution I) into a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 100-500 μg/ml. Triplicate dilutions of each of the above mentioned concentrations were prepared separately and from these triplicate solutions, 20 μl of each concentration of the drug was injected into the HPLC system two times separately and their chromatograms were recorded under the same

chromatographic conditions as described above. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

Analysis of formulation

10ml of the formulation of Propofol (Diprivan) was pipetted out and transferred to 100 mL volumetric flask, dissolved in sufficient quantity of mobile phase. The solution was filtered through 0.4 μm membrane filter paper. The contents were sonicated for 20 minutes and the final volume was made up to the mark with mobile phase (1000 μg/ml)

Appropriate aliquots were pipetted out from the sample stock A solution (1000 μg/ml) in to a series of 10 mL volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range of 100, 200, 300, 400 and 500 μg/ml of PROP. A 20 μl volume of each sample was injected in to the sample injector of HPLC system and their chromatograms were recorded under the same chromatographic conditions as described above. The area of each peak was determined at 254 nm and the amount of drug present in the sample was determined.

Method validation

The developed analytical method was subjected to various validation parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, precision, recovery studies and reproducibility as per the ICH guidelines.

RESULT AND DICUSSION

The present manuscript deals with estimation of PROP in pharmaceutical dosage form by RP-HPLC method using mobile phase as the solvent. The developed method is based upon estimation of the drug by determining the area under curve of the chromatogram at selected analytical wavelength.

The linearity of the proposed method was established by least square regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 100-500 μg/ml (r2 = 0.9999), along with the summary of validation and System suitability parameters as shown in the Table 1.

Recovery studies were also performed to determine the accuracy and precision of the proposed method. Recovery experiments were performed at three levels, 80%, 100% and 120% of the labeled amount of the drug as shown in Table 2.

Three replicate samples of each concentration levels were prepared and the percentage

recovery at each level (n = 3), and mean % recovery (n = 3) were determined and Statistical validation data for accuracy determination summarized in Table 3.

Intra-day precision as estimated by assaying samples containing 300 μg/ml of propofol six times and the results were averaged for statistical evaluation. The assay results and statistical validation data for intra-day precision are summarized in Table 4 and 5.

Inter-day precision was evaluated by analyzing a set of quality control samples containing 300 μg/ml of propofol, replicates were analyzed on three consecutive days. The determination of inter-day precision and statistical validation data for inter-day precision is summarized in Table 6 and 7.

Both intra-day and inter-day variation showed less % RSD value indicating high grade of precision of the method.

The robustness was evaluated by analyzing the samples by varying few parameters like wavelength and flow rate. The determination of robustness and statistical validation data is summarized in Table 8 and 9.

The validation results obtained confirm the suitability of the proposed RP-HPLC method for simple, accurate and precise analysis of propofol in pharmaceutical preparations. The proposed method does not need any prior separation of propofol before analysis. In addition it is suitable for application without interference of excipients and can be applied directly to the commercial preparations without previous treatment.

CONCLUSION
Proposed study describes a new RP-HPLC method for the estimation of Propofol in formulations using simple mobile phase. The method gives good resolution with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise. So the developed method can be used conveniently for analysis of Propofol in combined pharmaceutical dosage forms.
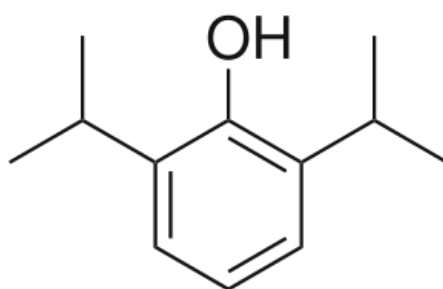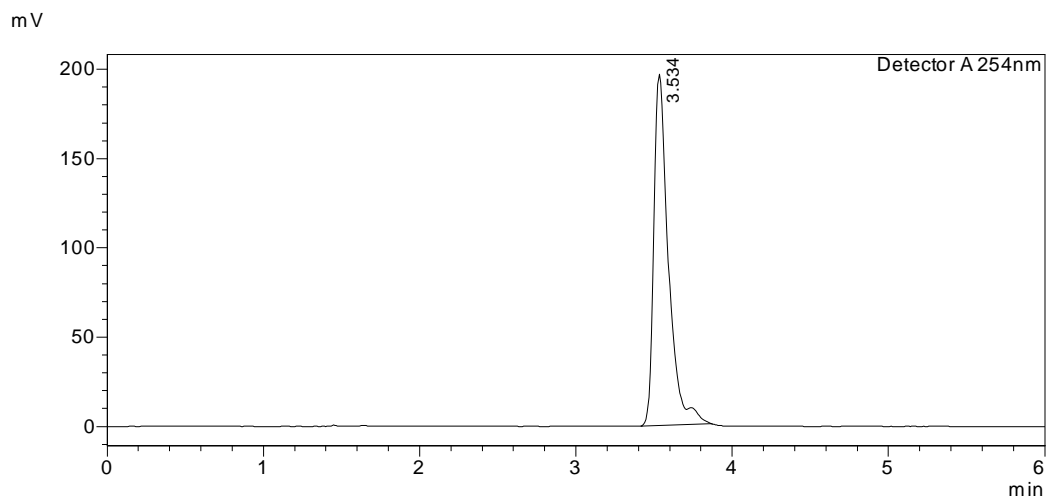
Fig. 1: Chemical Structure of Propofol

Fig. 2: Chromatogram showing the retention time of Propofol
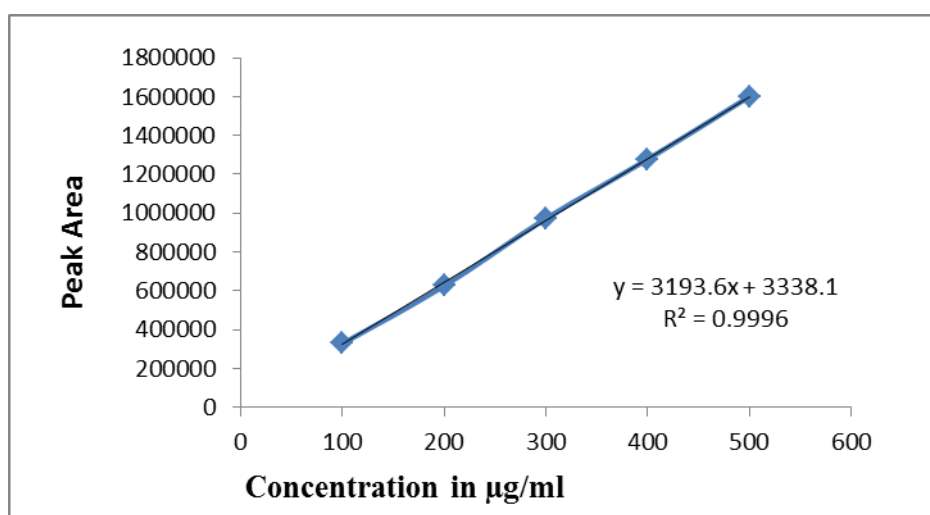

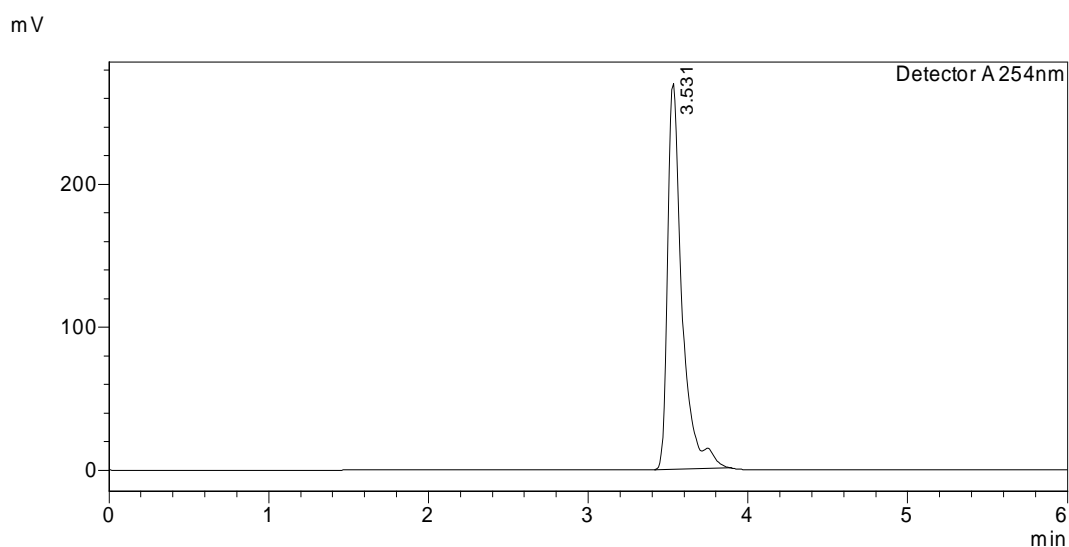**Fig. 3: Calibration curve of Propofol at 254nm by RP-HPLC method**

Fig. 4: Chromatogram of Propofol from formulation

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**Table 1: Summary of validation and System
suitability parameters of Propofol**

Parameters	Propofol
Linearity / Range µg/ml	100 - 500
Slope	3193.6
Intercept	3338.1
Regression Co efficient (r²)	0.9996
Limit of Detection (µg/ml)	7.4379
Limit of Quantitation (µg/ml)	22.5391
Retention Time (min)	3.535
Tailing Factor	1.192

**Table 2: Recovery of PROP in
spiked standard drug solution**

Level of % recovery	Amount present In (µg/ml)	Amount of standard drug added (µg)	Total amount recovered (µg)	% Recovery
80%	200	100	303.93	101.31
	200	100	303.69	101.23
	200	100	303.76	101.25
100%	200	200	398.74	99.68
	200	200	399.01	99.75
	200	200	398.89	99.72
120%	200	300	500.15	100.03
	200	300	500.14	100.02
	200	300	500.17	100.03

Where n *= 3

Table 3: Statistical Validation Data for Accuracy determination

Components	Mean*(%)	Standard Deviation*	% Relative standard deviation*	Standard Error*
PROP	100.33	0.7079	0.7055	0.4087

Table 4: Determination of intra-day precision of PROP

Sr. no	Amount present(µg)	Amount found(µg)	% Recovery
1	300	303.93	101.31
2	300	303.69	101.23
3	300	303.76	101.25
4	300	303.95	101.31
5	300	303.24	101.08
6	300	303.17	101.05

Table 5: Statistical validation data for determination of intra-day precision

Components	Mean*(%)	Standard Deviation*	% Relative standard deviation*	Standard Error*
PROP	101.20	0.1131	0.1118	0.0462

*n=6

Table 6: Determination of inter-day precision of PROP

Sr. no	Amount present(μg)	Amount found(μg)	% Recovery
DAY - 1			
1	300	303.76	101.25
2	300	303.20	101.06
3	300	303.17	101.05
4	300	303.69	101.23
5	300	303.95	101.31
6	300	303.76	101.25
DAY - 2			
1	300	303.93	101.31
2	300	303.17	101.05
3	300	303.93	101.31
4	300	303.97	101.32
5	300	303.37	101.12
6	300	303.24	101.08
DAY - 3			
1	300	303.37	101.12
2	300	303.69	101.23
3	300	303.29	101.09
4	300	304.03	101.34
5	300	304.04	101.34
6	300	303.97	101.32

Table 7: Statistical validation data for determination of inter-day precision

Components	Mean*(%)	Standard Deviation*	% Relative standard deviation*	Standard Error*
PROP	101.21	0.1111	0.1097	0.0453

Table 8: Determination of Robustness of PROP

Levels	Retention time	Tailing factor
Flow Rate		
-0.2	3.562	1.188
0	3.535	1.192
+0.2	3.503	1.163
Wave Length		
-2	3.538	1.161
0	3.535	1.192
+2	3.524	1.188

Table 9: Statistical validation data of determination of Robustness for change in method parameters

Parameters	Mean*(%)	Standard Deviation*	% Relative standard deviation*
Flow Rate			
Retention Time	3.5333	0.0295	0.8349
Tailing Factor	1.181	0.0157	1.3293
Wave Length			
Retention Time	3.5323	0.0073	0.2086
Tailing Factor	1.1803	0.0168	1.4285

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