RESEARCH PAPER

Comparative AQbD-Based RP-UPLC Method Development and Validation of Ramucirumab and Bevacizumab in Injectable Dosage Forms

Sabbavarapu Santhi Bhushan*[1], Rajiv Kukkar[2]
[1]Research Scholar, School of Pharmacy, Raffles university, Neemrana- 301705.
[2]Professor, School of Pharmacy, Raffles University, Neemrana- 301705.
Corresponding Author Details: Sabbavarapu Santhi Bhushan
Email id: ssanthibhushan@gmail.com

Received: 12th Mar, 2026 | **Revised:** 24th Mar, 2026 | **Accepted:** 14th Apr, 2026 | **Available Online:** 30th Apr, 2026

Abstract
The present study describes the development and validation of AQbD-assisted RP-UPLC methods for the estimation of Ramucirumab and Bevacizumab in bulk and injectable dosage forms. Central Composite Design (CCD) was employed to optimize critical chromatographic parameters. Ramucirumab showed a retention time of 1.469 min using 0.01N Na_2HPO_4:Methanol (61.5:38.5 v/v), while Bevacizumab exhibited a retention time of 0.945 min using 0.1% OPA:Acetonitrile (60:40 v/v). Both methods demonstrated excellent linearity with correlation coefficients of 0.999. Accuracy studies showed recoveries of 99.79% for Ramucirumab and 99.75% for Bevacizumab with %RSD values below 2%, confirming precision and robustness. LOD and LOQ values for Ramucirumab were 0.94 µg/mL and 2.84 µg/mL, whereas Bevacizumab showed higher sensitivity with values of 0.05 µg/mL and 0.16 µg/mL, respectively. Forced degradation studies confirmed the stability-indicating nature of the methods, with maximum oxidative degradation of 5.14% and 6.67%, respectively. The developed AQbD-based RP-UPLC methods were found to be rapid, accurate, economical, robust, eco-friendly, and suitable for routine pharmaceutical quality control analysis.

Keywords: AQbD, RP-UPLC, Ramucirumab, Bevacizumab, Central Composite Design, Method Validation, Stability Studies, Monoclonal Antibodies, Injectable Dosage Form.
How to cite this article: Santhi Bhushan S, Kukkar R. Comparative AQbD-Based RP-UPLC Method Development and Validation of Ramucirumab and Bevacizumab in Injectable Dosage Forms. Int J Drug Deliv Technol. 2026;16(53s): 306-314. DOI: 10.25258/ijddt.16.53s.78

INTRODUCTION

Analytical Quality by Design (AQbD) has become an important systematic approach in pharmaceutical analytical method development. AQbD focuses on predefined analytical objectives, risk assessment, design space establishment, and process understanding to achieve robust analytical performance. The implementation of AQbD principles minimizes variability and improves method reproducibility through scientific optimization techniques such as Design of Experiments (DoE) and Central Composite Design (CCD).

Monoclonal antibodies have revolutionized cancer therapy due to their selective targeting mechanisms and improved therapeutic efficacy. Ramucirumab is a vascular endothelial growth factor receptor-2 (VEGFR-2) antagonist that inhibits angiogenesis in tumors, whereas Bevacizumab is a monoclonal anti-VEGF antibody that suppresses vascular endothelial growth factor-mediated tumor angiogenesis. Both drugs are widely employed in the treatment of colorectal cancer, gastric cancer, lung cancer, and several metastatic malignancies.

Due to the increasing therapeutic importance of these biological molecules, there is a growing demand for sensitive, rapid, precise, and stability-indicating analytical methods for routine pharmaceutical quality control. RP-UPLC has emerged as a superior analytical technique due to its high resolution, rapid analysis time, reduced solvent consumption, and excellent reproducibility. In the present investigation, a detailed comparative study was performed between AQbD-based RP-UPLC methods developed for Ramucirumab and Bevacizumab with respect to chromatographic optimization, validation characteristics, sensitivity, degradation behavior, and analytical applicability.

Ramucirumab is an antineoplastic agent and direct VEGFR2 (vascular endothelial growth factor receptor 2) antagonist that blocks the binding of natural VEGF ligands, which are secreted by solid tumors to promote angiogenesis and enhance tumor blood supply

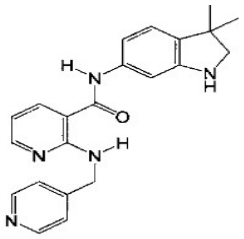

Figure 1 Structure of Ramucirumab

Bevacizumab is a monoclonal anti-vascular endothelial growth factor antibody used in combination with antineoplastic agents for the treatment of many types of cancer. The mechanism of action Transcription of the VEGF protein is induced by 'hypoxia inducible factor' (HIF) in a hypoxic environment. When circulating VEGF binds to VEGF receptors (VEGFR-1 and VEGFR-2) located on endothelial cells, various downstream effects are initiated. It should be noted that VEGF also binds to the neuropilin co-receptors (NRP-1 and NRP-1), leading to enhanced signaling.[4]

EXPERIMENTAL WORK

Materials and Methods:

Ramucirumab and Bevacizumab pure drug (API) and injectable formulation was obtained in pure form from Spectrum Pharma Research Laboratory, Hyderabad, were used for the analytical investigation. Analytical reagent (AR) grade hydrochloric acid (HCl) and sodium hydroxide (NaOH) were procured from Rankem, India. Hydrogen peroxide (H_2O_2) was supplied by Qualigens. Acetic acid, sodium dihydrogen orthophosphate, orthophosphoric acid, phosphate buffer, potassium dihydrogen orthophosphate buffer, methanol, acetonitrile, and other analytical and chromatographic grade solvents were procured from Fisher Scientific, S.D. Fine Chem Ltd., Merck India Pvt. Ltd., and Rankem. UPLC-grade acetonitrile (ACN) and methanol (MeOH) were employed for chromatographic analysis, while ultrapure water used throughout the study was prepared using a Merck Milli-Q water purification system.

The analytical studies were carried out using a WATERS ACQUITY UPLC system equipped with binary pumps, an auto sampler, and a TUV detector integrated with Empower 2 software. Additional instruments employed during the investigation included a Denver electronic analytical balance, a pH meter and ultrasonicator from BVK Enterprises, India, and a PG Instruments T60 UV–Visible spectrophotometer fitted with matched 2 mm and 10 mm quartz cells integrated with UV Win 6 software for absorbance measurements.

Methodology

Comparative Method Development and Optimization

AQbD Optimization Strategy

Both analytical methods were optimized using Central Composite Design (CCD) under AQbD principles. Critical Method Parameters (CMPs) such as flow rate, mobile phase composition, and column temperature were systematically varied to evaluate their influence on Critical Quality Attributes (CQAs), including retention time, theoretical plate count, and peak asymmetry.

Comparative CCD Experimental Conditions

Table 1: Comparative CCD Experimental Conditions for Ramucirumab and Bevacizumab

Parameter	Ramucirumab	Bevacizumab
Design Software	Design Expert 11.0	Design Expert 13.0
Design Type	Central Composite Design	Central Composite Design
Runs	20	20
Independent variables	Flow rate, organic ratio, temperature	Flow rate, organic ratio, temperature
Responses	RT, plate count, asymmetry	RT, plate count, tailing factor

The optimization process demonstrated significant influence of flow rate and mobile phase composition on chromatographic performance for both drugs. The desirability function generated optimized analytical conditions with excellent chromatographic efficiency.

AQbD Results for Ramucirumab and Bevacizumab

Table: 2Comparative AQbD Experimental Design and Optimization Results

Parameter	Ramucirumab	Bevacizumab
AQbD Software	Design Expert® 11.0	Design Expert® 13.0
Experimental Design	Central Composite Design (CCD)	Central Composite Design (CCD)
Study Type	Response Surface Methodology	Response Surface Methodology
Design Model	Quadratic Model	Quadratic Model
Number of Runs	20	20
Critical Method Parameters (CMPs)	Flow rate, Organic ratio, Temperature	Flow rate, Organic ratio, Temperature
Critical Quality Attributes (CQAs)	Retention time, Theoretical plates, Asymmetry	Retention time, Theoretical plates, Tailing factor
Flow Rate Range	0.24–0.35 mL/min	0.2495–0.3505 mL/min
Organic Composition Range	31.59–48.41%	31.59–48.41%
Temperature Range	24.95–35.05°C	24.95–35.05°C
Optimized Flow Rate	0.2948 mL/min	0.30 mL/min
Optimized Organic Composition	38.5% Methanol	40% Acetonitrile
Optimized Temperature	30.70°C	30.74°C
Optimized Retention Time	1.469 min	0.945 min
Optimized Plate Count	~3991	~2715
Optimized Tailing/Asymmetry	1.23	1.38
Composite Desirability	1.0	1.0
Detector Wavelength	320 nm	215 nm
Mobile Phase	0.01N Na_2HPO_4:Methanol (61.5:38.5)	0.1% OPA:Acetonitrile (60:40)

Comparative ANOVA Results of AQbD Optimization

Table: 3 ANOVA Results for Retention Time

Parameter	Ramucirumab	Bevacizumab
Model F-value	119.76	129.25
p-value	<0.0001	<0.0001
Significant Factors	Flow rate, Organic ratio, Temperature	Flow rate, Organic ratio, Temperature
R^2 Value	0.991	0.9915
Adjusted R^2	0.983	0.9838
Predicted R^2	0.947	0.9475

Table: 4 ANOVA Results for Theoretical Plates

Parameter	Ramucirumab	Bevacizumab
Model F-value	114.96	164.74
p-value	<0.0001	<0.0001
Significant Factors	Flow rate, Temperature	Flow rate, Mobile phase, Temperature
R^2 Value	0.989	0.9933
Adjusted R^2	0.981	0.9873

Predicted R^2	0.956	0.9565

Table: 5ANOVA Results for Tailing Factor/Asymmetry

Parameter	Ramucirumab	Bevacizumab
Model F-value	114.82	150.89
p-value	<0.0001	<0.0001
Significant Factors	Flow rate, Mobile phase	Flow rate, Mobile phase, Temperature
R^2 Value	0.990	0.9118
Adjusted R^2	0.982	0.9015
Predicted R^2	0.952	0.9013

AQbD Polynomial Regression Equations

Ramucirumab

Retention Time

$$RT = 1.121 - 0.1146(A) - 0.0399(B) - 0.0477(C)$$

Theoretical Plates

$$TP = 2641 + 5.432(A) + 1.480(B) + 3.238(C)$$

Asymmetry

$$Asymmetry = 1.21 + 0.0037(A) + 0.0081(B) + 0.0039(C)$$

Bevacizumab

Retention Time

$$RT = 0.9522 - 0.0311(A) - 0.0404(B) - 0.0159(C)$$

Number of Theoretical Plates

$$NTP = 2553.39 - 31.58(A) + 22.35(B) - 8.13(C)$$

Tailing Factor

$$TF = 1.49 + 0.0167(A) + 0.1105(B) + 0.0355(C)$$

Overall AQbD Interpretation

The AQbD-assisted RP-UPLC optimization successfully established robust chromatographic methods for both Ramucirumab and Bevacizumab. The CCD experimental design demonstrated significant effects of flow rate, mobile phase composition, and temperature on chromatographic responses including retention time, theoretical plates, and tailing factor. Ramucirumab exhibited superior chromatographic efficiency with higher theoretical plate count (~3991), while Bevacizumab demonstrated faster elution with shorter retention time (0.945 min). Both methods achieved composite desirability values close to 1.0, confirming optimal analytical performance and robustness. The statistical ANOVA results with highly significant F-values and p-values (<0.0001) validated the suitability and predictability of the AQbD optimization models for routine pharmaceutical quality control analysis

Comparative Optimized Chromatographic Conditions of Ramucirumab and Bevacizumab

Table 6: Comparative Optimized Chromatographic Conditions of Ramucirumab and Bevacizumab

Parameter	Ramucirumab	Bevacizumab
Column	ACQUITY UPLC HSS C18 (100 × 2.1 mm, 1.8 µm)	ACQUITY UPLC HSS C18 (100 × 2.1 mm, 1.7 µm)
Mobile Phase	0.01N Na2HPO4:Methanol (61.5:38.5)	0.1% OPA:Acetonitrile (60:40)
Flow Rate	0.29 mL/min	0.30 mL/min

Detection Wavelength	320 nm	215 nm
Injection Volume	5 µL	1 µL
Temperature	30.7°C	30.7°C
Retention Time	1.469 min	0.945 min

The Bevacizumab method produced faster elution with reduced retention time and shorter analysis cycle. However, the Ramucirumab method exhibited improved chromatographic separation and higher theoretical plate count, suggesting enhanced column efficiency.

Comparative Validation Results

System Suitability Results for Ramucirumab and Bevacizumab

Table 7 System Suitability Results for Ramucirumab and Bevacizumab

Parameter	Ramucirumab	Bevacizumab
Retention time	1.469 min	0.945 min
USP Plate Count	~3991	~2715
Tailing factor	1.23	1.38
System suitability status	Passed	Passed

Both methods complied with ICH guidelines requiring plate count >2000 and tailing factor <2.0. Ramucirumab demonstrated superior chromatographic efficiency due to higher theoretical plate counts.

Specificity

Both methods showed excellent specificity without interference from blank or placebo peaks at the retention times of the analytes. The chromatograms confirmed the absence of co-eluting impurities or excipients.

Linearity

Ramucirumab

- Concentration range: 10–60 µg/mL
- Regression equation: y = 16457x + 6948.2
- Correlation coefficient: 0.999

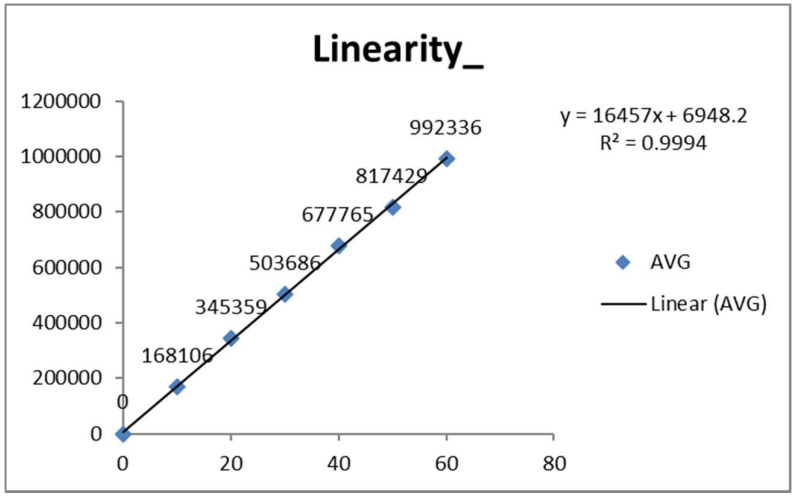

Figure 3: Linearity Curve for Ramucirumab

Bevacizumab

- Concentration range: 6.25–37.5 µg/mL
- Regression equation: y = 11549x + 1531.9
- Correlation coefficient: 0.999

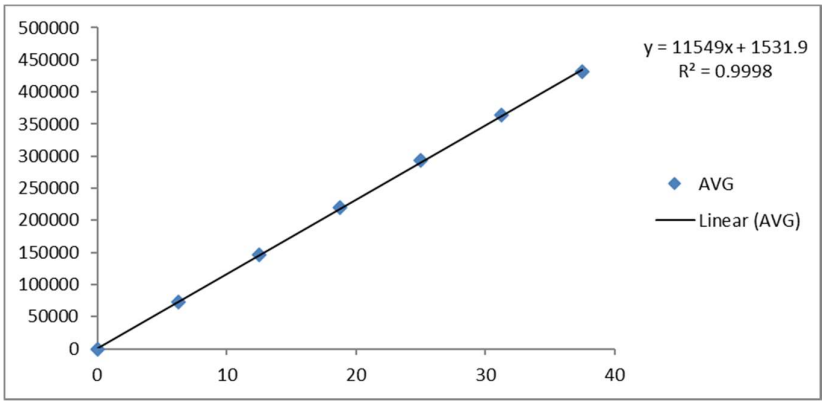

Figure 3: Linearity Curve for Bevacizumab

Both methods exhibited excellent linearity over the tested concentration ranges. Ramucirumab demonstrated a broader analytical range, whereas Bevacizumab showed greater sensitivity at lower concentrations.

Precision Studies

Table 8 Precision Studies for Ramucirumab and Bevacizumab

Precision Parameter	Ramucirumab (%RSD)	Bevacizumab (%RSD)
System Precision	0.3	0.8
Method Precision	0.3	0.2
Intermediate Precision	0.4	0.5

Both methods showed excellent reproducibility with %RSD values below 2%, satisfying ICH acceptance criteria. Ramucirumab exhibited slightly better intermediate precision, while Bevacizumab showed superior repeatability.

Accuracy Studies for Ramucirumab and Bevacizumab

Table 9 Accuracy Studies for Ramucirumab and Bevacizumab

Drug	Mean % Recovery
Ramucirumab	99.79%
Bevacizumab	99.75%

The recovery studies performed at 50%, 100%, and 150% concentration levels demonstrated excellent accuracy for both methods. The recovery values were within the acceptable range of 98–102%.

Sensitivity for Ramucirumab and Bevacizumab

Table 10 Sensitivity for Ramucirumab and Bevacizumab

Parameter	Ramucirumab	Bevacizumab
LOD	0.94 µg/mL	0.05 µg/mL
LOQ	2.84 µg/mL	0.16 µg/mL

The Bevacizumab method exhibited significantly lower LOD and LOQ values, indicating superior analytical sensitivity and suitability for trace-level quantification.

Comparative Robustness Studies for Ramucirumab and Bevacizumab

Table 11 Comparative Robustness Studies for Ramucirumab and Bevacizumab

Condition	Ramucirumab (%RSD)	Bevacizumab (%RSD)
Flow Minus	0.2	0.8

Flow Plus	0.4	0.9
Mobile Phase Minus	0.5	0.4
Mobile Phase Plus	0.7	0.9
Temperature Minus	0.3	0.8
Temperature Plus	0.6	0.8

Both methods remained unaffected by small deliberate changes in chromatographic conditions, indicating excellent robustness. Ramucirumab showed comparatively lower %RSD values under stress conditions, indicating better method ruggedness.

Comparative Assay Results for Ramucirumab and Bevacizumab

Table 12 Comparative Assay Results for Ramucirumab and Bevacizumab

Drug	Mean Assay (%)
Ramucirumab	99.94%
Bevacizumab	99.79%

The developed methods were successfully applied for estimation of marketed injectable formulations. Both formulations complied with label claim specifications and showed excellent assay accuracy.

Comparative Forced Degradation Studies for Ramucirumab and Bevacizumab

Table 13 Comparative Forced Degradation Studies for Ramucirumab and Bevacizumab

Degradation Condition	Ramucirumab (% degraded)	Bevacizumab (% degraded)
Acid degradation	1.33	4.38
Alkali degradation	0.97	4.35
Oxidative degradation	5.14	6.67
Thermal degradation	1.43	2.40
Photolytic degradation	0.78	1.66
Neutral degradation	1.03	0.68

Both drugs were highly susceptible to oxidative degradation. Bevacizumab showed comparatively higher degradation under acidic, alkaline, oxidative, and thermal conditions, indicating relatively lower stability than Ramucirumab. Both methods successfully separated degradation products from the principal analyte peaks, confirming their stability-indicating capability.

Summary

The present investigation successfully developed and validated AQbD-assisted RP-UPLC analytical methods for the quantitative estimation of Ramucirumab and Bevacizumab in bulk and injectable dosage forms. Analytical Quality by Design (AQbD) principles using Central Composite Design (CCD) were employed to optimize critical method parameters including flow rate, mobile phase composition, and column temperature. For Ramucirumab, the AQbD optimization was performed using Design Expert® 11.0 software with flow rate range of 0.24–0.35 mL/min, organic ratio range of 31.59–48.41%, and temperature range of 24.95–35.05°C. The optimized conditions obtained were flow rate 0.29 mL/min, mobile phase composition of 0.01N Na_2HPO_4:Methanol (61.5:38.5 v/v), and column temperature 30.7°C, producing a retention time of 1.469 min, theoretical plate count of approximately 3991, and asymmetry factor of 1.23. The ANOVA study showed significant model F-values for retention time (119.76), theoretical plates (114.96), and tailing factor (114.82), confirming the suitability of the AQbD optimization model.

Similarly, the AQbD optimization for Bevacizumab was carried out using Design Expert® 13.0 software with flow rate range of 0.2495–0.3505 mL/min, mobile phase composition range of 31.59–48.41%, and temperature range of 24.95–35.05°C. Optimized chromatographic conditions included 0.1% OPA:Acetonitrile (60:40 v/v), flow rate of 0.30 mL/min, and temperature of 30.7°C with detection at 215 nm. Under these conditions, Bevacizumab exhibited a retention time of 0.945 min, theoretical plate count of approximately 2715, and tailing factor

of 1.38. The AQbD statistical analysis showed excellent model significance with F-values of 129.25 for retention time, 164.74 for theoretical plate count, and 150.89 for tailing factor. The optimized desirability function confirmed robustness and reproducibility of the chromatographic method.

Both methods demonstrated excellent validation characteristics as per ICH Q2(R1) guidelines. Ramucirumab showed linearity over 10–60 µg/mL with regression equation y = 16457x + 6948.2 and correlation coefficient (R^2) of 0.999, while Bevacizumab exhibited linearity over 6.25–37.5 µg/mL with regression equation y = 11549x + 1531.9 and R^2 value of 0.999. Precision studies for Ramucirumab showed system precision, method precision, and intermediate precision %RSD values of 0.3%, 0.3%, and 0.4%, respectively, whereas Bevacizumab showed corresponding %RSD values of 0.8%, 0.2%, and 0.5%. Accuracy studies produced mean recoveries of 99.79% for Ramucirumab and 99.75% for Bevacizumab. Sensitivity evaluation revealed LOD and LOQ values of 0.94 µg/mL and 2.84 µg/mL for Ramucirumab, while Bevacizumab demonstrated superior sensitivity with LOD and LOQ values of 0.05 µg/mL and 0.16 µg/mL respectively. Assay values for marketed formulations were found to be 99.94% for Ramucirumab and 99.79% for Bevacizumab.

Forced degradation studies demonstrated that both monoclonal antibodies were more susceptible to oxidative degradation than acidic, alkaline, thermal, photolytic, and neutral stress conditions. Ramucirumab exhibited maximum degradation of 5.14% under oxidative stress, while Bevacizumab showed 6.67% degradation under similar conditions, indicating comparatively lower stability of Bevacizumab. Overall, the AQbD-assisted RP-UPLC methods were found to be rapid, robust, eco-friendly, economical, highly sensitive, and stability-indicating, making them highly suitable for routine pharmaceutical quality control analysis and stability studies of monoclonal antibody formulations

CONCLUSION

The present study successfully developed AQbD-assisted RP-UPLC methods for the estimation of Ramucirumab and Bevacizumab in bulk and injectable dosage forms. The optimized methods showed rapid retention times of 1.469 min for Ramucirumab and 0.945 min for Bevacizumab with excellent chromatographic performance. Both methods exhibited excellent linearity with correlation coefficients of 0.999, high accuracy with recoveries of 99.79% and 99.75%, and precision with %RSD values below 2%. The AQbD optimization using Central Composite Design demonstrated significant robustness and reproducibility with highly significant ANOVA F-values. Sensitivity studies revealed LOD and LOQ values of 0.94 µg/mL and 2.84 µg/mL for Ramucirumab, while Bevacizumab showed superior sensitivity with values of 0.05 µg/mL and 0.16 µg/mL, respectively. Forced degradation studies confirmed stability-indicating capability, with maximum oxidative degradation of 5.14% for Ramucirumab and 6.67% for Bevacizumab. Overall, the developed methods were found to be rapid, accurate, economical, robust, eco-friendly, and highly suitable for routine pharmaceutical quality control analysis..

Justification of Publication in IJDDT

The present AQbD-based RP-UPLC analytical study is suitable for publication in the International Journal of Drug Delivery Technology (IJDDT) because the journal focuses on pharmaceutical analysis, analytical method development, drug quality evaluation, and innovative pharmaceutical technologies. The developed methods for Ramucirumab and Bevacizumab demonstrated excellent precision, accuracy, robustness, rapid analysis time, and stability-indicating capability in accordance with ICH guidelines. The incorporation of AQbD principles and Central Composite Design (CCD) provides significant scientific and industrial relevance, making the work highly appropriate for IJDDT readership and scope.

REFERENCES

1. Bhushan, S. S., & Rao, C. M. M. P. (2026). *AQbD analytical method development and validation of Ramucirumab in bulk and injectable dosage form by using RP-UPLC*. School of Pharmacy, Raffles University, Neemrana, India.
2. Bhushan, S. S., & Rao, C. M. M. P. (2026). *Eco friendly and economical AQbD analytical method development and validation of Bevacizumab in bulk and injectable dosage form by using RP-UPLC*. School of Pharmacy, Raffles University, Neemrana, India.

3. Ahuja, S., & Scypinski, S. (2001). *Handbook of modern pharmaceutical analysis* (Vol. 3). Academic Press.
4. Antonio Martínez-Ortega, Cabeza, L., Perazzoli, G., Mesas, C., Ramírez-Tortosa, C., Melguizo, C., & Prados, J. (2018). Validated reverse phase HPLC diode array method for the quantification of intact bevacizumab, infliximab and trastuzumab for long-term stability study. *International Journal of Biological Macromolecules, 116*, 993–1003.
5. Beckett, A. H., & Stenlake, J. B. (1997). *Practical pharmaceutical chemistry* (Vol. 2). CBS Publishers and Distributors.
6. Green, J. M. (1996). A practical guide to analytical method validation. *Analytical Chemistry News & Features*, 305A–309A.
7. National Center for Biotechnology Information (2026). PubChem Compound Summary for Ramucirumab. Retrieved May 24, 2026 from https://pubchem.ncbi.nlm.nih.gov/compound/Ramucirumab.
8. National Center for Biotechnology Information (2026). PubChem Compound Summary for Bevacizumab. Retrieved May 24, 2026 from https://pubchem.ncbi.nlm.nih.gov/compound/Bevacizumab.
9. Hokanson, G. C. (1994). A life-cycle approach to the validation of analytical methods during pharmaceutical product development. *Pharmaceutical Technology*, 92–100.
10. International Conference on Harmonisation. (2005). *ICH Q2(R1): Validation of analytical procedures: Text and methodology*. ICH Harmonised Tripartite Guideline.
11. International Conference on Harmonisation. (2009). *ICH Q8(R2): Pharmaceutical development*. ICH Harmonised Tripartite Guideline.
12. Juran, J. M. (1992). *Juran on quality by design: The new steps for planning quality into goods and services*. Free Press.
13. Kaushal, C., & Srivastava, B. (2010). A process of method development: A chromatographic approach. *Journal of Chemical and Pharmaceutical Research, 2*(2), 519–545.
14. Lindholm, J. (2004). *Development and validation of UPLC method for analytical and preparative purposes*. Acta Universitatis Upsaliensis.
15. Liu, A., et al. (2008). Determination of Ramucirumab in human plasma by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Journal of Chromatography B, 867*(1), 144–148.
16. Malvia, R., Bansal, V., Pal, O. P., & Sharma, P. K. (2010). A review of high-performance liquid chromatography. *Journal of Global Pharma Technology*.
17. Montgomery, D. C. (2013). *Design and analysis of experiments* (8th ed.). John Wiley & Sons.
18. Rashmin, P. (2012). An introduction to analytical method development for pharmaceutical formulations. *Indo Global Journal of Pharmaceutical Sciences, 2*(2), 191–196.
19. Shanthi Priya, D. K., & Mukthinuthalapati Mathrusri Annapurna. (2024). Stability indicating RP-HPLC method for simultaneous estimation of Olaparib and Bevacizumab in pharmaceutical dosage forms. *Research Journal of Pharmacy and Technology, 17*(2), 910–914.
20. Vibha Gupta, Jain, A. D. K., Gill, N. S., & Kapil. (2012). Development and validation of UPLC method. *International Research Journal of Pharmaceutical and Applied Sciences, 2*(4).