RESEARCH PAPER

DEVELOPMENT AND VALIDATION OF A SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND METOPROLOL SUCCINATE IN A SYNTHETIC MIXTURE OF BILAYER TABLET FORMULATION

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ABSTRACT

A simple, accurate and precise UV spectrophotometric method was developed and validated for the simultaneous estimation of Rosuvastatin calcium (ROS) and Metoprolol succinate (METO) in a bilayer tablet formulation. The analysis was performed using the simultaneous equation method at the absorption maxima (λmax) of 243 nm and 222 nm for ROS and METO, respectively, using phosphate buffer (pH 6.8) as a solvent. The method was validated according to ICH Q2(R1) guidelines. Linearity was observed in the concentration range of 5–30 µg/mL for both drugs with correlation coefficients (R^2) of 0.9999 and 0.9997. The mean recovery for ROS and METO was found to be 99.78% and 100.24%, respectively. The developed method is suitable for routine quality control analysis of these drugs in combined dosage forms.

Keywords: Rosuvastatin calcium, Metoprolol succinate, Simultaneous Equation Method, UV Spectrophotometry, Method Validation, Bilayer Tablet

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1. Introduction

Dyslipidemia and hypertension are the two most significant risk factors for cardiovascular disease[1]. They often occur together, a condition sometimes referred to as dyslipidemia hypertension[2]. The presence of both conditions at the same time has an impact on a person's risk for heart attack, stroke, and other cardiovascular problems[3] and highlights the need for integrated care of these two significant cardiovascular disease (CVD) risk factors [4].

Rosuvastatin calcium is a hypolipidemic agent classified as an HMG-CoA reductase inhibitor, a rate-limiting enzyme. HMG-CoA reductase catalyzes the conversion of 3 - hydroxyl - 3 – methyl glutaryl coenzyme A to mevalonate, a precursor of cholesterol. It increases the catabolism of LDL and inhibits the hepatic synthesis of VLDL and increases the synthesis of HDL[5]. Immediate release formulations of Rosuvastatin calcium usually result in fast initial start of the therapeutic effect[6]. Metoprolol succinate is a selective β1 receptor blocker used in cardiovascular therapy. It acts by blocking receptors that are responsible for increasing heart rate and force of contraction leading to a decrease in blood pressure[7]. The physical and pharmacological features of metoprolol succinate make it a suitable option for such persistent matrix modifications [8].

Combination therapy is a treatment modality that involves the use of two or more different drugs to treat a coexisting disease or condition. Bilayer tablets are one of the best ways to deliver fixed dose combination where one layer acts as immediate release and the other layer for sustained release of the drug. A single drug or two different drugs can be combined as one tablet with different release profiles. Bilayer tablets have many advantages over single-layer tablets; most importantly, they stabilize incompatible APIs and modify their release in a combined formulation[9]. Bilayer tablets reduce the dose regime and increase patient convenience. Combining these agents in a bilayer tablet is an effective strategy for managing co-morbidities [10].

In pharmaceutical quality control, reliable analytical procedures are needed to ensure content uniformity and dissolution kinetics of multi-layered fixed-dose combinations. Traditional approaches like High-Performance Liquid Chromatography (HPLC) and LC-

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MS are sensitive but have disadvantages such as high cost and complicated sample preparation. Hence, there is a demand for simple, inexpensive and speedy analytical instruments. UV-Visible spectrophotometry with the help of mathematical models, especially Vierordt's method, provides an alternative technique that allows the simultaneous determination of pharmaceuticals in a single sample without the need for prior separation. The present paper deals with the development of a simple and efficient UV-spectroscopic method for the estimation of Rosuvastatin calcium and Metoprolol succinate in synthetic bilayer tablet matrix.

2. Materials and Methods

2.1 Instrumentation and Material:

All spectrophotometric absorbance measurements were carried out using a UV-Visible spectrophotometer (Model: UV-1800, Shimadzu Co., Japan). The weight of the samples was accurately weighed by an electronic analytical weighing balance (Model: SECURA225D-10IN, Sartorius, Germany). Rosuvastatin calcium and metoprolol succinate were supplied from Hetero Labs Ltd., Hyderabad, India and Granules India Ltd., Hyderabad, India respectively. All the other formulation excipients were obtained from Hetero Healthcare Ltd., Guwahati, India.

2.2 Bilayer tablet formulation:

A bilayer tablet formulation was designed with rosuvastatin calcium as immediate release and metoprolol succinate as sustained release layer. The composition of rosuvastatin and metoprolol layer is shown in Tables 1 and 2 respectively where 10.42 mg Rosuvastatin calcium is equivalent to 10 mg of rosuvastatin, and 47.5 mg of metoprolol succinate is equivalent to 50 mg of metoprolol tartrate

Table 1: Formulation of Rosuvastatin immediate release layer.

Ingredients	**Unit formula (mg)**
Rosuvastatin calcium	10.42
Microcrystalline cellulose	70.00
Lactose monohydrate	17.00
Crospovidone XL 10	2.00
Lake sunset yellow	0.58
Magnesium stearate	1.00
Tablet Weight (mg)	**101.00**

Table 2: Formulation of Metoprolol succinate sustained release layer.

Ingredients	**Unit formula (mg)**
Metoprolol succinate	47.5
HPMC K100M	90
Ethyl cellulose	10
PVP K30	7.2
Colloidal silicone dioxide	0.9
Microcrystalline cellulose PH102	22.6
Magnesium stearate	1.8
Tablet Weight (mg)	**180**

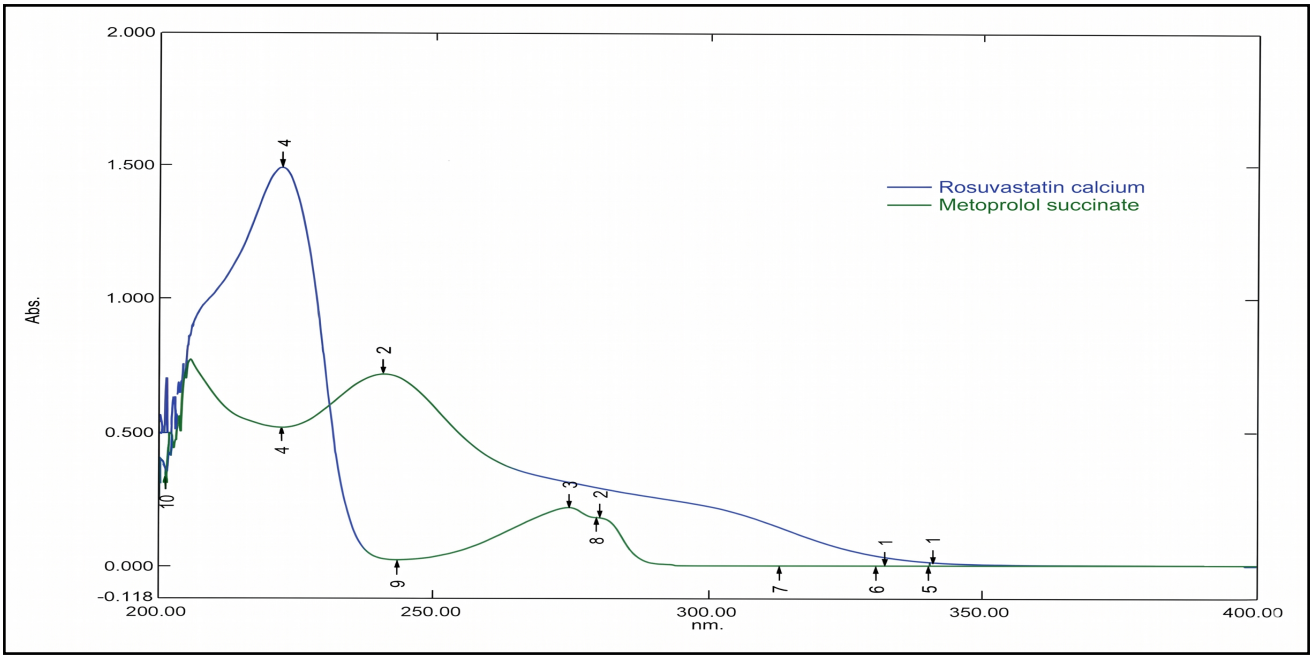


Figure 1: Overlay spectra of 20 µg/mL Rosuvastatin calcium and 50 µg/mL Metoprolol succinate solution

2.3 Method development:

2.3.1 Preparation of Stock Solutions:

Primary stock solutions with a concentration 1000 µg/mL were prepared for both drugs by dissolving required quantities of drugs in a small quantity of methanol and diluted with phosphate buffer (pH 6.8). Secondary stock solutions of 20 µg/mL and 50 µg/mL solutions of rosuvastatin calcium and metoprolol succinate were prepared in Phosphate buffer with suitable dilutions from the Primary stock solution.

2.3.2 Determination of wavelength of maximum absorbance (λmax) and iso-absorptive Point:

The prepared secondary stock solution 20 µg/mL and 50 µg/mL of rosuvastatin calcium and metoprolol succinate were scanned using a UV spectrophotometer at wavelengths ranging from 400nm to 200 nm to obtain the λmax. The λmax of rosuvastatin calcium was found to be 243 nm and metoprolol succinate 222 nm as shown in Figure 1.

2.3.3 Preparation of Test Sample Mixture:

A mixture of Rosuvastatin calcium and metoprolol succinate was made separately as per the formulation mentioned in table 1 and table 2 and finally both the formulations were combined and mixed properly. The combination was dissolved in a small quantity of methanol, sonicated for 15 minutes and then diluted with phosphate buffer pH 6.8 to 100 ml. The mixture containing 104.2 µg/mL of rosuvastatin calcium and 475 µg/mL Metoprolol succinate was filtered through Whatman No. 41 paper to remove the undissolved excipients. 1 mL of the mixture was then diluted with phosphate buffer pH 6.8 to prepare a final dilution of 10.42 µg/mL Rosuvastatin calcium and 47.5 µg/mL Metoprolol succinate for further analysis.

2.3.4 Simultaneous Equation Method:

Simultaneous estimation of rosuvastatin calcium and metoprolol succinate in bilayer tablet formulation was done by simultaneous equation method[11, 12]. The adoption of Vierordt's mathematical approach enables us to calculate accurately in overlapping binary mixtures without prior physical or chromatographic separation processes[13]. Standard Calibration curve plotted from the above linearity data; absorptivity values were determined for both the drugs at selected wavelength. Using the absorptivity coefficient values, two simultaneous equations were obtained and used for further analysis.

$$A_1 = ax_1Cx + ay_1Cy \quad \text{--- eq (1)}$$
$$A_2 = ax_2Cx + ay_2Cy \quad \text{--- eq (2)}$$

Where A_1 and A_2 are the total absorbances of the mixture at two different wavelength λ_1 & λ_2, ax_1 and ax_2 absorptivity of x at wavelength 1 and 2, ay_1 and ay_2 the absorptivity coefficient y at wavelength 1 and 2, Cx and Cy the unknown concentration of component x and component y.

$$Cx = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2 \quad \text{--- eq (3)}$$
$$Cy = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2 \quad \text{--- eq (4)}$$

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2.4 Method Validation:

The analytical procedure was thoroughly validated in compliance with the International Conference on Harmonization (ICH) Q2 (R1) guidelines to ensure accuracy, precision, linearity, and sensitivity (ICH, 2005).

2.4.1 Linearity:

Linearity of analytical method is the ability to generate test results which are directly proportional to the concentration of analyte in the sample throughout a certain range. In the study, six-point calibration curve was used to establish the linearity profiles of both Rosuvastatin calcium and Metoprolol succinate in the concentration range of 5–30 µg/mL. Calibration equations were generated using linear regression (y = mx + c) analysis of the standard results, correlation coefficients (R^2) were obtained, and compliance with the systemic validation criteria was confirmed.

2.4.2 Accuracy:

The accuracy of an analytical method is defined as the closeness confirming between a recognized true value and the experimental value obtained. In the present study, recovery tests were carried out using the standard addition method at three different levels (80%, 100% and 120%) as per the ICH guidelines. The framework includes the testing of five replicates at each level corresponding to target working concentrations of Rosuvastatin calcium and Metoprolol succinate. The percentage of drug recovered was calculated as follows:

$$\text{Accuracy (\% recovery)} = (\text{Measured value} / \text{True value}) \times 100 \text{ --- } eq\ (5)$$

2.4.3 Precision:

Method precision is a measure of the variation between replicate results and is used to determine the overall reproducibility of the system. To establish this parameter, intra-day and inter-day precision study was carried out for the standard solutions of Rosuvastatin calcium and Metoprolol succinate at three different concentration levels (10, 20 and 30 µg/mL). Intra-day repeatability was tested by measuring absorbance of samples in duplicate at different time points during a day. The inter-day precision was evaluated by measuring these absorbances on three separate days. To determine the low variability and robust analytical repeatability of the proposed spectrophotometric method, percent relative standard deviation (% RSD) was calculated from these data according to published validation protocols (Wankhede et al., 2010).

2.4.4 Sensitivity (LOD & LOQ):

The method was validated for sensitivity by determining Limit of Detection (LOD) and Limit of Quantitation (LOQ) as per ICH standards. LOD is the minimal amount of analyte that can be distinguished from the baseline noise without strict quantification. LOQ means the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy. These parameters were extracted from the standard deviation of the intercepts of the regression lines (σ) and the mean slope (S) of the calibration curves:

$$LOD = (3.3 \times \sigma) / S \quad \text{--- } eq\ (6)$$
$$LOQ = (10 \times \sigma) / S \quad \text{--- } eq\ (7)$$

3. Results and Discussion

3.1 Simultaneous Equation Development

UV absorption spectra of Rosuvastatin calcium (ROS) and Metoprolol Succinate (METO) in phosphate buffer (pH 6.8) showed absorption maxima at 243 nm and 222 nm respectively with considerable overlap in the 200–300 nm region. The overlap restricts any direct measurement by single wavelength spectrophotometry. The simultaneous equation method of Vierordt was evaluated to solve this which provides accurate quantification if the ratios of the absorptivities of the two components are sufficiently different. The ratio of the absorptivity of ROS at 243 nm and METO at 222 nm showed their different spectral features, which enabled accurate analysis by simultaneous linear equations based on the mean absorptivity coefficients A_1 and A_2 where A_1, A_2 are the absorptivity value of both the drug at 222 nm and 243 nm respectively. Using the calculated absorptivity values, the following simultaneous equations were established for concentration (C) determination:

$$A1 = 0.0175\ C(ROS) + 0.0358\ C(METO) \quad \text{------ } eq\ (8)$$

$$A2 = 0.0496\ C(ROS) + 0.0124\ C(METO) \quad \text{------ } eq\ (9)$$

The proposed simultaneous equation method was successfully applied to the synthetic bilayer tablet powder mixture, and the results for the assay and percentage recovery are summarized in Table 3.

Table 3: Analysis of synthetic powder mixture

Synthetic	Name of the drug	Label claim (mg)	Amount of drug detected	% Recovery

powder Mixture	Rosuvastatin calcium	10	9.963	99.63
	Metoprolol succinate	47.5	47.082	99.12

3.2 Validation

3.2.1 Linearity:

A linear relationship was observed for both Rosuvastatin calcium and Metoprolol Succinate over the concentration range of 5 –30 µg/ml. The statistical and regression data representing this linearity are documented in Tables 4 and 5. The individual calibration curves for Rosuvastatin calcium and Metoprolol succinate are illustrated in Figures 2, 3, 4, and 5 respectively. Both drugs followed Beer-Lambert law in the range of 5–30 µg/mL.

Table 4: Linear regression analysis of Rosuvastatin calcium

Concentration (µg/mL)	Absorbance at 222 nm	Absorbance at 243 nm
5	0.086	0.252
10	0.172	0.502
15	0.258	0.757
20	0.347	0.996
25	0.426	1.248
30	0.532	1.487
Regression Equation	**y = 0.01751*x - 0.002464**	**y = 0.04962*x + 0.004536**
Correlation Coefficient (R^2)	**0.9991**	**0.9999**

Table 5: Linear regression analysis of Metoprolol succinate

Concentration (µg/mL)	Absorbance at 222 nm	Absorbance at 243 nm
5	0.179	0.068
10	0.367	0.124
15	0.538	0.187
20	0.72	0.251
25	0.892	0.3127
30	1.076	0.372
Regression Equation	**y = 0.03576*x + 0.002393**	**y = 0.01237*x + 0.002200**
Correlation Coefficient (R^2)	**0.9999**	**0.9997**

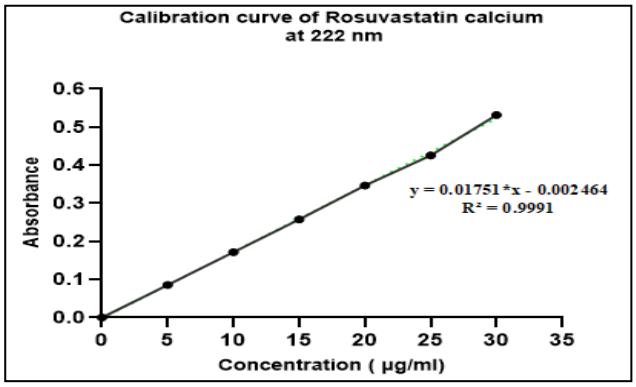


Figure 2: Absorbance of various concentrations of Rosuvastatin calcium at 222 nm.

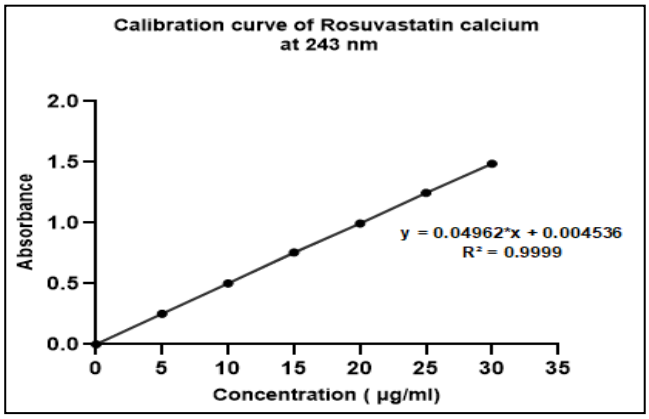


Figure 3: Absorbance of various concentrations of Rosuvastatin calcium at 243 nm.

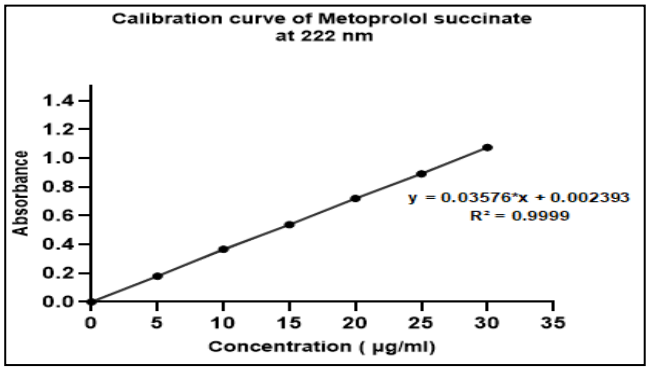


Figure 4: Absorbance of various concentrations of Metoprolol succinate at 222 nm.

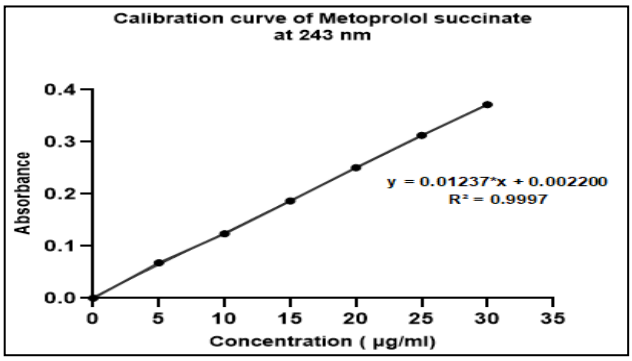


Figure 5: Absorbance of various concentrations of Metoprolol succinate at 243 nm.

3.2.2 Accuracy:

Recovery tests were carried out by the standard addition procedure in accordance with ICH guidelines to determine the accuracy and reliability of the proposed spectrophotometric method. The synthetic matrix samples were pre-analyzed and had a baseline concentration of 10.42 µg/mL Rosuvastatin calcium and 47.50 µg/mL Metoprolol succinate. The samples were then spiked with pure active pharmaceutical ingredients (API) standards at three different concentration levels (80%, 100% and 120%). The quantitative recovery data are given in Table 6. The average percentage recovery of Rosuvastatin calcium ranged from 99.32%±0.078% to 100.15%±0.083%, and the recovery of Metoprolol succinate ranged from 99.60%±0.483% to 100.77%±0.642%. Precision of the method was excellent with % RSD for every level of fortification being less than 1.0% showing strong accuracy for the two analytes. The near-ideal recovery results demonstrate that the complex polymeric excipients contained in the synthetic matrix (HPMC K100M, ethyl cellulose and crosspovidone) do not exert considerable spectrum interference on the simultaneous estimation of either drug. Thus, the new approach shows excellent performance, sensitivity and applicability for the routine quality control assay testing of this dual-drug combination.

Table 6: Accuracy parameter for Rosuvastatin calcium and Metoprolol succinate

Drug	Level (%)	Expected (µg/mL)	Found (µg/mL)	Mean Recovery ± SD (%)	% RSD
Rosuvastatin calcium	80%	18	17.63	99.32±0.078	0.42
	100%	20	20.03	100.15±0.083	0.40
	120%	22	21.85	99.70±0.096	0.42
Metoprolol succinate	80%	85.5	85.8	100.35±0.506	0.59
	100%	95	94.62	99.60±0.483	0.51
	120%	104.5	105.3	100.77±0.642	0.61

The mean recovery was found to be 99.72% for ROS and 100.24% for METO. The strong recovery patterns suggest that standard multi-layer tableting excipients do not introduce significant spectral baseline interference or chemical extraction limitations [11].

3.2.3 Precision:

Method precision was assessed by studying repeatability and intra-day and inter-day analytical variations. Table 7 presents statistical evaluation metrics. The system repeatability profile (n = 6) showed an optimal mean assay of 100.00% for both active pharmaceutical constituents. The mean assay values observed in the intra-day and inter-day variability studies (n = 3) were very near to the nominal baseline targets with the highest percentage relative standard deviation (%RSD) values observed well below 2.0 %. The very low variance of these measures is well within the typical analytical validation parameters, confirming the high operational dependability and reproducibility of the established spectrophotometric assay method.

Table 7: Precision parameters for Rosuvastatin calcium and Metoprolol succinate.

Study Parameter	Drug Name	Mean Assay ± SD (%)	% RSD
Repeatability (n = 6)	Rosuvastatin calcium	100.13±0.542	0.5414
	Metoprolol succinate	99.86±0.725	0.7261
Intra-Day Precision (n = 3)	Rosuvastatin calcium	99.93±0.621	0.6215
	Metoprolol succinate	98.93±0.814	0.8229
Inter-Day Precision (n = 3)	Rosuvastatin calcium	100.16±0.943	0.9416
	Metoprolol succinate	99.97±1.124	1.1242

3.2.4 LOD and LOQ:

Table 8 shows the data of LOD and LOQ. Based on Slopes: 0.04962 for ROS and 0.03576 for MET, these low LOD/LOQ values confirm that the method has very low "noise" or background interference.

Table 8: LOD and LOQ data

Parameter	Rosuvastatin calcium	Metoprolol succinate
LOD (µg/mL)	0.655	0.619
LOQ (µg/mL)	1.984	1.877

4. Conclusion

The simple, rapid, highly accurate and cost-effective UV spectrophotometric method was developed for the simultaneous determination of Rosuvastatin calcium and Metoprolol succinate by applying Vierordt's simultaneous equation model. The mathematical method is based on the difference in the absorptivity values of the two drugs at their respective absorption maxima (λ max) of 243 and 222 nm. This method was successfully used for the analysis of the overlapped spectra of the drugs without any prior chemical or physical separation. The very high processing throughput of the presented method, which reduces the time of sample preparation and analysis to only a small part of the time needed for conventional chromatographic procedures, underlines its effectiveness.

The analytical performance confirms the excellent efficiency and robustness of the approach under the ICH Q2 (R1) regulatory framework. Excellent correlation values (R^2 =0.9999 for ROS and 0.9997 for METO) were obtained, with the linear correlations being carefully followed over a large working concentration range of 5–30 µg/mL for both the analytes. The approach showed good sensitivity with low Limits of Detection (LOD of 0.655 µg/mL for ROS and 0.619 µg/mL for METO) and Quantitation. Moreover, the high recovery percentages (99.72% for ROS and 100.24% for METO) and the well-controlled precision values (%RSD<1.0%, well below the acceptable 2.0% regulatory threshold) prove that the multi-layer polymeric matrix binders (e.g. HPMC K100, ethyl cellulose and crosspovidone) do not

cause any background spectral distortion or chemical extraction restrictions.

This non-destructive and economically sustainable method from an industrial and environmental point of view offers a very efficient alternative to expensive, maintenance intensive and time consuming High-Performance Liquid Chromatography (HPLC) systems. Hence, this validated spectrophotometric method is highly recommended for routine application in industrial quality control laboratories in the area of rapid content uniformity, raw material assay and extended in vitro dissolution profiling of advanced bilayer tablet formulations.

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Author's Contribution

MG: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing the original draft. **JMK**: Validation, Resources. **BN**: Data Curation, Validation. **DL**: Methodology, Investigation, Data curation, Validation. **NB**: Formal analysis, Visualization, Validation, Writing & editing. **AS**: Conceptualization, Supervision, Project administration, Validation, Writing & editing, Resources **BPS**: Supervision, Resources, Funding acquisition, Writing & editing.

Consent to Participate

Not applicable

Consent for Publication

Not applicable

Conflict of Interest

Author declares no conflict of interest financially or otherwise

Ethical Approval

Not applicable

Funding

None

Informed Consent

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