International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

A Novel UV Spectrophotometric Approach Using Absorbance Correction for Concurrent Quantification of Meloxicam and Rizatriptan Benzoate

Harsh K. Patel[1], Satish A. Patel[2]

[1]Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Ganpat Vidyanagar, Kherva, 384012, Mehsana, Gujarat, India
Email: *patelhk2910[at]gmail.com*

Abstract: ***A novel and highly accurate and cost-effective method for determining the concentrations of the two medications in solution by means of their UV absorbances correction was developed and validated based on the distinctively different UV properties of Meloxicam and Rizatriptan Benzoate. Although the spectra of absorbance for each of the two compounds overlapped, the developed method overcame the overlap (interference) by making use of a unique way to quantify each compound without the need for either separation or isolation. Validation of the proposed method, as per the ICH guidelines (linearity, limit of detection, limit of quantification, precision and accuracy) has successfully been accomplished with the use of methanol as the solvent for all of the studies performed. The calibration graphs for both Meloxicam (2-12 µg/ml) and Rizatriptan Benzoate (2-12 µg/ml) show significant linear regressions with all coefficients of determination greater than 0.9900. The recovery rates of 98-102% for both compounds correspond to the accurate quantification of both compounds. Precision studies indicated an %RSD value of <2% for intra-day and inter-day precision which further supports the precision and accuracy of the developed method. The method can be recommended as a viable alternative to chromatographic techniques and can be used for routine Quality Control analysis for both mixed preparations and product formulations.***

Keywords: Meloxicam, Rizatriptan Benzoate, UV Spectrophotometry, Absorbance Correction, Validation

1. Introduction

Modern medicine now focuses on combination treatments that produce synergistic effects through their ability to treat various disease mechanisms. In migraine management the combination of Meloxicam (MELO) a selective cyclooxygenase-2 COX-2 inhibiting NSAID from the oxicam family with Rizatriptan Benzoate (RIZA) a second-generation triptan that strongly binds to 5-HT$_1$B/$_1$D receptors creates an effective treatment approach which targets both inflammatory and neurovascular systems during acute migraine attacks.

The molecular structure of MELO ($C_{14}H_{13}N_3O_4S_2$; MW: 351.4 g/mol) exists as a benzothiazine scaffold which contains a thiazole-substituted carboxamide feature and presents itself as a pale yellow microcrystalline solid that shows minimal water solubility but sufficient solubility in methanol. Rizatriptan Benzoate (RIZA) ($C_{22}H_{25}N_5O_2$; MW: 391.5 g/mol) presents an indole-based architecture bearing a triazolymethyl substituent and a tertiary amine chain which exists as a benzoate salt to enhance its water solubility of 42 milligrams per milliliter and its pharmaceutical stability [1].

The analytical assessment of multiple active ingredients in pharmaceutical products requires testing methods which can separate overlapping spectral patterns while remaining affordable and easy to use and meeting all necessary regulatory standards. Pharmaceutical quality control relies on ultraviolet spectrophotometry as its primary testing method because this technique provides affordable and fast results with minimal need for sample handling. There arises the need for advanced mathematical solutions to analyze compounds that cause spectral interference, for it is becoming very complex to experimentally design testing conditions.

The absorbance correction method, which scientists also call the absorption correction factor technique, provides an effective solution for solving spectral overlap issues that arise in binary mixtures. The method uses the principle which states that at an analyte's maximum absorption wavelength (λmax) the other component will display different measurable absorbance values. The method establishes correction factors through absorptivity ratio measurements taken at two specific wavelength points which allow researchers to mathematically separate individual component concentrations from total absorbance measurements without requiring chromatographic testing [2].

MELO displays its typical absorption characteristics at 360 nanometers and RIZA reaches its highest absorption point at 225 nanometers in the current analysis process. The two components show distinct wavelength separation which results in predictable absorptivity patterns that serve as a foundation for building an absorbance correction-based method to simultaneously estimate different components. The mathematical method computes correction coefficients which show how much each drug absorbs at the specific wavelength needed to analyze its partner drug. These factors are then used to determine the separate concentration levels from the combined spectral information [3].

The existing literature contains detailed descriptions of different analytical techniques which enable researchers to measure these agents without using shared methods. The researchers have not yet published a validated spectrophotometric method which uses absorbance correction to measure both substances at the same time. The combination formulation currently undergoes clinical testing because it is being evaluated in Phase 3 trials to treat acute migraine. The development of a cost-effective and laboratory-accessible analytical method needs to proceed because it is essential for developing the formulation and assessing its stability and conducting routine quality assurance activities [4].

The researchers aimed to create and assess a new UV spectrophotometric method which applies absorbance correction techniques to simultaneously measure MELO and RIZA in synthetic mixtures and pharmaceutical products. The proposed methodology was comprehensively validated following International Conference on Harmonisation (ICH) Q2(R2) guidelines, evaluating parameters including specificity, linearity, range, accuracy, precision, robustness, and solution stability to ensure its suitability for intended pharmaceutical application [5].

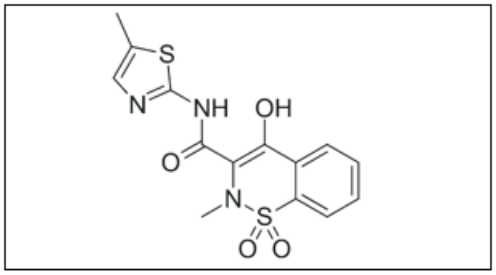

Figure 1: MELO

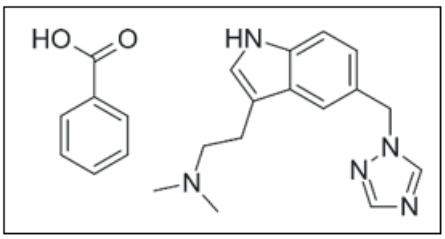

Figure 2: RIZA

2. Materials and Methods

2.1 Instruments

The present investigation was carried out using a UV–Visible double-beam spectrophotometer (Shimadzu UV-1800, Japan) with a spectral bandwidth of 2 nm and wavelength accuracy of ±0.5 nm. Measurements were performed using a pair of matched quartz cuvettes with a path length of 10 mm. Spectral data were acquired and processed through UV-Probe 2.0 software. An electronic analytical balance (Sartorius CP224S) was employed for precise weighing, and an ultrasonic bath was used for sample preparation during the experimental procedure [6].

2.2 Materials

Pure standards of MELO and RIZA were obtained as gift samples from Intas Pharmaceuticals Ltd., Ahmedabad, India, and were used as received. Analytical reagent (AR) grade methanol was used as solvent throughout the study. All chemicals used were of analytical grade [7].

2.3 Preparation of Solution

2.3.1 Preparation of Standard Stock Solutions

Stock solutions of both analytes were prepared independently by dissolving exactly 10.0 mg of each reference standard (MELO and RIZA) in separate 100 mL calibrated volumetric flasks. Complete dissolution was achieved by adding approximately 50 mL of methanol followed by ultrasonication for 5 min. After cooling to ambient temperature, the volume in each flask was adjusted to 100 mL with methanol, resulting in stock solutions with a final concentration of 100 µg/mL. [8]

2.3.2 Preparation of Standard Solution

Working standards were prepared by appropriate dilution of stock solutions with methanol in 100 mL volumetric flasks. MELO concentrations of 2, 5, 10, 15, and 20 µg/mL and RIZA concentrations of 1, 2, 4, 6, 8, and 10 µg/mL were obtained by transferring suitable aliquots from respective stock solutions. All working solutions were prepared fresh daily and analyzed immediately.

2.4 Preparation of Synthetic Mixture

A laboratory-prepared synthetic blend totaling 100 mg was formulated by accurately weighing 10 mg of RIZA and 20 mg of MELO, and combining them with 70 mg of selected excipients, namely Povidone K30, magnesium stearate, and mannitol. All components were mixed uniformly to achieve a well-homogenized powder blend. This prepared mixture was subsequently used as the sample for absorbance correction spectrophotometric analysis.

2.5 Preparation of Calibration Curve

The researchers created standard solutions which contained concentrations between 2 and 12 µg/mL through the process of transferring 0.2 to 1.2 mL samples from MELO and RIZA stock solutions, which had a concentration of 100 µg/mL, into 10 mL volumetric flasks to create a final solution through methanol. The researchers measured the UV absorption spectra of each standard against a methanol blank to determine the two most suitable wavelengths for testing which were 225 nm and 234 nm because they demonstrated maximum absorption and least spectral interference.

For the absorbance correction method, absorbance measurements were performed at both wavelengths for all concentration levels of each drug. Calibration graphs were plotted between absorbance and concentration at 360 nm and 225 nm for both analytes. The linearity was assessed through correlation coefficients obtained from regression analysis.

2.6 Selection of Analytical Wavelength

Standard stock solutions of RIZA and MELOwere prepared in a suitable solvent and scanned individually in the wavelength range of 200–400 nm using a UV–Visible spectrophotometer. The zero-order spectra of both drugs were recorded and overlaid to evaluate their spectral characteristics and interference pattern.

The overlay spectra indicated spectral overlap in the lower wavelength region, while MELO showed a well-defined absorption maximum at 360 nm where RIZA exhibited negligible absorbance. Therefore, 360 nm was selected for the direct estimation of MELO without interference from RIZA. A second wavelength at 225 nm, corresponding to the absorption maximum of RIZA, was selected for combined measurement, where MELO also showed measurable absorbance.

For quantification, the absorbance measured at 360 nm was used to determine the concentration of MELO directly. The contribution of MELO at 225 nm was then calculated using its absorptivity coefficient and subtracted from the total absorbance at 225 nm. The corrected absorbance value was used to estimate the concentration of RIZA. The suitability of the selected wavelengths (225 nm and 360 nm) was confirmed through overlay spectral analysis and absorptivity calculations to ensure specificity and accuracy of the absorbance correction method.

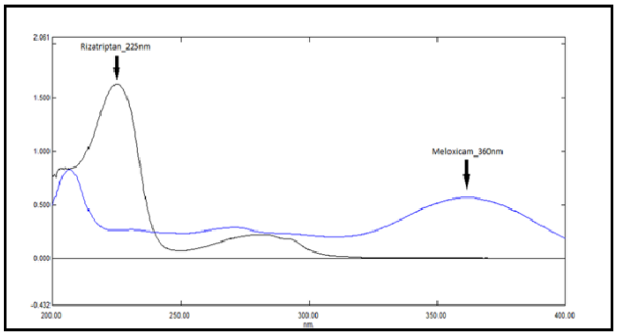

Figure 3: Zero Order Overlay Spectra RIZA and MELO

3. Absorbance Correction Spectrophotometric Method

The absorbance correction UV spectrophotometric technique was applied for the simultaneous quantification of MELO and RIZA. This approach is based on measuring absorbance at two selected wavelengths and mathematically correcting the contribution of one drug to accurately determine the other.

In this method, the zero-order UV spectra of both drugs were recorded in a suitable solvent. MELO showed significant absorbance at 360 nm, where RIZA exhibited negligible or no absorbance. Therefore, MELO was directly estimated at 360 nm without interference. RIZA showed strong absorbance at 225 nm; however, MELO also absorbs at this wavelength. Hence, the absorbance of MELO at 225 nm was calculated using its absorptivity ratio and subtracted from the total absorbance measured at 225 nm to obtain the corrected absorbance of RIZA.

Calibration curves for both drugs were established by plotting absorbance versus concentration at their respective analytical wavelengths. The corrected absorbance values were then used to determine the concentrations of MELO and RIZA in synthetic mixtures.

(A) Concentration of MELO at 360nm
A= abc
Where, A=Absorbance of Mixture at 360nm
a = A (1%, 1cm) of MELO at 360nm
b = Path length = 1cm
c = Concentration in gm/100mL
Calculate concentration and convert it in µg/mL

(B) Absorbance of MELO at 225nm
A= abc
Where, A=Absorbance of MELO at 225nm
a = A (1%, 1cm) of MELO at 225nm
b = Path length = 1cm
c = Concentration of MELO 360nm

(C) Calculation of concentration of RIZA from the corrected Absorbance at 225nm
Corrected absorbance = A225 (Mixture) – A225 (RIZA)
Concentration of RIZA from corrected absorbance,
C=A/ab
Where, A= Corrected absorbance
a = A (1%, 1cm) of MELO at 225nm
b = Path length = 1cm
c = Concentration of RIZA in gm/100mL

4. Calibration Curve

The calibration range for both RIZA and MELO was established between 2–12 µg/mL. The researchers prepared working standards by using appropriate aliquots from the standard stock solutions. The researchers transferred drug solutions by measuring 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL volumes into separate 10mL volumetric flasks. The researchers used methanol to bring each flask up to the required concentration level. The solutions served as the basis for creating the calibration curve and conducting subsequent analytical tests.

5. Method Validation

The absorbance correction spectrophotometric technique was validated following ICH Q2 (R2) recommendations by evaluating key performance characteristics such as linearity, working range, precision, accuracy, as well as the limit of detection (LOD) and limit of quantification (LOQ).

5.1 Linearity

The calibration curves were plotted over a concentration range of 2-12 µg/mL for MELO and RIZA. Separate 10 mL volumetric flasks contained the accurately measured standard stock solutions ofMELO and RIZA at concentrations of2, 4, 6, 8, 10 and 12 µg/mL. The absorbance was measured at the respective wavelengths for MELO and RIZA. The calibration curves were constructed by plotting absorbance versus concentration and equations were calculated.

5.2 Limit of Detection (LOD)

The lowest detectable concentration refers to the minimal amount of analyte that yields a distinguishable signal from the blank under the established analytical conditions, without requiring accurate quantification. For this absorbance correction methodology, the limit of detection was computed as per ICH guidelines utilizing the expression:

$$LOD = 3.3\sigma/S$$

where σ corresponds to the response standard deviation and S indicates the gradient of the calibration graph.

5.3 Limit of Quantification (LOQ)

The lowest quantifiable concentration corresponds to the minimal analyte amount that yields measurements with adequate precision and trueness. For this absorbance correction technique, the limit of quantification was calculated following ICH recommendations using the relationship:

$$LOQ = 10\sigma/S$$

wherein σ represents the response standard deviation and S indicates the gradient of the linearity curve.

5.4 Range

The analytical range refers to the span of concentrations between the minimum and maximum levels over which the method demonstrates a reliable linear response. In the developed procedure, a satisfactory linear relationship was observed within the concentration range of 2–12 µg/mL for MELO and 2–12 µg/mL for RIZA.

5.5 Precision

5.5.1 Repeatability

Repeatability represents the closeness of agreement among results obtained under identical experimental conditions within a short time interval. In this study, suitable aliquots of standard solutions ofMELO and RIZA were prepared and examined on the same day while keeping all analytical and instrumental settings unchanged. Measurements were carried out using a UV spectrophotometer, and the method precision was evaluated in terms of percentage relative standard deviation (%RSD).

5.5.2 Intermediate Precision:

The precision of the developed analytical procedure was additionally assessed through both intraday and interday variation studies. Intraday precision was evaluated by measuring three separate concentration levels of each drug at multiple time points within the same day. Interday precision was determined by performing the analysis on three successive days using identical concentration levels. The obtained results were calculated and reported as percentage relative standard deviation (%RSD).

5.6 Accuracy (% Drug Recovery)

The conventional addition method was used to measure MELO and RIZA recoveries which created a testing method that determined measurement accuracy. The three accuracy levels tested show results of 75%, 100%, and 125%. The researchers tested the solution which contained pre-measured amounts of MELO (4-12 µg/mL) and RIZA (2-6 µg/mL) together with known quantities of MELO (4 µg/mL) and RIZA (2 µg/mL) reference solutions. The researchers measured the solution's absorbance at 360 nm and 225 nm to determine MELO and RIZA concentrations. The researchers used the absorbance correction equation method to find MELO and RIZA quantities at every level while they presented percentage recoveries as results.

6. Results and Discussion

6.1 Linearity

An absorbance correction UV spectrophotometric method was developed for the simultaneous quantification of MELO and RIZA in pharmaceutical dosage forms. In this approach, the absorbance of each drug was measured at its selected analytical wavelength, and appropriate absorbance correction factors were applied to eliminate spectral interference from the other component, enabling accurate estimation in the combined formulation.

Table1: Result of Linearity

Parameters	*MELO*	*RIZA*
Wavelength (nm)	360	225
Concentration range (µg/mL)	2-12	2-12
Regression Equation y=mx+c Slope (m) Intercept(c)	y=0.1005x+0.0499 0.1005 0.0499	y=0.2806x+0.0031 0.2806 0.0031
Corelation coefficient	0.9991	0.9990

The method followed Beer–Lambert's law over the concentration range of 2–12 µg/mL for both MELO and RIZA. Excellent linear behavior was obtained within this range, with correlation coefficient values exceeding R^2 = 0.9900, demonstrating a strong linear relationship between absorbance and concentration. The linearity of the procedure was further verified through the calibration plots constructed for both drugs.

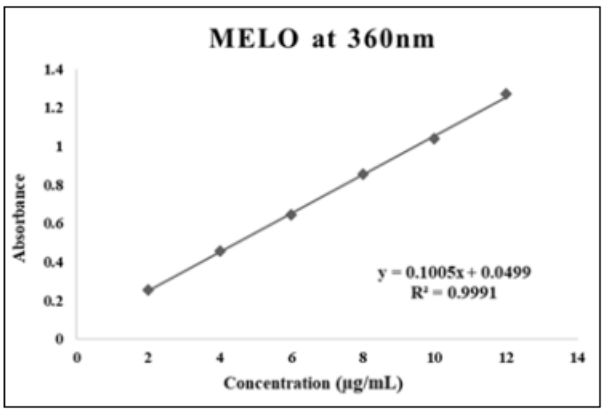

Figure 4: Linearity (MELO)

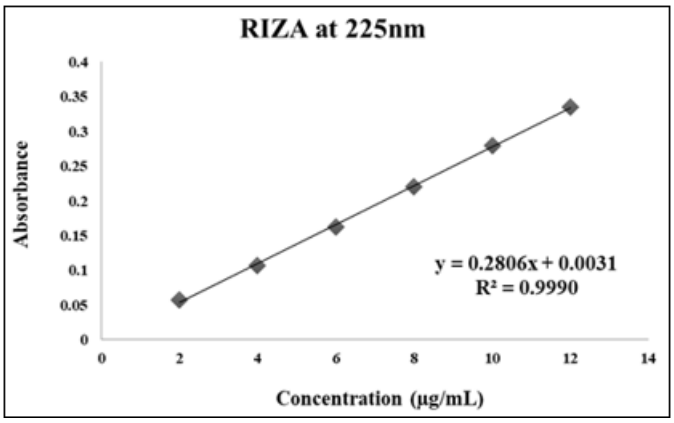

Figure 5: Linearity (RIZA)

6.2 LOD and LOQ

The detection and quantification limits were determined using mathematical calculations following ICH Q2(R2) guidelines. The results demonstrated that this analytical method possesses adequate sensitivity for measuring both active pharmaceutical ingredients.

For MELO analysis at 360nm wavelength, the limit of detection was determined to be 0.24 µg/mL, while the limit of quantification was established at 0.55 µg/mL. In the case of RIZA measured at 225nm, the detection limit was calculated as 1.23 µg/mL, with a quantification limit of 3.74 µg/mL. These relatively low threshold values confirm the method's capability to detect and accurately measure small quantities of both compounds, thereby validating its sensitivity for this application.

6.3 Precision

6.3.1 Repeatability

Table 2: Result of Repeatability

S. No.	*RIZA*	*MELO*
1	0.162	0.340
2	0.163	0.346
3	0.161	0.342
4	0.163	0.346
5	0.161	0.342
6	0.164	0.344
Mean	0.162	0.343
SD	0.001	0.002
%RSD	0.746	0.705

6.3.2 Intermediate Precision

The precision of the developed absorbance correction spectrophotometric technique was assessed through intra-day and inter-day repeatability studies to establish intermediate precision. Analysis of the obtained %RSD values for both MELO and RIZA revealed minimal variation, thereby confirming the method's reliability and reproducibility for routine pharmaceutical analysis.

Table 3: Result of Intermediate Precision

Sr. No	Drug	*%RSD (n=3)*	
		Intraday	*Interday*
1	MELO	0.621-0.248	0.488-0.171
2	RIZA	0.408-0.138	0.206-0.248

6.4 Accuracy (% Drug Recovery)

The accuracy of the absorbance correction UV spectrophotometric method was assessed using a standard addition approach at three concentration levels through recovery studies. The obtained percentage recoveries for MELO and RIZA ranged between 98% and 102%, demonstrating that the proposed method provides reliable and accurate results and is not affected by interference from formulation excipients.

Table 4: Result of Accuracy (MELO & RIZA)

(% Recovery Level)	*Sample Amt. Taken (µg/mL)*		*Standard Amt. Added (µg/mL)*		*Amount recovered (µg/mL)*		*% Recovery ± SD (n=3)*		*%RSD (n=3)*
	MELO	*RIZA*	*MELO*	*RIZA*	*MELO*	*RIZA*	*MELO*	*RIZA*	
I (75%)	4	2	3	1.5	2.97	1.51	99.52 ± 1.65	100.9 ± 1.93	1.37
II (100%)	4	2	4	2	4.08	1.99	101.0 ± 1.31	99.83 ± 1.04	1.04
III (125%)	4	2	5	2.5	5.06	2.49	100.7 ± 1.60	99.47 ± 1.22	1.22

6.5 Assay of synthetic mixture

The content of MELO and RIZA in the synthetic mixture was determined using the absorbance correction UV spectrophotometric technique. The obtained assay results confirm that the developed method is reliable, reproducible, and appropriate for routine quantitative analysis of MELO and RIZA in combined pharmaceutical formulations.

Table 5: Result of Analysis of MELO & RIZA in synthetic mixture (Assay)

Sample No.	Drug Amount (mg)		Amount found (mg)		%Assay	
	MELO	RIZA	MELO	RIZA	MELO	RIZA
1	20	10	19.63	9.82	98.15	98.20
2	20	10	20.27	9.94	101.3	99.40
3	20	10	19.85	10.09	99.25	100.9
4	20	10	20.22	10.15	101.1	101.5
5	20	10	20.19	9.81	100.9	98.10
6	20	10	19.65	10.18	98.25	101.8
Mean					99.84	99.98
SD					1.473	1.643

7. Conclusion

The developed absorbance correction spectrophotometric method for the simultaneous estimation of MELO and RIZA demonstrate good linearity over the concentration range of 2–12 µg/mL for both drugs. The validation results confirm that the method provides both accurate results and precise measurements since its recovery values approach 100 percent while its relative standard deviation stays within acceptable boundaries. The studies for both intra-day and inter-day precision demonstrated that the system maintained constant performance which produced identical results throughout the testing period. The method enables quantitative analysis through its simple design which requires short processing time and incurs low operational costs while delivering dependable results. The system effectively handles routine quality control testing and assay procedures for MELO and RIZA which exist in combined mixtures.

Acknowledgement
The authors are highly gratefully acknowledging the Intas Pharmaceuticals Ltd. for their generous contribution to provide pure drug standards of MELO and RIZA, which were essential for this research. We extend our sincere thanks to the Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, for granting access to their facilities and providing the necessary infrastructure to conduct this study.

References

[1] Bhatia V, Vikram V, Rattan A, “consolidated activity of rizatriptan and MELO in suppressing CGRP-induced nociception and COX-mediated inflammation,” I, pp 3871-3883, 2025.
[2] D. S. Wishart, Y. D. Feunang, A. C. Guo, E. J. Lo, A. Marcu, J. R. Grant, et al., “DrugBank 5.0: a major update to the DrugBank database for 2018,” Nucleic Acids Research, 46 (D1), pp. D1074–D1082, 2018.
[3] D. A. Skoog, F. J. Holler, S. R. Crouch, Principles of Instrumental Analysis, 6th ed., Thomson Brooks/Cole, Belmont (CA), 2007.
[4] Beckett AH, Stenlake JB, Practical Pharmaceutical Chemistry, 4th ed., Part II, New Delhi: CBS Publishers & Distributors, 2002.
[5] Chatwal GR, Anand SK, Instrumental Methods of Chemical Analysis. 5th ed. Mumbai: Himalaya Publishing House, 2007.
[6] “International Council on Harmonisation (ICH),” ICH Q2(R2), Validation of Analytical Procedures, Text and Methodology, Geneva: ICH; 2024.
[7] Snyder LR, Kirkland JJ, Introduction to Modern Liquid Chromatography, 3rd ed., Hoboken (NJ), John Wiley & Sons; 2010.
[8] Willard HH, Merritt LL, Instrumental Methods of Analysis. 7th ed., New Delhi: CBS Publishers & Distributors, 2024.