WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.453
Volume 15, Issue 1, 1413-1421. Research Article ISSN 2277–7105

ANALYTICAL METHOD DEVELOPEMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ANTIHYPERLIPIDEMIC COMBINATION BY RP-HPLC METHOD

[1]Dr. Deshpande A. N., *[2]Ms. Shinde P. S., [3]Ms. Chanshetty S. S., [4]Ms. Hande A. S.

*[1]Channabasveshvar Pharmacy College, Latur, Dist. Latur, Maharashtra-413512.

[2]Tatyaraoji More College of Pharmacy, Umarga, Dist. Dharashiv, Maharashtra-413606.

[3]Tatyaraoji More College of Pharmacy, Umarga, Dist. Dharashiv, Maharashtra-413606.

[4]Tatyaraoji More College of Pharmacy, Umarga, Dist. Dharashiv, Maharashtra-413606.

Article Received on 05 Dec. 2025,
Article Revised on 25 Dec. 2025,
Article Published on 05 Jan. 2026,

https://doi.org/10.5281/zenodo.18152669

***Corresponding Author**

Ms. Shinde P. S.

Channabasveshvar Pharmacy College, Latur, Dist. Latur, Maharashtra-413512.

How to cite this Article: [1]Dr. Deshpande A. N., *[2]Ms. Shinde P. S., [3]Ms. Chanshetty S. S., [4]Ms. Hande A. S. (2026). THE MRNA VACCINE: A COMPREHENSIVE REVIEW OF DEVELOPMENT, ADVANCED TECHNOLOGIES, THERAPEUTIC APPLICATIONS, AND SAFETY CONSIDERATIONS. "World Journal of Pharmaceutical Research, 15(1), 1413-1421.

ABSTRACT

A simple, rapid, economical, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Bempedoic acid and Ezetimibe in bulk drug and combined tablet dosage form. Chromatographic separation was achieved using a Kromosil C18 (150 × 4.6 mm, 3.0 µm) column with an isocratic mobile phase consisting of 0.01 N potassium dihydrogen phosphate (KH_2PO_4) buffer and methanol in the ratio of 80:20 v/v, delivered at a flow rate of 0.9 mL/min. The column was maintained at 30 °C, and detection was performed at 229 nm. The retention times of Bempedoic acid and Ezetimibe were found to be 2.132 min and 2.586 min, respectively, indicating a short run time of less than 3 minutes. The method exhibited excellent linearity over the concentration ranges of 22.5–135 µg/mL for Bempedoic acid and 1.25–7.5 µg/mL for Ezetimibe, with correlation coefficients (R^2) of 0.9999 for both analytes. Accuracy studies showed mean recoveries of 100.00% for Bempedoic acid and 100.14% for Ezetimibe. Precision, robustness, specificity, and sensitivity parameters complied with ICH Q2(R1) guidelines. LOD/LOQ values were 1.01/3.38 µg/mL for Bempedoic acid and 0.02/0.05 µg/mL for Ezetimibe. Forced degradation studies under acid, base, oxidative, thermal, UV, and water stress conditions confirmed the stability-

indicating nature of the method. Overall, the proposed RP-HPLC method is accurate, reliable, reproducible, and suitable for routine quality-control analysis of combined Bempedoic acid and Ezetimibe formulations.

KEYWARDS: Bempedoic Acid, Ezetimibe, RP-HPLC Method Development, Method Validation (ICH Q2(R1)), Simultaneous Estimation.

INTRODUCTION

Analytical method development is a critical aspect of pharmaceutical research to ensure the quality, safety, and efficacy of drug substances and formulations. Reverse-phase high-performance liquid chromatography (RP-HPLC) is widely employed for pharmaceutical analysis owing to its sensitivity, reproducibility, high resolution, and suitability for a wide range of compounds.[1] RP-HPLC is particularly advantageous for the simultaneous estimation of multiple active pharmaceutical ingredients in combination products.

Bempedoic acid and Ezetimibe are used in fixed-dose combination therapy for the management of hypercholesterolemia. Bempedoic acid inhibits ATP citrate lyase, reducing hepatic cholesterol synthesis, while Ezetimibe decreases intestinal cholesterol absorption by blocking the NPC1L1 transporter. Their complementary mechanisms result in enhanced lowering of low-density lipoprotein cholesterol, making the combination clinically important, especially for statin-intolerant patients.[2]

Although several analytical methods have been reported for the estimation of these drugs individually and in combination, many suffer from limitations such as high solvent consumption, longer retention times, expensive instrumentation, or incomplete validation.[3-4] These limitations highlight the need for a simple, economical, and fully validated analytical method.

The present study aims to develop and validate a rapid, accurate, precise, robust, and stability-indicating RP-HPLC method for the simultaneous estimation of Bempedoic acid and Ezetimibe in bulk and tablet dosage forms. The method employs a cost-effective mobile phase, achieves short retention times (<3 min), and complies with ICH Q2(R1) guidelines, making it suitable for routine quality-control analysis.[5]

MATERIALS AND METHODS

MATERIALS

Bempedoic Acid (API) and Ezetimibe (API) were purchased Dr. Reddy's LAb Hydrabad. Methanol (HPLC grade) and other analytical-grade solvents were purchased from Merck Life Sciences Pvt. Ltd. (Mumbai, India). Potassium dihydrogen phosphate (KH_2PO_4), sodium hydroxide, hydrochloric acid, and hydrogen peroxide (30%) were procured from SD Fine Chemicals Ltd., Mumbai, India. Milli-Q water used throughout the analysis was produced using a Millipore purification system.

The chromatographic analysis was performed on a Waters Alliance e2695 HPLC system equipped with a UV detector and an autosampler. A Kromosil C18 column (150 × 4.6 mm, 3.0 µm) was utilized for all separations.

METHODS

Preparation of Buffers

Potassium dihydrogen phosphate buffer (0.01 N, pH 4.0) was prepared by accurately dissolving 1.36 g of potassium dihydrogen phosphate (KH_2PO_4) in 1000 mL of purified water, followed by pH adjustment to 4.0 using dilute orthophosphoric acid. A 0.1% (v/v) orthophosphoric acid solution was prepared by diluting 1 mL of concentrated orthophosphoric acid to 1000 mL with purified water. All solutions were filtered through a 0.45 µm membrane filter and degassed prior to use.[6]

Standard and Sample Preparation

Standard stock solutions of Ezetimibe and Bempedoic acid were prepared separately at concentrations of 50 µg/mL and 900 µg/mL, respectively, using the selected diluent. Appropriate aliquots of these stock solutions were further diluted to obtain working standard solutions.

For sample preparation, twenty tablets were weighed, finely powdered, and an amount equivalent to one tablet was transferred to a volumetric flask containing diluent. The mixture was sonicated to ensure complete extraction of the analytes, filtered through an HPLC membrane filter, and suitably diluted to obtain final working concentrations of 5 µg/mL Ezetimibe and 90 µg/mL Bempedoic acid.[7-8]

Method Validation

The developed RP-HPLC method was validated in accordance with ICH Q2(R1) guidelines. Validation parameters included system suitability, specificity, precision (repeatability), linearity, accuracy (recovery studies), robustness, and sensitivity in terms of limit of detection (LOD) and limit of quantification (LOQ).[9-12]

Stress Degradation Studies

Forced degradation studies were conducted under acidic, alkaline, oxidative, and thermal stress conditions to evaluate the stability-indicating capability of the method. Degraded samples were analysed after appropriate dilution, and chromatograms were examined to ensure adequate separation of degradation products from the analyte peaks.[13]

RESULT AND DISCUSSION

Development and Optimization of the Method

Method development aimed to achieve adequate retention, baseline separation, symmetrical peak shape, and a short run time for the simultaneous estimation of Bempedoic acid and Ezetimibe using RP-HPLC. Various C18 columns and mobile phase compositions were evaluated. Methanol-based systems produced broader peaks and higher back pressure, while higher organic content led to poor retention; therefore, acetonitrile was selected as the organic modifier. A 0.01 N potassium dihydrogen phosphate buffer adjusted to pH 4.0 provided optimal peak symmetry, selectivity, and reproducibility. A flow rate of 1.0 mL/min was chosen to balance resolution and analysis time. Detection wavelength selection based on UV spectra ensured adequate sensitivity. Under optimized conditions, Bempedoic acid and Ezetimibe eluted at approximately 2.13 and 2.59 min, respectively, with acceptable system suitability parameters, confirming the method's suitability for routine quality-control analysis as shows in fig.1.

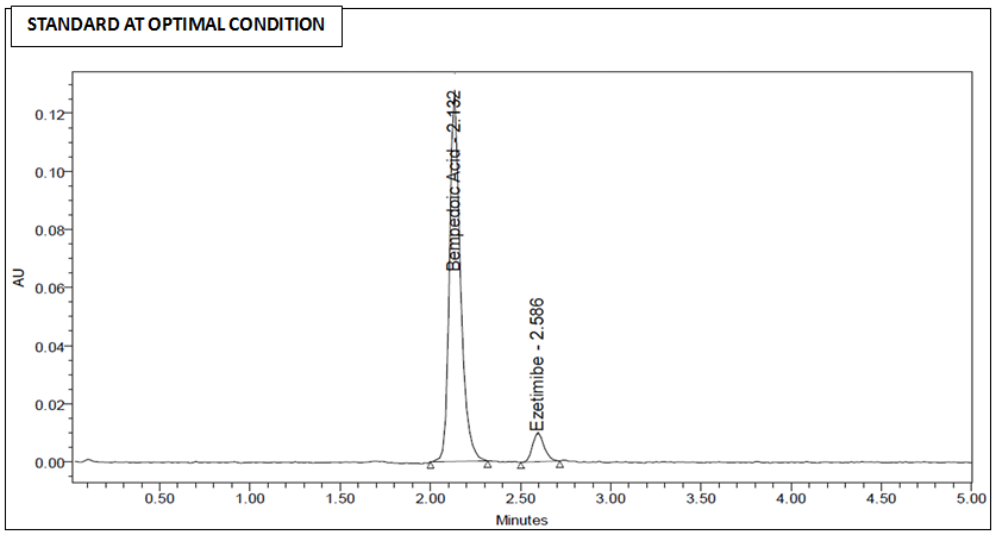


Fig.1: Represented chromatogram at the optimum condition.

Validation of the method

The developed RP-HPLC method was validated in accordance with ICH Q2(R1) guidelines. System suitability parameters demonstrated acceptable performance, with %RSD of peak areas below 2%, tailing factors within limits, adequate theoretical plate counts, and satisfactory resolution between Bempedoic acid and Ezetimibe peaks.

The method exhibited good specificity, as no interfering peaks were observed at the retention times of the analytes in blank and placebo chromatograms as shows in fig.2. Precision studies showed %RSD values within acceptable limits, indicating good repeatability mentioned in table no.1. Linearity was established over the concentration range of 1.25–7.5 µg/mL for Ezetimibe and 22.5–135 µg/mL for Bempedoic acid, with correlation coefficients (R^2) greater than 0.999 for both drugs.

Table No. 1: Intra and Inter-day precision data.

Drug	Concentration (µg/mL)	Precision Type	Mean Peak Area (n = 6)	SD	%RSD
Ezetimib	5	Intra-day	352146	4125	1.17
		Inter-day	351284	4598	1.31
Bempedoic acid	90	Intra-day	1289643	13685	1.06
		Inter-day	1284721	15421	1.20

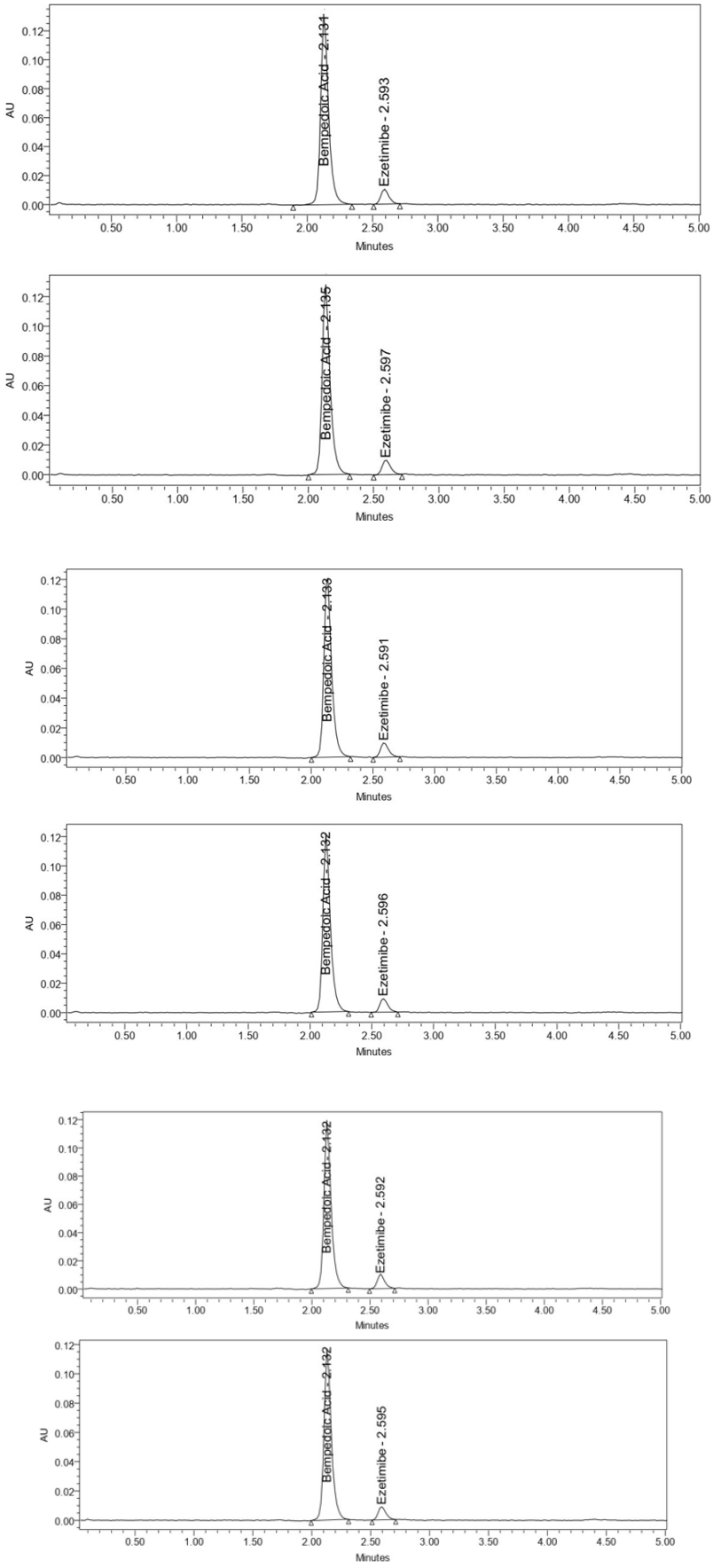


Fig.2: Chromatograms of precision study.

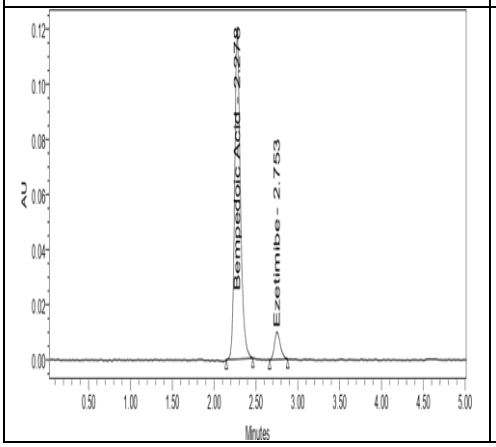
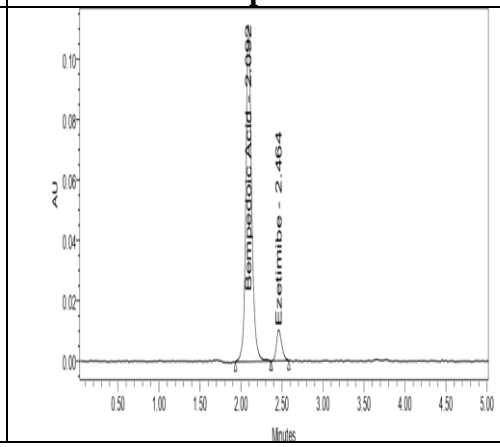
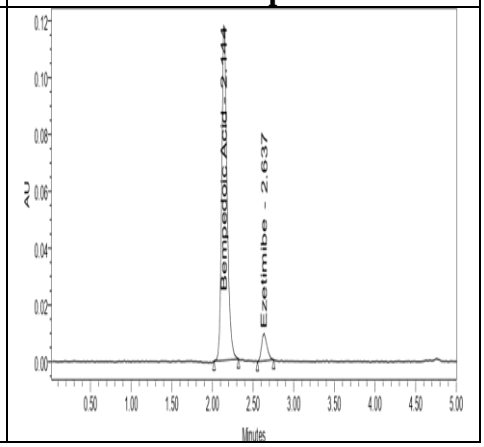
Accuracy studies demonstrated mean recoveries within 98.0–102.0%, confirming the reliability of the method. Robustness testing revealed no significant effect on chromatographic performance upon small deliberate changes in flow rate, mobile phase composition, and column temperature. The limits of detection (LOD) and quantification (LOQ) indicated adequate method sensitivity. Overall, the validation results confirm that the method is precise, accurate, robust, and suitable for routine quality-control analysis as in table no.2.

Table No. 2: Accuracy study of Ezetimibe and Bempedoic acid.

Drug	Recovery Level	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery	%RSD
Ezetimibe	50%	2.5	2.48	99.2	0.85
	100%	5.0	5.03	100.6	0.72
	150%	7.5	7.46	99.5	0.68
Bempedoic acid	50%	45	44.6	99.1	0.79
	100%	90	91.2	101.3	0.66
	150%	135	133.9	99.2	0.71

Robustness Studies

The robustness of the developed RP-HPLC method was evaluated by introducing small, deliberate variations in chromatographic conditions, including flow rate, mobile phase composition, and column temperature. Each modified condition was tested in duplicate at the working concentration of Ezetimibe (5 µg/mL) and Bempedoic acid (90 µg/mL). System suitability parameters such as retention time, peak area, and %RSD were monitored to assess the impact of these changes mentioned in table no.3. No significant changes were observed in chromatographic performance refer Fig.3.

Flow rate	Mobile phase ratio	Column temperature
		

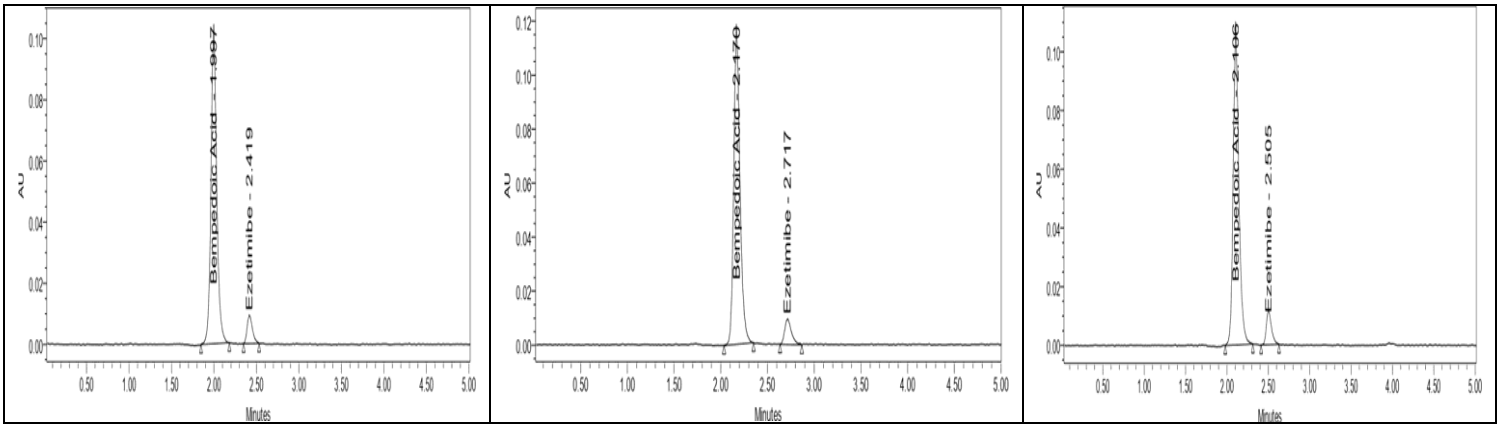


Fig. 3: Robustness chromatogram study.

Table No. 3: Robustness study.

Parameter Varied	Condition	Ezetimibe (%RSD)	Bempedoic Acid (%RSD)
Flow rate	0.8 mL/min	1.28	1.34
	1.0 mL/min (normal)	1.17	1.06
	1.2 mL/min	1.31	1.38
Mobile phase ratio	−2% organic	1.22	1.29
	+2% organic	1.35	1.41
Column temperature	23 °C	1.26	1.33
	34 °C	1.30	1.36

System Suitability Study

System suitability tests were performed to verify the adequacy of the chromatographic system prior to analysis. A standard solution containing Ezetimibe (5 µg/mL) and Bempedoic acid (90 µg/mL) was injected six times under optimized chromatographic conditions. Parameters such as retention time, theoretical plate count, tailing factor, resolution, and percentage relative standard deviation (%RSD) of peak areas were evaluated and indicating good system performance, reproducibility, and suitability of the method for routine analysis in table no.4.

Table No. 4: System suitability parameters.

Parameter	Ezetimibe	Bempedoic Acid	Acceptance Criteria
Retention time (min)	2.59	2.13	—
Theoretical plates (N)	4520	4985	≥ 2000
Tailing factor	1.12	1.09	≤ 2.0
Resolution (Rs)	-	2.35*	≥ 2.0
%RSD of peak area (n = 6)	1.17	1.06	≤ 2.0

CONCLUSION

A simple, rapid, accurate, and stability-indicating RP-HPLC method was successfully developed and validated for the simultaneous estimation of Bempedoic acid and Ezetimibe in

bulk drug and combined tablet dosage form. The method demonstrated excellent chromatographic performance with short retention times, good resolution, and symmetrical peak shapes. Validation studies performed as per ICH Q2(R1) guidelines confirmed that the method is linear, precise, accurate, robust, and sensitive over the selected concentration ranges. Forced degradation studies established the stability-indicating nature of the method, with effective separation of degradation products from the analyte peaks. Owing to its simplicity, cost-effectiveness, and reliability, the proposed RP-HPLC method is suitable for routine quality-control analysis and stability testing of Bempedoic acid and Ezetimibe in pharmaceutical formulations.

REFERENCE'S

1. Sharma BK. Instrumental Methods of Chemical Analysis. Goel Publishing House, 2007.
2. Lindholm J. Development and Validation of HPLC Method for Analytical and Preparative Purpose. Acta Universitatis Upsaliensis, 2004.
3. Rashmin. Introduction to Analytical Method Development for Pharmaceutical Formulations. Indoglobal J Pharm Sci., 2010.
4. Teja Yarra US, et al. Stability-indicating RP-UPLC method for Bempedoic acid and Ezetimibe, 2021.
5. Jain V, et al. RP-HPLC method for simultaneous estimation of lipid-lowering agents, 2022.
6. Maheshwari K, et al. Simultaneous estimation of Bempedoic acid and Ezetimibe using RP-HPLC, 2022.
7. ICH Q2 (R1). Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation, 2005.
8. ICH Q1A (R2). Stability Testing of New Drug Substances and Products. ICH, 2003.
9. Lindholm J. Development and Validation of HPLC Methods for Pharmaceutical Analysis. Acta Universitatis Upsaliensis, 2004.
10. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Wiley, 2010.
11. Shrivastava A., Gupta V. Methods for validation of analytical assays. Chron Young Sci., 2012.
12. United States Pharmacopeia (USP). USP–NF, 2020.
13. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th ed. Cengage Learning, 2014.