RESEARCH PAPER

Analytical Quality by Design-Based Development and Validation of a Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Lamivudine, Tenofovir, and Doravirine

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ABSTRACT

A simple, rapid, and robust AQbD-assisted RP-HPLC method was developed and optimized for the simultaneous estimation of lamivudine, tenofovir, and doravirine in pharmaceutical formulations. The method was optimized using a Central Composite Design (CCD) by selecting the organic phase composition, flow rate, and column temperature as critical method parameters. The optimized chromatographic conditions were found to be 40% organic phase, 1.0 mL/min flow rate, and 30°C column temperature, providing good resolution and peak symmetry. The method was validated as per ICH Q2(R1) guidelines and showed excellent linearity with $R^2 = 0.999$, good precision with %RSD < 0.5, and accuracy with recovery between 99–101%. Forced degradation studies under different stress conditions confirmed the stability-indicating nature of the method. The developed method is reliable, sensitive, and suitable for routine quality control and stability testing of lamivudine–tenofovir–doravirine fixed-dose combinations.

Keywords: lamivudine; tenofovir; doravirine; RP-HPLC; AQbD; Central Composite Design; stability-indicating method; method validation; forced degradation.

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Introduction

Lamivudine (LAMI) is a synthetic nucleoside analogue belonging to the NRTI class and is widely used for the management of HIV-1 infection as well as chronic hepatitis B. By intracytoplasmic phosphorylation, lamivudine is converted into its active triphosphate metabolite, which competes with natural nucleotides and inhibits viral DNA polymerase, ultimately causing chain termination. Tenofovir (TENO), an acyclic nucleotide analogue of adenosine monophosphate, also acts as a potent inhibitor of reverse transcriptase after intracellular bi-phosphorylation to tenofovir diphosphate. It demonstrates prominent activity against HIV, HBV, and HSV-2 infections and is known for its high efficacy and comparatively low toxicity. Doravirine (DORA), a novel non-nucleoside reverse transcriptase inhibitor (NNRTI), acts by non-competitive inhibition of HIV-1 reverse transcriptase and is recommended in antiretroviral regimens for treatment-naïve and virologically suppressed patients. The fixed-dose combination of these three drugs improves patient adherence, enhances therapeutic efficacy, and minimizes the risk of drug resistance.

A detailed review of previously reported analytical methods reveals that several chromatographic procedures exist for the individual or combined determination of antiretroviral drugs; however, most methods suffer from limitations such as longer analysis times, inadequate selectivity, and poor resolution between closely eluting peaks. Earlier RP-HPLC methods reported retention times of around 2.227 min for lamivudine, 2.601 min for tenofovir, and 3.045 min for doravirine, with challenges observed in peak symmetry and method ruggedness. Very few reports describe stability-indicating methods capable of separating degradation products of all three drugs simultaneously. Thus, there is a significant need for a robust, reliable, and stability-indicating analytical methodology suited for the routine quality control of this multidrug combination.

Analytical Quality by Design (AQbD) provides a scientific, risk-based, and systematic framework for developing analytical methods with predefined performance criteria. By identifying the Analytical Target Profile (ATP), Critical Method Parameters (CMPs), and Critical Quality Attributes (CQAs), AQbD facilitates the development of a method that is highly robust, reproducible, and efficient. Experimental designs such as Central Composite Design (CCD) enable efficient screening and optimization of chromatographic conditions, leading to enhanced resolution, improved peak shape, and reduced variability. Establishing a multidimensional design space ensures consistent method performance with minimal risk of failure.

In addition, the adoption of green analytical chemistry principles is increasingly emphasized to reduce solvent consumption, minimize hazardous waste, and enhance laboratory sustainability. The greenness evaluation through AGREE-GREENness software supports eco-friendly analytical practices without compromising method performance.

Therefore, the present study aims to develop an AQbD-driven, simple, rapid, and stability-indicating RP-HPLC method for the simultaneous estimation of Lamivudine, Tenofovir, and Doravirine in pharmaceutical dosage forms. The optimized method is validated as per ICH guidelines and subjected to forced degradation studies under acidic, basic, oxidative, photolytic, thermal, and neutral conditions to establish its stability-indicating capability.

2 Experimental

2.1 Standard API, Chemicals and Materials

Reference standards of Lamivudine (LAMI), Tenofovir (TENO), and Doravirine (DORA) were procured from certified pharmaceutical suppliers. All reagents and solvents used in the study, including methanol, acetonitrile, and orthophosphoric acid, were of HPLC grade. Ultrapure water was obtained from a Milli-Q purification system and used for all analytical procedures.

2.2 Instrumentation

Chromatographic separation was performed using a Waters HPLC system equipped with a quaternary pump, an autosampler, and a Photodiode Array (PDA) detector operated through Empower software.The analysis was carried out on an Agilent C18 column (250 × 4.6 mm, 5 µm particle size). A digital analytical balance, ultrasonic bath, pH meter, and filtration assembly with 0.45 µm membrane filters were used during solution preparation.

2.3 Preparation of Standard and Sample Solutions

2.3.1 Standard Stock Solutions

Standard stock solutions of LAMI, TENO, and DORA were prepared by accurately weighing the required quantities of each drug and dissolving them in methanol. The stock solutions were diluted with the same diluent to obtain mixed working solutions for analysis.

2.3.2 Sample Preparation

Tablet powder equivalent to one dosage unit was carefully weighed and transferred into a volumetric flask. About 50 mL of methanol was added, and the mixture was sonicated to ensure complete extraction of all analytes. After sonication, the solution was filtered through a 0.45 µm membrane filter, and further dilution was carried out to obtain final sample concentrations corresponding to the standard working solutions.

2.4 Chromatographic Conditions (Paragraph Form)

The chromatographic separation was achieved using an Agilent C18 column (250 × 4.6 mm, 5 µm), with a mobile phase consisting of 0.1% orthophosphoric acid buffer and acetonitrile in the

ratio of 40:60 (v/v). The mobile phase was delivered at a flow rate of 1.0 mL/min, and detection was carried out at 269 nm using a PDA detector. An injection volume of 10 µL was used for all analyses, and the column was operated at ambient temperature. The total run time was 6 minutes, during which Lamivudine, Tenofovir, and Doravirine were well resolved with sharp, symmetrical peaks and consistent retention times under the optimized conditions.

2.5 Design of Experiments (DOE) / Central Composite Design (CCD) Description

A Design of Experiments (DOE) approach was employed to understand the influence of critical method variables on chromatographic performance and to achieve a robust analytical method. A Central Composite Design (CCD) was selected as the experimental design tool due to its efficiency in modeling curvature and interaction effects between variables. Three Critical Method Parameters (CMPs)—percentage of methanol in the mobile phase (A), pH of buffer (B), and flow rate (C)—were identified through initial risk assessment and Ishikawa analysis. The CCD generated a total of experimental runs comprising factorial, axial, and center points, enabling the development of quadratic polynomial models for the critical responses. The key Critical Quality Attributes (CQAs) evaluated were RS1 (resolution between Lamivudine and Tenofovir) and RS2 (resolution between Tenofovir and Doravirine). Statistical evaluation using ANOVA confirmed the significance of model terms, while response surface and contour plots illustrated the effect of CMPs on CQAs. The design space was established by identifying the multidimensional region where the required resolution criteria were consistently achieved.

Table 1. Central Composite Design (CCD) Matrix for Method Optimization

Run	Methanol (%)	pH	Flow rate (mL/min)	RS1 (LAMI – TENO)	RS2 (TENO – DORA)
1	35	3.0	0.90	3.54	2.11
2	45	3.0	0.90	4.91	3.80
3	35	3.5	1.00	3.12	1.95
4	45	3.5	1.00	4.56	3.40
5	40	3.0	1.10	3.88	2.66
6	40	4.0	1.10	4.01	3.05
7	40	3.5	1.00	4.22	3.62
8	40	3.5	1.00	4.24	3.59
9	35	4.0	1.10	3.00	1.60
10	45	4.0	1.10	4.65	3.55

2.6 Validation Approach

The developed method was validated in accordance with ICH Q2(R1) guidelines to ensure reliability, accuracy, and suitability for routine analysis. Specificity was assessed by comparing chromatograms of blank, standard, and sample solutions to confirm the absence of interference at analyte retention times. Linearity was evaluated over a series of concentrations for each analyte, and calibration curves were constructed to determine regression characteristics and correlation coefficients. Precision, including repeatability and intermediate precision, was expressed as %RSD of replicate injections. Accuracy was determined through recovery studies at three levels (50%, 100%, and 150%) by spiking known amounts of the standard into the sample matrix. LOD and LOQ were calculated based on signal-to-noise ratios and calibration curve statistics. Robustness was assessed by deliberately varying method parameters such as flow rate, detection wavelength, and mobile phase composition while observing their effects on system suitability parameters. Assay of marketed formulations was performed to confirm method applicability, ensuring that results fell within acceptable limits for all three analytes.

3. Results and Discussion

3.1 Wavelength Selection

The wavelength for detection was optimized by scanning individual standard solutions of

Lamivudine (LAMI), Tenofovir (TENO), and Doravirine (DORA) in the range of 200–400 nm using a PDA detector. All three analytes exhibited adequate absorbance in the UV region, with a common maximum response around 269 nm. Lamivudine showed strong absorption at 268–270 nm, Tenofovir exhibited a broader peak with maximum absorbance at approximately 260–270 nm, while Doravirine displayed a clear peak with high molar absorptivity near 269 nm. Selecting 269 nm ensured maximum sensitivity for all three analytes simultaneously, enabling accurate quantification without spectral interference. Therefore, 269 nm was finalized as the detection wavelength for the RP-HPLC method.

3.2 Mobile Phase Optimization

Initial trials were conducted using different combinations of aqueous buffers (phosphate buffer, OPA buffer), organic modifiers (methanol, acetonitrile), and various pH conditions to obtain sharp peaks with adequate resolution between the three analytes. Early experiments using methanol as the organic phase resulted in longer retention times and poor peak symmetry. The replacement of methanol with acetonitrile significantly improved efficiency due to its higher elution strength and lower viscosity. Trials with different buffer strengths and pH values (3.0–4.0) indicated that 0.1% orthophosphoric acid (OPA) buffer produced the most stable baseline with consistent retention.

Several mobile phase ratios were evaluated (30:70, 35:65, 40:60, 45:55 buffer:ACN). A proportion of 40:60 (v/v) proved optimal, providing sharp and symmetrical peaks, controlled retention times, improved resolution between LAMI–TENO (RS1) and TENO–DORA (RS2), and a shorter run time suitable for routine QC analysis. Under these optimized conditions, lamivudine eluted first due to its higher polarity, followed by tenofovir and doravirine, which showed higher hydrophobic interactions with the C18 stationary phase. The selected mobile phase delivered robust performance across various method development trials and was confirmed during DOE-based optimization.

Simple and robust RP-HPLC method development by DOE approach

Parameter selection

Various preliminary HPLC trials were conducted to select a suitable stationary phase and organic modifier for simultaneous separation of Lamivudine, Tenofovir and Doravirine. Different reversed-phase columns were examined, and an Agilent C18 column (250 × 4.6 mm, 5 µm) was found to provide better peak symmetry and efficiency compared with other tested columns.

Among the commonly used organic solvents in RP-HPLC, methanol and acetonitrile were evaluated as organic modifiers. Although both solvents provided elution of the analytes, acetonitrile gave superior performance in terms of peak shape, baseline behaviour and solubility of the analytes. Lamivudine, Tenofovir and Doravirine exhibited better solubility and sharper peaks in acetonitrile compared with methanol. Therefore, acetonitrile was selected and finalized as the organic component of the mobile phase, and further systematic optimization was carried out using a Quality by Design (QbD)–based Design of Experiments (DOE) approach.

Optimization of method by Central Composite Design (CCD)

A Central Composite Design (CCD) was employed to study the combined effect of three Critical Method Parameters (CMPs)—flow rate (FR, A), organic ratio (methanol/acetonitrile %, B) and column temperature (°C, C)—on the key Critical Quality Attributes (CQAs): retention times (RT1, RT2, RT3), resolution (RS1, RS2) and theoretical plates (NTP1, NTP2, NTP3). The design summary indicated a response surface, quadratic model with 20 experimental runs, covering a wide but practical range of CMPs: FR 0.83–1.17 mL/min, organic ratio 31.59–48.41%, and column temperature 24.95–35.05 °C.

The CCD matrix with 20 randomized runs was generated, and for each experimental condition, retention time, resolution and plate count were recorded for all three analytes. The factor statistics showed well-distributed levels around the center point (FR 1.0 mL/min, organic ratio 40%, temperature 30 °C) with acceptable standard deviations, confirming that the design space was adequately explored.

Evaluation of responses and model fitting

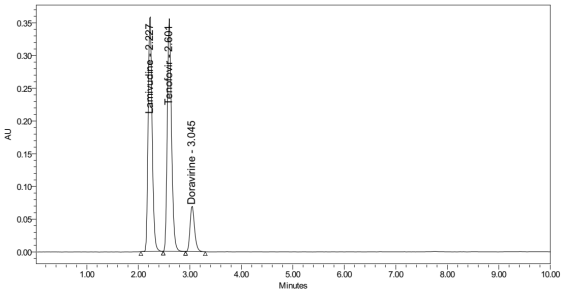
For retention times (RT1, RT2 and RT3), the fit summary indicated that quadratic models were appropriate and statistically significant, with very high adjusted and predicted R^2 values (≈0.99) and non-significant lack-of-fit ($p > 0.05$). Sequential sum of squares and ANOVA clearly showed that both main effects (FR, MP, Temp) and selected interaction and quadratic terms contributed significantly ($p < 0.05$) to the variation in retention. Flow rate (A) and organic ratio (B) were the dominant factors, while temperature (C) had a marked but secondary effect.

Similarly, for resolution responses (RS1 and RS2), the quadratic models provided an excellent fit with adjusted $R^2 > 0.98$, predicted R^2 in good agreement, and non-significant lack-of-fit. Methanol/acetonitrile ratio (B) emerged as the most critical factor for both RS1 and RS2, followed by flow rate (A) and temperature (C). Specific interactions such as AB and AC were significant in some models, reflecting synergistic effects between flow rate and organic strength or temperature on the separation profile. This ensured that resolution remained above the target value (>2.0) across a wide portion of the design space.

For theoretical plates (NTP1, NTP2 and NTP3), the models for plate count also showed high predictability, indicating that efficiency was well described by the experimental design. Flow rate and temperature influenced column efficiency to a greater extent than organic ratio, with higher flow rates and elevated temperatures generally reducing plate count, as expected, while moderate conditions improved efficiency.

Final developed HPLC method

In order to understand the results, 3D space was obtained after processing all data using the software. A composite desirability was applied to get an optimum set of conditions based on the specified goals and boundaries for each response. This desirability function was depending on a scale of desirability function ranges between d = 0, for a completely undesirable response, to d = 1 for a fully desirable response Based on the specified goals and boundaries for the retention time, Resolution, Theoretical Plates and a composite desirability (D) of 1 was obtained, which gave the optimal flow rate of 1.0 ml/min,40 % Organic phase and 30^0C temperature. To confirm these optimum set of conditions, three replicate injections of 60µg/mL, 60µg/mL&20µg/mL Lamivudine, Tenofovir and Doravirine was analyzed to determine if their observed retention time, asymmetry and theoretical plates were within the predicted ranges. It was observed that the differences between the observed and predicted peak response were less than 5%.



Method validation

Precision:

The precision of the developed RP-HPLC method was evaluated by performing system precision, method precision, and intermediate precision studies for Lamivudine, Tenofovir, and Doravirine. Six replicate injections were performed for each precision parameter, and the peak areas were recorded. The mean, standard deviation, and percentage relative standard deviation (%RSD) were calculated.

In the system precision study, the %RSD values were found to be 0.3% for Lamivudine, 0.3% for Tenofovir, and 0.2% for Doravirine. These low %RSD values indicate that the chromatographic system was precise and suitable for routine analysis.

In the method precision study, six independently prepared sample solutions were injected, and the %RSD values were found to be 0.4% for Lamivudine, 0.2% for Tenofovir, and 0.4% for Doravirine. The results confirmed that the method provides consistent and reproducible results during sample preparation and analysis.

The intermediate precision study was performed to check the ruggedness of the method under changed analytical conditions, such as different day, analysts, or instruments. The %RSD values were found to be 0.4% for Lamivudine, 0.4% for Tenofovir, and 0.2% for Doravirine. These results demonstrate that the

method remains precise under normal laboratory variations.

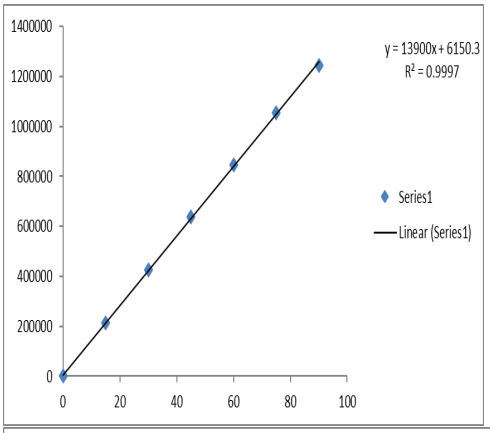
Overall, all the %RSD values were found to be less than 2.0%, which is within the acceptable limit as per ICH guidelines. Therefore, the developed analytical method is precise, reproducible, and suitable for the simultaneous estimation of Lamivudine, Tenofovir, and Doravirine in bulk and pharmaceutical dosage forms.

LINEARITY:

To demonstrate the linearity of the assay method, inject 6 standard solutions with different concentrations of Lamivudine, Tenofovir, and Doravirine. Plot a graph of concentration versus peak area. Slope, Linearity plot are shown in Fig.

Table Linearity, Concentration, and Response

	Lamivudine		Tenofovir		Doravirine	
s.no	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak ar
1	0	0	0	0	0	0
2	15	213652	15	208913	5	4373
3	30	424674	30	414817	10	8649
4	45	636384	45	621219	15	13051
5	60	847674	60	837510	20	17345
6	75	1055211	75	1046490	25	21675
7	90	1243963	90	1235660	30	25310
Conc range (µg/mL)	15-90		15-90		5-30	
Regression Equation	y = 13900x + 6150.3		y = 13821x + 1570		y = 8516.5x + 1403.8	
Co-relation	0.999		0.999		0.999	



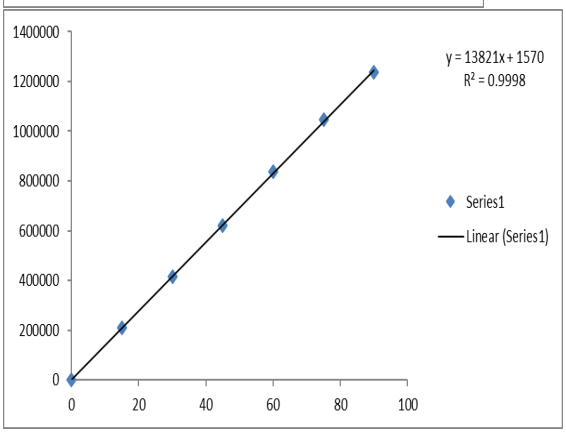


Fig. Linearity Plot: Lamivudine. Fig Linearity Plot Tenofovir

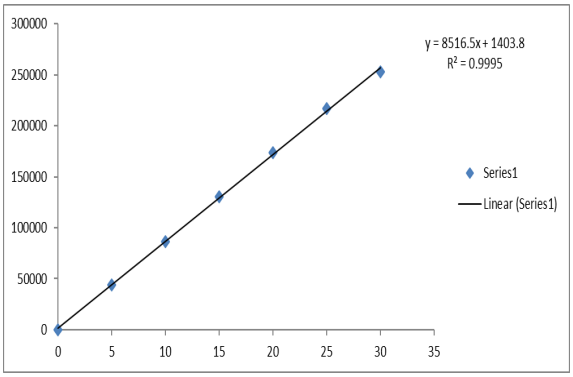


Fig Linearity Plot Doravirine

Accuracy

The accuracy of the developed method was evaluated by recovery studies at 50%, 100%, and 150% levels. Known amounts of Lamivudine, Tenofovir, and Doravirine were spiked into the sample solution, and the recovered amounts were calculated. The percentage recovery was found to be 99.59% for Lamivudine, 99.40% for Tenofovir,

and 99.63% for Doravirine. The recovery results were within the acceptable limit, indicating that the developed method is accurate and suitable for the simultaneous estimation of all three drugs in pharmaceutical dosage form.

LOD and LOQ

The sensitivity of the method was confirmed by LOD and LOQ studies. The LOD values were 0.29, 0.21, and 0.12 µg/mL, while the LOQ values were 0.88, 0.64, and 0.37 µg/mL for lamivudine, tenofovir, and doravirine, respectively. The low values indicate that the developed method is sensitive and suitable for quantification of all three drugs at low concentration levels.

Robustness

Robustness of the developed method was evaluated by making small deliberate changes in flow rate, mobile phase composition, and temperature. The %RSD values for Lamivudine, Tenofovir, and Doravirine were found within the acceptable limit, ranging from **0.2% to 1.0%**. Since all values were below **2%**, the method was found to be robust and reliable under minor variations in chromatographic conditions.

Assay of Marketed Formulation

The assay of the marketed formulation was performed by injecting standard and sample solutions separately into the chromatographic system. The amount of drug present in the sample was calculated using the standard assay formula. The average % assay was found to be 99.58% for Lamivudine, 99.54% for Tenofovir, and 99.96% for Doravirine. The %RSD values were 0.4%, 0.2%, and 0.4%, respectively. All assay results were within the acceptable limits, confirming that the developed method is accurate, precise, and suitable for routine estimation of Lamivudine, Tenofovir, and Doravirine in pharmaceutical formulations.

Stress Degradation Study

Forced degradation studies were performed to evaluate the stability-indicating ability of the developed RP-HPLC method. Lamivudine, Tenofovir, and Doravirine were exposed to different stress conditions, including acid, base, neutral, oxidative, thermal, and photolytic degradation. Under acid hydrolysis with 2N HCl at 60°C for 60 minutes, degradation was found to be 3.96%, 3.71%, and 5.31%, respectively. Under base hydrolysis with 2N NaOH at 60°C for 60 minutes, degradation was 4.63%, 5.94%, and 7.40%, respectively, with two degradation products observed. In neutral hydrolysis with water at 60°C for 6 hours, low degradation of 0.86%, 0.88%, and 0.95% was observed. Oxidative degradation with 20% H_2O_2 at 60°C for 60 minutes showed degradation of 4.88%, 6.40%, and 7.41%, respectively. Thermal degradation at 105°C showed 2.37%, 2.21%, and 1.94% degradation, while photolytic degradation showed 2.09%, 1.90%, and 1.21% degradation, respectively. The highest degradation was observed under base and oxidative conditions, whereas the drugs were comparatively stable under neutral, thermal, and photolytic conditions. These results confirm that the developed method is stability-indicating and suitable for separation of the drugs from their degradation products.

Conclusion

A rapid, robust, and stability-indicating RP-HPLC method for the simultaneous quantification of Lamivudine, Tenofovir, and Doravirine was successfully developed using a QbD-based DOE approach. The application of CCD enabled efficient optimization of chromatographic conditions, leading to improved resolution, reduced analysis time, and enhanced method reliability. Validation results confirmed that the method is linear, precise, accurate, specific, and robust, meeting all regulatory expectations. The forced degradation studies demonstrated that the method is capable of clearly separating all degradation products, confirming its suitability as a stability-indicating analytical procedure. The method is therefore highly suitable for routine quality control, stability testing, and regulatory submission of Lamivudine–Tenofovir–Doravirine fixed-dose combinations.

References

1. International Council for Harmonisation. ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. ICH Harmonised Tripartite Guideline; 2005.
2. International Council for Harmonisation. ICH Q14: Analytical Procedure Development. ICH Harmonised Guideline; 2023.

3. International Council for Harmonisation. ICH Q1A(R2): Stability Testing of New Drug Substances and Products. ICH Harmonised Guideline; 2003.
4. Food and Drug Administration. DELSTRIGO® prescribing information: Doravirine, Lamivudine and Tenofovir disoproxil fumarate tablets. FDA; 2025.)
5. European Medicines Agency. ICH guideline Q2(R1): Validation of analytical procedures: text and methodology. EMA; 2006. (European Medicines Agency (EMA))
6. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. Journal of Pharmaceutical Analysis. 2014;4(3):159–165.
7. Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. Journal of Pharmaceutical and Biomedical Analysis. 2002;28(6):1011–1040.
8. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Hoboken: John Wiley & Sons; 2010.
9. Kazakevich Y, Lobrutto R. HPLC for Pharmaceutical Scientists. Hoboken: John Wiley & Sons; 2007.
10. Dong MW. Modern HPLC for Practicing Scientists. Hoboken: John Wiley & Sons; 2006.
11. Montgomery DC. Design and Analysis of Experiments. 9th ed. Hoboken: John Wiley & Sons; 2017.
12. Orlandini S, Pinzauti S, Furlanetto S. Application of quality by design to analytical methods. Analytical and Bioanalytical Chemistry. 2013;405:443–450.
13. Tome T, Žigart N, Časar Z, Obreza A. Development and optimization of liquid chromatography analytical methods by using AQbD principles. Organic Process Research & Development. 2019;23(9):1784–1802.
14. Peraman R, Bhadraya K, Padmanabha Reddy Y. Analytical quality by design: A tool for regulatory flexibility and robust analytics. International Journal of Analytical Chemistry. 2015;2015:868727.
15. Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE—Analytical GREEnness metric approach and software. Analytical Chemistry. 2020;92(14):10076–10082. (ACS Publications)
16. Gałuszka A, Migaszewski Z, Namieśnik J. The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. TrAC Trends in Analytical Chemistry. 2013;50:78–84.
17. Tobiszewski M, Marć M, Gałuszka A, Namieśnik J. Green chemistry metrics with special reference to green analytical chemistry. Molecules. 2015;20(6):10928–10946.
18. Swartz ME, Krull IS. Analytical Method Development and Validation. New York: Marcel Dekker; 1997.
19. Ahuja S, Dong MW. Handbook of Pharmaceutical Analysis by HPLC. Amsterdam: Elsevier Academic Press; 2005.
20. United States Pharmacopeial Convention. United States Pharmacopeia and National Formulary USP–NF: General Chapter <621> Chromatography. Rockville, MD: USP; latest official version.