Original Article

High-Performance Liquid Chromatography (HPLC): A Cost-Effective and Accurate Method for Propofol Plasma Level Monitoring

Uzma Naeem[1], Fareeha Farooq[2], Muhammad Nazir Awan[4], Salma Salim[5], Jawairia Iftikhar[3] and Mahreen Mirza[6]

ABSTRACT

Objective: To determine the propofol plasma levels by using a validated HPLC method in the Pakistani population.
Study Design: Descriptive cross-sectional study
Place and Duration of Study: This study was conducted at the IIMC-Pakistan Railway Journal Hospital, Rawalpindi in collaboration with the Centralized Resource Laboratory, Peshawar from August 2022 to December 2022.
Materials and Methods: One hundred and twenty Pakistani patients who received propofol for induction of anesthesia (2 mg/kg) were selected for this study. A customized HPLC method measured propofol plasma concentrations after five and ten minutes of propofol induction. We used an already validated technique of HPLC in our study with some modifications which made it more cost-effective.
Results: A linear calibration curve of the propofol was obtained within the range of 0.2 to 1.0 mg/L, while a value of the correlation coefficient (R^2 = 0.993) indicated good linearity for the drug. We observed marked inter-individual variability in the propofol serum levels in the Pakistani population ($p<0.05$).
Conclusion: The estimation of propofol serum levels by this customized HPLC method was meticulous and relatively cost-effective. So, this method can be helpful in propofol pharmacokinetic studies to minimize its adverse effects, especially in countries where resources are limited.
Key Words: Propofol, Serum concentration, High-Performance Liquid Chromatography, precision, cost-effectiveness.

Citation of article: Naeem U, Farooq F, Awan MN, Salim S, Iftikhar J, Mirza M. High-Performance Liquid Chromatography (HPLC): A Cost-Effective and Accurate Method for Propofol Plasma Level Monitoring. Med Forum 2023;34(5):20-23.

INTRODUCTION

Propofol is one the most widely used intravenous soporific drug mainly used for anesthesia induction.[1] Propofol has several benefits, including quick induction, simple depth control, quick recovery of consciousness, and lower incidences of postoperative nausea and vomiting. Propofol has been the most widely used substance for the induction of anesthesia because of these qualities.[2,3,4]. Propofol's illogical or misuse may result in injection site discomfort, respiratory depression, hypotension, and bradycardia. [6,7] Propofol-induced cardiovascular depression is the most common adverse effect of this anesthetic at therapeutic doses and can be lethal even after a single induction dose of propofol.[8,9] Allegedly, vast inter-individual inconsistency is displayed in different dose requirements, serum levels, and ultimately in the propofol-induced adverse effects.[10,11] So, monitoring propofol serum levels during anesthesia is important to minimize the adverse effects. For the monitoring, it is critical to introduce a swift, delicate, and trustworthy method for the measurement of propofol blood concentrations during and after anesthesia. Propofol concentrations in the blood can be measured commonly by High-Performance Liquid Chromatography, liquid chromatography-mass spectrometry, and gas chromatography.[12] HPLC is possibly the most used technique for the detection and quantification of propofol. Nowadays it is considered the gold standard

[1.] Department of Pharmacology, IIMC, Riphah University.
[2.] Department of Biochemistry / Pharmacology[3], IIMCT, Rawalpindi.
[4.] Department of Anesthesia, Muhammad Nazir Awan, Pakistan Railway Hospital, Rawalpindi.
[5.] Department of Pharmacology, Mohi-Ud-Din Islamic Medical College, Mirpur.
[6.] Department of Pharmacology, Islamabad Medical and Dental College, IMDC, Islamabad.

Correspondence: Dr. Uzma Naeem, Associate Professor Pharmacology, IIMC, Riphah University, Rawalpindi
Contact No: 0334-9157316
Email: uzma.naeem@riphah.edu.pk

Received: February, 2023
Accepted: March, 2023
Printed: May, 2023

for multiple propofol authentication research.[13] HPLC provides drug analysis with exactitude, particularity, and swiftness.[14] According to my knowledge, no data on propofol serum concentrations in our population is available. So, this cross-sectional study was intended to estimate the serum levels of an average dose of propofol in Pakistani patients undergoing laparoscopic cholecystectomy, by customized high-performance liquid chromatography (HPLC) method.

MATERIALS AND METHODS

This descriptive cross-sectional study was held at IIMC (Islamic International Medical College)- Pakistan Railway Hospital (PRH), Rawalpindi, in collaboration with the Centralized Resource Laboratory, University of Peshawar, Peshawar. After approval from the ethical review board of IIMC (Riphah/IIMC/IRC/20/002 and the clinical trial number NCT05383534), sample collection was started in the PRH. One hundred and twenty Pakistani patients aged 25 to 45 (without gender discrimination) undergoing laparoscopic cholecystectomy were selected for this research after taking informed written consent. Patients of this study were healthy according to the American Society of Anesthesiology (ASA class I & II). The sample size was based on a previously published propofol pharmacokinetic study.[15] Anesthesia was induced with propofol at a dose of 2 mg/kg. For the estimation of plasma propofol concentration, two blood samples were taken after 5, & 10 minutes of propofol injection, the blood was then centrifuged at 5,000 rpm, and serum was collected and stored at -8 ºC in eppendorf tubes to estimate propofol concentration.

HPLC for measuring propofol concentrations: Many studies employ high-performance liquid chromatography as the gold standard for the identification and measurement of propofol for the purpose of validating propofol.[16] HPLC is combined with several measuring methods, with ultraviolet detection being the most used.[13]

1. **Samples preparation:** A 0.5 mL of the plasma sample was added into 5 mL polypropylene followed by adding 1 mL of acetonitrile and vortexing for 30 seconds to precipitate the proteins. A high nitrogen flow was employed at room temperature to deposit the mixture in a gas chamber until the tube was dry. With 1.5 mL of the mobile phase, the extract was reconstituted and vortexed. The precipitated proteins were removed from the liquid part by centrifugation. A 0.2 µm syringe was used to refilter the liquid supernatant. Five microliters of the final sample were injected into the HPLC system soon after the purification process.
2. **Preparation of standard solutions:** A calibration curve was developed by running five calibration standard solutions. 100 ppm standard solution of the drug was prepared by dissolving an appropriate amount of the drug in the methanol-acetonitrile mixture (70:30). Then, working standard solutions (0.2, 0.4, 0.6, 0.8, and 1 ppm) were obtained by diluting a suitable volume of the stock standard solution with the mobile phase.
3. **Chromatographic conditions:** A high-performance liquid chromatographic technique (reverse-phase, Waters e2695 separation module, USA) supplied with a 2489 UV/Vis detector and autosampler were used for the analysis of the samples. A C8 column (25 cm x 4.6 mm) with a particle size of 5 µm was employed. The mobile phase consisted of methanol and acetonitrile in a V/V ratio of 10:90. The flow rate was one ml per minute and the analysis time was 8 minutes. The ultraviolet detector wavelength was set to 270 nm.

Calibration curve: A calibration curve was developed by running five calibration standard solutions. A standard stock solution (50 mg/L) of propofol was prepared by dissolving a suitable amount of the drug in acetonitrile and then diluting up to the mark. The working standard solutions for the propofol (0.2, 0.4, 0.6, 0.8, and 1 mg/L) were created by diluting the stock standard solution with the mobile phase at the proper volume. By comparing the peak area for each concentration to the drug concentration, the calibration curve was created (Figure No. 1).

RESULTS

The calibration curve of propofol in plasma using the least square regression equation was linear within the range of 0.2 to 1.0 mg/L, displayed in Figure 1.

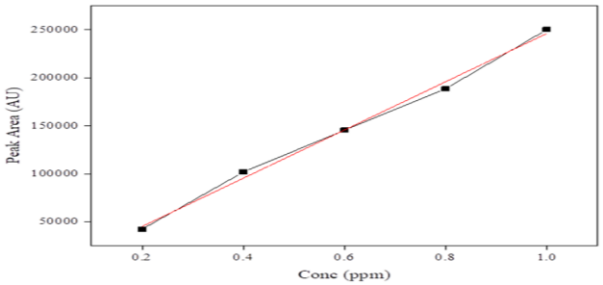
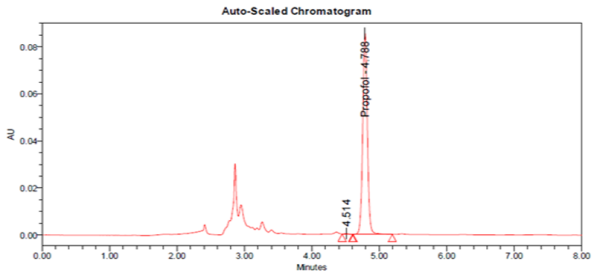


Figure No 1: Calibration Curve

Table No. 1: Comparison of propofol serum levels at two points.

Propofol serum levels	After 5 minutes of induction	After 10 minutes of induction	Sig. (2-tailed)	95% Confidence Interval of the Difference	
				Upper	Lower
Mean	2.582	1.392	<0.001	1.097	1.282
N	120	120			
Std. Deviation	1.183	.878			
Minimum	.97	.00			
Maximum	8.35	5.22			



Peak Results

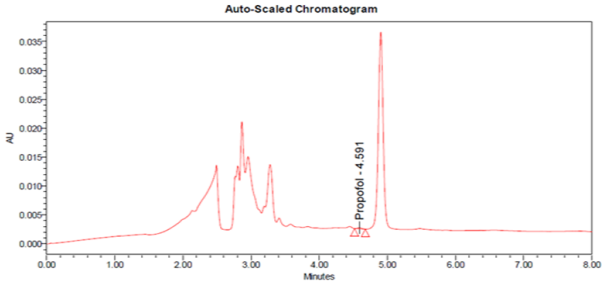
	Name	RT	Area	Height	Amount	Units
1		4.514	1071	263		
2	Propof ol	4.788	408245	85445	5.222	ppm

Figure No. 1: ppm = plasma concentration in mg/L, RT = retention time

The correlation coefficient was obtained by plotting this calibration curve and the obtained value of the correlation coefficient R^2 = 0.993 indicated good linearity for the drug.

Propofol serum levels were measured using this validated high-performance liquid chromatography and an ultraviolet detector (Figures 1 & 2). The levels were measured at two points after 5 and 10 minutes of propofol induction, results are shown in Table 1.

Auto-scaled chromatogram showing retention time and serum concentration of propofol after 5 minutes of induction**.**



Peak Results

	Name	RT	Area	Height	Amount	Units
2	Propof ol	4.591	747	188		ppm

Figure No. 2: ppm = plasma concentration in mg/L, RT = retention time. Auto-scaled chromatogram showing retention time and serum concentration of propofol after 10 minutes of induction.

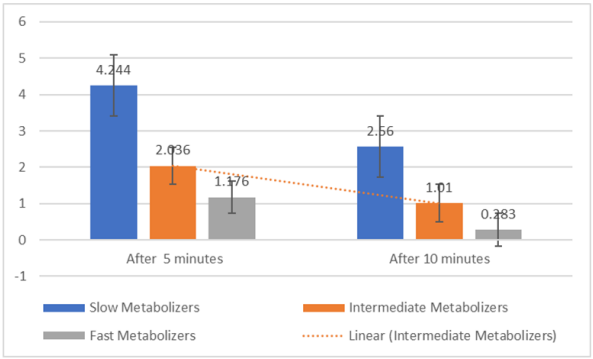


Figure No. 3: Categorization of patients in three groups according to their serum levels

The half-life of propofol after a bolus injection of propofol is about 3 minutes and propofol concentration should decline up to 80% of its original concentration after ten minutes of propofol injection.[17,18] A marked difference in the serum levels among patients was observed and according to their serum levels, they were divided into three groups: slow, intermediate, and fast metabolizers.[19]

DISCUSSION

Serum propofol concentrations were measured by an already validated simple, sensitive, and rapid high-performance liquid chromatographic (HPLC) with some modifications. Our study revealed that the method was specific, accurate, precise, and reliable (R^2 = 0.993). Recently in 2022, Sajida *et al* presented a review of 23 studies and compare different techniques for the measurement of propofol concentration in the blood. They concluded that HPLC is in use since 1990 and many researchers confirmed its reliability, accuracy, and precision.[5] The UV detector was used to measure the ultraviolet absorbance of the HPLC eluent and it also act as a constant detector, used to quantify the propofol.[20] To avoid any incongruity, the measurements of propofol were done at two points, after 5 and 10 minutes of propofol induction. These two points were selected because five minutes is the time of peak effects and propofol's duration of action is up to ten minutes after a single bolus injection.[22] In this study the mean propofol concentrations were 2.58 mg/L (range 0.97 to 8.35) and 1.39 mg/L (ranges between 0.00 to 5.22) respectively at 5, & 10 minutes with a significant difference of less than 0.001. The findings of our study are consistent with the observations of Kanaya and co-workers, their 5, and 10-minute measurements after 2 mg/kg bolus dose was 2.2 & 0.99 mg/L respectively.[11] A previous study conducted back in 2012 also showed the same decline in serum propofol levels after induction with the same dose.[22] It is evident from the standard deviations that a wide range of interindividual variability is present in our population. So, according to their serum levels participants were alienated among three groups: slow, intermediate, and rapid metabolizers. Mikstacki et al also showed the same findings in the Polish population, they also find a positive correlation between their findings with the genetic makeup of that population.[19]

CONCLUSION

The estimation of propofol serum levels by this customized HPLC method is meticulous and relatively cost-effective. So, this method can be helpful in propofol pharmacokinetic studies to minimize its adverse effects because wide interindividual variability in propofol serum levels is present in our population.

Author's Contribution:

Concept & Design of Study:	Uzma Naeem
Drafting:	Fareeha Farooq, Muhammad Nazir Awan
Data Analysis:	Salma Salim, Jawairia Iftikhar, Mahreen Mirza
Revisiting Critically:	Uzma Naeem, Fareeha Farooq
Final Approval of version:	Uzma Naeem

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Doi M, Morita K, Takeda J, Sakamoto A, Yamakage M, Suzuki T. Efficacy, and safety of remimazolam versus propofol for general anesthesia: a multicenter, single-blind, randomized, parallel-group, phase IIb/III trial. J Anesth 2020;34(4):543-553.
2. Folino TB, Muco E, Safadi AO, Parks LJ. Propofol. In: Stat Pearls. Treasure Island (FL): StatPearls Publishing; July 25, 2022.
3. Tian D, Tian M, Ma ZM, Zhang LL, Cui YF, Li JL. Anesthetic propofol epigenetically regulates breast cancer trastuzumab resistance through the IL-6/miR-149-5p axis. Scientific Reports 2020;10(1):1-8.
4. Dinis-Oliveira RJ. Metabolic Profiles of Propofol and Fospropofol: Clinical and Forensic Interpretative Aspects. Biomed Res Int 2018; 6852857.
5. Sajeeda S, Kumar L, Verma R. An Overview of Analytical Methods for the Estimation of Propofol in Pharmaceutical Formulations, Biological Matrices, and Hair Marker. Crit Rev Anal Chem 2022;52(7):1694-1701.
6. Yang E, Bu W, Suma A, et al. Binding Sites and the Mechanism of Action of Propofol and a Photoreactive Analogue in Prokaryotic Voltage-Gated Sodium Channels. ACS Chem Neurosci 2021;12(20):3898-3914.
7. Li Z, Liu J, Liang H. Biophysical Model: A Promising Method in the Study of the Mechanism of Propofol: A Narrative Review. Comput Intell Neurosci 2022:8202869.
8. Liu Y, Zhang XH, Mi WD, Zhou YL, Zhang CS, Zhang XX. Rapid determination and continuous monitoring of propofol in microliter whole blood sample during anesthesia by paper spray ionization-mass spectrometry. Anal Bioanal Chem 2021;413(1):279-287.
9. Irwin MG, Chung CKE, Ip KY, Wiles MD. Influence of propofol-based total intravenous anaesthesia on peri-operative outcome measures: a narrative review. Anaesthesia 2020;75 Suppl 1: e90-e100.
10. Sneyd JR, Absalom AR, Barends CRM, Jones JB. Hypotension during propofol sedation for colonoscopy: a retrospective exploratory analysis and meta-analysis. Br J Anaesth 2022;128(4): 610-622.
11. Kanaya A, Sato T, Fuse N, Yamaguchi H, Mano N, Yamauchi M. Impact of clinical factors and UGT1A9 and CYP2B6 genotype on inter-individual differences in propofol pharma-cokinetics. J Anesth 2018;32(2):236-243.
12. Cowley NJ, Laitenberger P, Liu B, Jarvis J, Clutton-Brock TH. Evaluation of a new analyser for rapid measurement of blood propofol concentration during cardiac surgery. Anaesthesia 2012;67(8):870-874.
13. Ferrier DC, Kiely J, Luxton R. Propofol detection for monitoring of intravenous anesthesia: a review. J Clin Monit Comput 2022;36(2):315-323.
14. Birajdar AS. New Method Development by HPLC and Validation as per ICH Guidelines. Acta Scient Pharmaceutic Sci 2020;4(4): 55-60.
15. Komarova T, McKeating D, Perkins AV, Tinggi U. Trace Element Analysis in Whole Blood and Plasma for Reference Levels in a Selected Queensland Population, Australia. Int J Environ Res Public Health 2021;18(5):2652.
16. Müller DJ, Rizhanovsky Z. From the Origins of Pharmacogenetics to First Applications in Psychiatry. Pharmacopsychiatr 2020;53(4): 155-161.
17. Pavlovic D, Budic I, Jevtovic Stoimenov T, Stokanovic D, Marjanovic V, Stevic M, et al. The Effect of UGT1A9, CYP2B6 and CYP2C9 Genes Polymorphism on Propofol Pharmacokinetics in Children. Pharmgenomics Pers Med 2020;13: 13-27.
18. Morse JD, Cortinez LI, Meneely S, Anderson BJ. Propofol context-sensitive decrement times in children. Paediatr Anaesth 2022;32(3):396-403.
19. Mikstacki A, Zakerska-Banaszak O, Skrzypczak-Zielinska M, Tamowicz B, Prendecki M, Dorszewska J, et al. The effect of UGT1A9, CYP2B6, and CYP2C9 genes polymorphism on individual differences in propofol pharma-cokinetics among Polish patients undergoing general anesthesia. J Appl Genet 2017;58(2): 213-20.
20. Wysocki J, Dong M. Ultraviolet detectors: perspectives, principles, and practices. LCGC North Am 2019;37(10):750-9.
21. Lundström S, Twycross R, Mihalyo M, Wilcock A. Propofol. J Pain Symptom Manage 2010;40(3): 466-70.
22. Loryan I, Lindqvist M, Johansson I, Hiratsuka M, Van Der Heiden I, Van Schaik RH, et al. Influence of sex on propofol metabolism, a pilot study: implications for propofol anesthesia. Eur J Clin Pharmacol 2012;68(4):397-406.