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Uv Spectrophotometric Simultaneous Estimation of Nebivolol Hydrochloride and Ramipril: Method Development and Validation

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ABSTRACT:
A reliable, accurate, and precise First order derivative UV-spectrophotometric method was developed and validated for the simultaneous estimation of Nebivolol Hydrochloride and Ramipril in a synthetic mixture. Methanol was selected as the solvent for analysis due to its good solubility and spectral compatibility with both drugs. The absorption spectra of Nebivolol HCl and Ramipril were recorded in the wavelength range of 200-400 nm using a UV-visible spectrophotometer to determine suitable analytical wavelengths. The first-derivative spectrophotometric technique was employed. Selective estimation was achieved by measuring Nebivolol HCl at 237 nm, where Ramipril showed zero absorbance, and Ramipril at 218 nm, where Nebivolol HCl exhibited zero absorbance. This approach ensured accurate quantification of both drugs in the presence of each other. The developed method was validated according to standard ICH Q2 (R2) guideline with respect to linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and assay. The results demonstrated good linear relationships, high precision, and acceptable recovery values. The percentage assay obtained from the derivative method was 99.95% for Nebivolol HCl and 99.90% for Ramipril, indicating excellent result. Hence, the proposed UV-spectrophotometric method was simple, economical, and suitable for routine quality control analysis of Nebivolol HCl and Ramipril in synthetic mixture.

INTRODUCTION:
Hypertension is a major public health concern and a leading contributor to cardiovascular morbidity and mortality worldwide. According to the World Health Organization (WHO), elevated blood pressure is one of the most significant modifiable risk factors for cardiovascular diseases such as stroke, myocardial infarction, heart failure, and renal disorders [1]. The increasing prevalence of hypertension, particularly in low- and middle-income countries, has necessitated effective pharmacological interventions, often in the form of combination therapy, to achieve optimal blood pressure control and improve patient outcomes [1]. The Combination of

Nebivolol HCl with Ramipril was studied under clinical trial phase [2] and it was proved that this combination is safe and effective in reducing systolic blood pressure and diastolic blood pressure in hypertensive patients uncontrolled by monotherapy. Hypertensive patient with SBP ranging from ≥140 to ≤179 mmHg and DBP ranging from ≥90 to ≤109 mmHg on treatment, for at least 30 days prior to screening, with Nebivolol HCl 5 mg and Ramipril 5 mg or any other ACE-1 will be screened for eligibility [3]. The synergistic antihypertensive effect of Nebivolol hydrochloride and Ramipril is attributed to combined β_1-adrenergic blockade with nitric oxide–mediated vasodilation and inhibition of the renin angiotensin aldosterone system [2-3]. There is robust evidence on the benefits of beta-blocker and ACE inhibitor use in patients with hypertension and elevated heart rate, CAD, AF, and heart failure. In combination, these two classes provide a comprehensive neuroendocrine blockade targeting both the heart, where beta blockade reduces cardiac output, and the vessels, where ACE inhibition induces vasodilation among other actions [3]. Nebivolol hydrochloride is a third-generation, highly selective β_1-adrenergic receptor blocker with nitric oxide–mediated vasodilatory activity, offering effective blood pressure reduction with improved tolerability. The chemical formula for Nebivolol HCl (Figure 1A) is (1R)-1-[(2R)-6-fluoro-3, 4-dihydro-2H-1 benzopyran-2-yl]-2-[(2s)-6-fluoro-3, 4-dihydro-2H-1-benzopyran-2-yl]-2- hydroxyethyl] amino} ethan-1-ol hydrochloride [4-5]. Ramipril, an angiotensin-converting enzyme (ACE) inhibitor, is widely prescribed for the management of hypertension and cardiovascular risk reduction. The chemical formula for Ramipril (Figure 1B) (2S,3aS,6aS)-1-[(2S)-2-[[(2S)-1-Ethoxy-1-oxo-4-phenylbutan-2-yl] amino] propanoyl]-3,3a,4,5,6,6a- hexahydro-2H-cyclopenta [b] pyrrole-2-carboxylic acid. ACE inhibitors work by blocking the action of a compound in the body called angiotensin converting enzyme (ACE) [5-6].

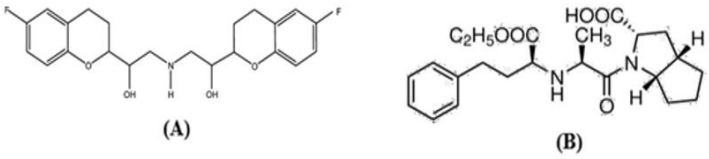

Figure 1: Chemical Structure of (A) Nebivolol HCl (B) Ramipril

The combination of Nebivolol hydrochloride and Ramipril was provided complementary mechanisms of action, resulting in enhanced antihypertensive efficacy, reduced cardiovascular complications, and improved patient compliance. Consequently, such combinations are increasingly utilized in clinical practice. Both drugs are official in Indian Pharmacopoeia which includes Liquid Chromatography [5].

The literature reported numerous analytical methods for the estimation of Nebivolol Hydrochloride and Ramipril, both individually and in combination with other drugs. Various UV spectrophotometric methods have been developed for the determination of Nebivolol Hydrochloride in bulk and pharmaceutical dosage forms [7], as well as for its simultaneous estimation in combination with Hydrochlorothiazide [8]. Reverse-phase high-performance liquid chromatographic (RP-HPLC) methods have also been reported for the analysis of Nebivolol in individual form [9] and in combination with Amlodipine Besylate [10]. Similarly, several analytical methods [11] have been documented. These included UV spectrophotometric techniques such as absorption maxima and area under curve (AUC) methods for the estimation of Ramipril in pharmaceutical dosage forms [12]. Simultaneous estimation of Ramipril with Amlodipine using UV spectrophotometric methods had also been reported [13]. Novel UV spectrophotometric approaches employing simultaneous equation and AUC methods have been developed for the estimation of Ramipril and Hydrochlorothiazide in combined dosage forms [14]. In addition, stability-indicating RP-HPLC methods have been established for the determination of Ramipril in pure and pharmaceutical formulations [15]. Furthermore, a validated stability-indicating RP-UPLC method has been reported for the simultaneous quantitative determination of Metoprolol, Atorvastatin, and Ramipril in capsule dosage forms [16]. Despite this extensive literature work, no any UV spectrophotometric method had been reported for the simultaneous estimation of Nebivolol Hydrochloride and Ramipril in synthetic mixture. Several analytical methods have been reported for the estimation of Nebivolol hydrochloride and Ramipril, either individually or in combination. However, these methods are often associated with higher cost, longer analysis time, and complex sample preparation, which may limit their routine application, particularly in resource-limited settings. UV-spectrophotometric technique offers a simple, rapid, economical, and reproducible alternative for routine pharmaceutical analysis. Among these, first-order derivative UV-spectrophotometry enhances spectral resolution and minimizes interference between overlapping spectra, making it suitable for simultaneous estimation of drugs in synthetic mixture. To address this gap, a first-order derivative UV spectrophotometric method was employed, offering a rapid and economical alternative to chromatographic

techniques [17-18]. This approach avoids expensive columns, large volumes of organic solvents, and extensive sample preparation, while enabling reliable quantification of compounds with overlapping absorption profiles. The objective of the present work therefore undertaken to develop and validate a linear, simple, accurate, precise, and sensitive first-order derivative UV-spectrophotometric method for the simultaneous estimation of Nebivolol hydrochloride and Ramipril in a synthetic mixture. The method was validated in accordance with ICH Q2 (R2) [19] guideline and was intended to be suitable for routine quality control analysis.

2. EXPERIMENTAL MATERIALS AND METHODOLGY:

2.1 Chemicals and reagents

Nebivolol Hydrochloride was obtained as a gift sample from Zydus Pharmaceuticals Ltd., Ahmedabad, India, while Ramipril was kindly supplied by Cadila Pharmaceuticals Ltd., Ahmedabad, India. Methanol (HPLC grade) was procured from Finar Chemicals Pvt. Ltd., India, and used as the solvent throughout the study. All materials employed in the experimental work were of appropriate quality to ensure accuracy, reliability, and reproducibility of the UV-spectrophotometric analysis.

2.2 Instrument & Software

The spectrophotometric measurements were performed using a UV-Visible spectrophotometer (Shimadzu-1900, UV Probe 2.7 version software) with a spectral bandwidth of 1 nm was employed for all spectroscopic measurements, using a pair of 1.0 cm matched quartz cells over the range of 200-400 nm. The Scale-Tec analytical balance was utilized to weigh the samples. Digital Pro^{+} PS-10A Sonicator from broleo was used for sonication purpose.

2.2.1 Analytical Conditions

Methanol used as a Solvent. As detection wavelength, 270 nm was selected for Nebivolol HCl and 210 nm for Ramipril. In accordance with ICH Q2 (R2) [19] requirements, the analytical conditions for a simultaneous technique for the measurement of Nebivolol HCl and Ramipril in UV were optimized and validated.

2.3 Preparation of Solutions

2.3.1 Preparation of standard stock and working standard solutions

Standard stock solutions of Nebivolol HCl and Ramipril were prepared separately by accurately weighing 10 mg of both drugs and dissolving them in 100 mL of water in volumetric flasks to obtain concentration of 100 µg/mL for Nebivolol HCl and Ramipril. The solutions were sonicated for 5 min to ensure complete dissolution. 0.2 ml standard stock of Nebivolol HCl (100 µg/ml) and 0.2 ml standard stock solution of Ramipril (100 µg/ml) were pipetted out into different 10 ml volumetric flask and diluted up to mark with water to get the 2 µg/ml of Nebivolol HCl and 2 µg/ml of Ramipril. Each solution was scanned in the range of 200-400 nm.

2.3.2 Preparation of Calibration curves of Nebivolol HCl (1-5 µg/ml) and Ramipril (1-5 µg/ml)

Aliquots 0.1, 0.2, 0.3, 0.4 and 0.5 ml of solution for both drugs from standard stock solution (100 µg/mL) were pipetted out into ten different 10 ml volumetric flasks and made up to mark with Methanol to obtained 1, 2, 3, 4 and 5 µg/mL of Nebivolol HCl at 218 nm and Ramipril at 237 nm. Absorbance of each solution was measured using Methanol as blank. Graph of Absorbance v/s. Concentration was plotted.

2.4 *Methodology*

Each working standard solution was scanned individually over the wavelength range of 200-400 nm. The zero-order absorption UV spectra were converted to first-order derivative UV spectra. Calibration functions were established by plotting first-order derivative absorbance against corresponding concentrations for each analyte.

2.4.1 Procedure of selection of wavelength

Pipetted out 0.2 ml solution of Nebivolol HCl (100 µg/ml) and 0.2 ml standard stock solution of Ramipril (100 µg/ml) into different 10 ml volumetric flask and diluted up to mark with Methanol to get the 2 µg/ml of Nebivolol and 2 µg/ml of Ramipril. Each solution was scanned in the range of 200-400 nm. The absorption maxima of both drugs in methanol showed slight shifts under the experimental conditions. Nebivolol HCl exhibited an absorption maximum at 226 nm, 265 nm & 272 nm (Figure 2), while Ramipril showed an absorption maximum at 210 nm & 254 nm (Figure 3). All zero-order spectrum (D0) were converted to first derivative spectrum (D1) using delta lambda 4.0 and scaling factor 2. The overlain first derivative spectrums of Nebivolol HCl and Ramipril at different concentration were recorded. The zero-crossing point (ZCP) of

Nebivolol HCl was found to be 218 nm and ZCP Ramipril was found to be 237 nm (Figure 5).

3. METHOD VALIDATION

The developed method was validated with respect to linearity, Precision, limit of detection, limit of quantification, accuracy and, assay in accordance with the ICH Q2 (R2) [19] guideline. All validation parameters [20-22] performed.

3.1 Linearity and Range: (n=6)

Linearity was studied by preparing standard solution at 5 different concentrations. The linearity range for Nebivolol HCl and Ramipril were found to be 1-5 µg/ml and 1-5 µg/ml respectively. Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

3.2 Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, reproducibility (Interday precision), repeatability.

Intraday Precision (n=3): Solutions containing 1, 2, 3 µg/ml of Nebivolol HCl and 1, 2, 3 µg/mL of Ramipril were analyzed three times on the same day and % R. S. D was calculated.
Interday Precision (n=3): Solutions containing 1, 2, 3 µg/ml of Nebivolol HCl and 1, 2, 3 µg/mL of Ramipril were analyzed on three different successive days and % R. S. D was calculated.
Repeatability (n=6): Solutions containing 2 µg/mL of Nebivolol and 2 µg/mL of Ramipril were analyzed for six times and %R.S.D. was calculated. % R.S.D was not more than 2%.

3.3 Limit of Detection (LOD):

Limit of detection can be calculated using following equation as per ICH guidelines.

$$\mathrm{LOD} = 3.3 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve
S = slope of the calibration curve

3.4 Limit of Quantification (LOQ):

Limit of quantification can be calculated using following equation using the standard deviation of the Y-intercept (σ) and the mean slope (S) of the calibration curve according to ICH Q2 (R2) guideline.

$$\mathrm{LOQ} = 10 * \frac{\sigma}{S}$$

3.5 Accuracy (Recovery study) (n=3):

The accuracy of an analytical procedure expressed the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50 %, 100 %, 150 % and the values were measured for Nebivolol HCl (2 µg/ml) and Ramipril (2 µg/ml). This performance was done in triplicate.

3.6 Preparation and Application of Method to Synthetic Mixture

A synthetic mixture equivalent to a 100 mg containing Nebivolol Hydrochloride (5 mg) and Ramipril (5 mg) was prepared to simulate the combined synthetic form in the ratio of 1:1. Accurately weighed quantities of Nebivolol Hydrochloride and Ramipril were mixed with microcrystalline cellulose (53 mg), lactose (20 mg), croscarmellose sodium (5 mg), talc (10 mg), and magnesium stearate (2 mg). All ingredients were thoroughly blended using a mortar and pestle to obtain a homogeneous mixture. This mixture was transferred in 100 ml volumetric flask and allowed to sonicate and made up to mark with Methanol. This solution was filtered through Whatman filter paper. The filtrate was diluted to the mark with Methanol. The mixture produced 50 µg/ml of Nebivolol HCl and 50 µg/ml of Ramipril in combination.

3.6.1 Preparation of sample solution

Accurately 0.4 ml of the above [mixture solution of Nebivolol HCl (50 µg/ml) and Ramipril (50 µg/ml)] was

pipetted out into 10 ml volumetric flask, and the volume was adjusted up to the mark with Methanol. Final concentration of Nebivolol HCl was 2 µg/ml and Ramipril 2 µg/ml was run into UV. The absorbance was measured and calculated its amount using regression equation.

4. RESULTS

4.1 Selection of wavelength for Nebivolol HCl and Ramipril

Nebivolol HCl (2 µg/ml) and Ramipril (2 µg/ml) solutions were scanned between 200-400 nm. The remarkable absorbance of Nebivolol HCl was identified at 226 nm, 265 nm and 272 nm showed in Figure 2 whereas Ramipril at 210 nm and 254 nm showed in Figure 3.

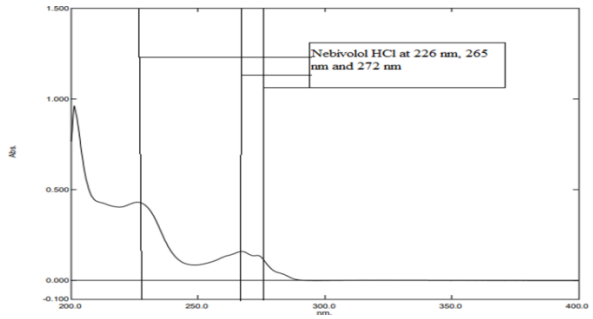

Figure 2: UV Spectrum of Nebivolol HCl at 226 nm, 265 nm and 272 nm

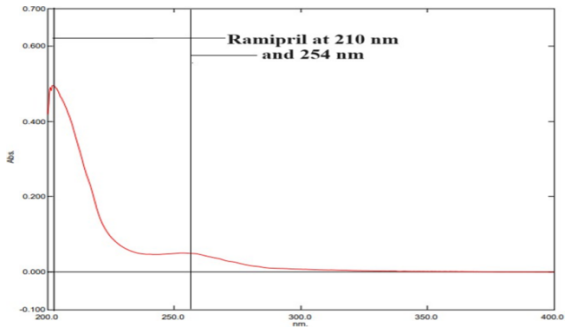

Figure 3: UV Spectrum of Ramipril at 210 nm and 254 nm

For determination of detection wavelength Zero order Overlay UV Spectra of Nebivolol HCl (2 µg/ml) and Ramipril (2 µg/ml) in Methanol showed in Figure 4.

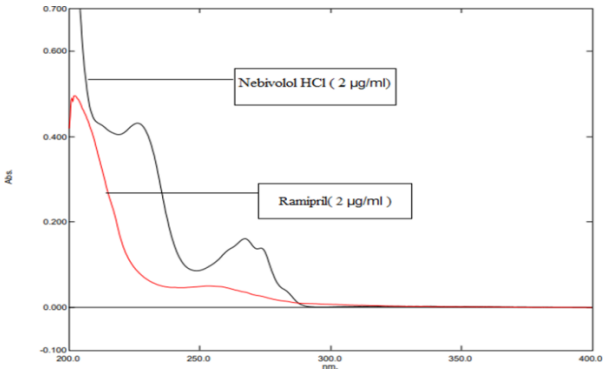

Figure 4: Overlain UV Spectra of Nebivolol HCl (2 µg/ml) And Ramipril (2 µg/ml) In Methanol (Zero Order)

4.1.1 First order derivative UV method development

All zero-order spectrum (D^0) were converted to first derivative spectrum (D^1) using delta lambda 4 and scaling factor 2. The overlain first derivative UV spectrums of Nebivolol HCl and Ramipril at different concentration were recorded. The zero-crossing point (ZCP) of Nebivolol HCl was found to be 218 nm and ZCP of Ramipril was found to be 237 nm.

Overlay First order derivative UV Spectra of Nebivolol HCl (2 µg/ml) and Ramipril (2 µg/ml) in methanol showed in figure 5.

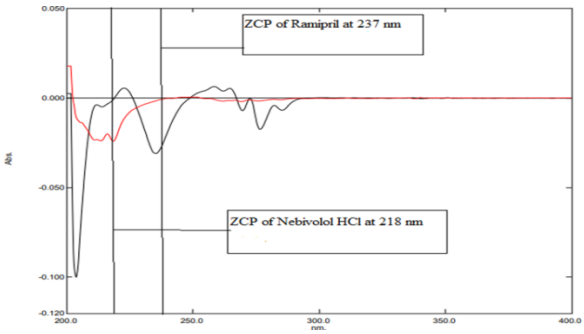

Figure 5: Overlain UV Spectra of Nebivolol HCl (2 µg/ml) And Ramipril (2 µg/ml) in Methanol (First Order)

4.2 METHOD VALIDATION

4.2.1 Linearity and range

The absorbance was measured for Nebivolol HCl (1-5 µg/ml) at 237 nm and for Ramipril (1-5 µg/ml) at 218 nm, using methanol as the blank. The corresponding UV spectra over the linearity range are shown in Figures 6 and 7, respectively.

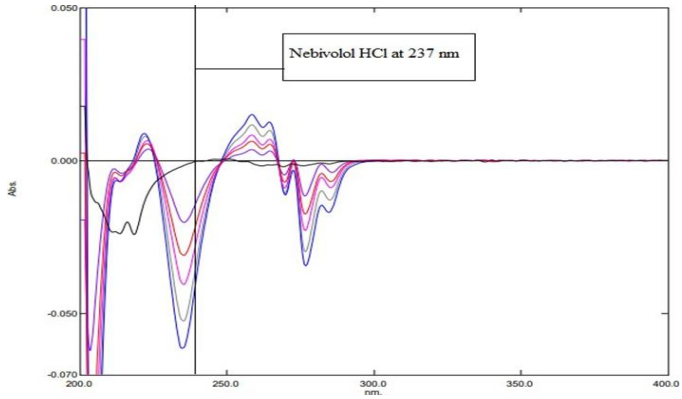

Figure 6: Overlain UV Spectra of Nebivolol HCl (1-5 µg/ml) at 237 nm

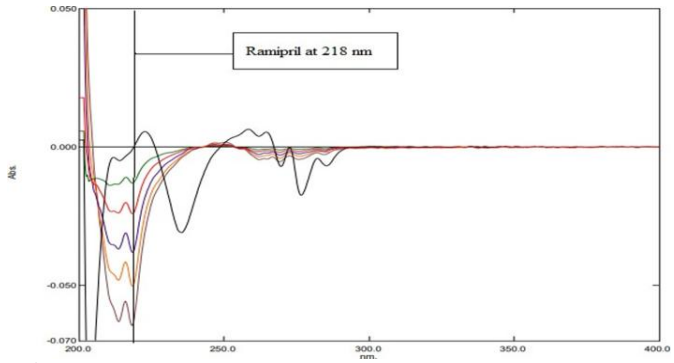

Figure 7: Overlain UV spectra of Ramipril (1-5 µg/ml) at 218 nm

The concentration graph was plotted for concentration and absorbance. For Nebivolol HCl, the calibration curve equation y = 0.0102x + 0.0085, while for Ramipril, it was y = 0.0122x + 0.0014. Results showed that the correlation coefficient (R^2) was between 0.9991 and 0.999 (table 1).

Table 1: Linearity data of Nebivolol HCl and Ramipril

	Nebivolol HCl at 237 nm	**Ramipril at 218 nm**
Linearity Range	1.0 - 5.0 µg/mL	1.0 - 5.0 µg/mL
Regression Equation	y = 0.0098x + 0.0072	y = 0.0017x + 0.0003
Correlation Coefficient	0.999	0.998
LOD	0.10	0.06
LOQ	0.33	0.20

4.2.2 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling. The precision of an analytical method is the closeness of a series of individual analyte measurements applied repeatedly to multiple aliquots of the same sample. It is calculated as a Relative Standard Deviation are homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility and its measurements were conducted at three distinct concentrations 1.0, 2.0 and 3.0 µg/mL for Nebivolol HCl at 237 nm and 1.0, 2.0 and 3.0 µg/mL for Ramipril at 218 nm in triplicate on the same day for intraday, over three different days for interday and 2.0 µg/mL for both drugs were measured for repeatability. The resulting RSD values for Inter-day, Intraday precision were less than 2 and showed in table 2, respectively.

Table 2: Precision study of Nebivolol HCl & Ramipril

Intraday precision					
Conc. (µg/ml)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
Nebivolol HCl	**Ramipril**	**Nebivolol HCl**	**Ramipril**	**Nebivolol HCl**	**Ramipril**
1.0	1.0	|-0.0168| ± 0.00020	|-0.012| ± 0.00014	1.18	1.17
2.0	2.0	|-0.0270| ± 0.00026	|-0.023| ± 0.00023	0.95	1.00
3.0	3.0	|-0.0366| ± 0.00033	|-0.036| ± 0.00035	0.89	0.96
Interday precision					
Conc. (µg/ml)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
Nebivolol HCl	**Ramipril**	**Nebivolol HCl**	**Ramipril**	**Nebivolol HCl**	**Ramipril**
1.0	1.0	|-0.0168| ± 0.00021	|-0.0119|±0.00014	1.22	1.20
2.0	2.0	|-0.0270| ± 0.00026	|-0.0231|±0.00025	0.97	1.10
3.0	3.0	|-0.0366| ± 0.00034	|-0.0366|±0.00037	0.92	1.00
Repeatability					
Conc. (µg/ml)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
Nebivolol HCl	**Ramipril**	**Nebivolol HCl**	**Ramipril**	**Nebivolol HCl**	**Ramipril**
2.0	2.0	|-0.0270| ± 0.00025	|-0.0229|±0.00022	0.92	0.96

4.2.3 LOD and LOQ

The limits of detection (LOD) and quantification (LOQ) were calculated in accordance with ICH Q2 (R2) guideline using the standard deviation of the response and the slope of the calibration curve. The minimum detectable quantity of an analyte within a sample by an analytical method was determined to be 2.0 µg/mL for Nebivolol HCl and 2.0 µg/mL for Ramipril, The quantitation limit for a specific analytical method refers to the minimum quantity of the substance in a sample that can be accurately and precisely measured which was found to be 2.0 µg/mL for Nebivolol HCl and 2.0 µg/mL for Ramipril (Table 1). The limit of detection (LOD) was found to be 0.10 µg/mL for Nebivolol Hydrochloride and 0.06 µg/mL for Ramipril, whereas the limit of quantification (LOQ) was found to be 0.33 µg/mL and 0.20 µg/mL for Nebivolol Hydrochloride and Ramipril, respectively were showed in Table 1. The low LOD and LOQ values obtained at the selected wavelengths indicated the adequate sensitivity of the proposed UV spectrophotometric method for the estimation of both drugs.

4.2.4 Accuracy

To decide the accuracy of the technique recuperation, change into accomplished by means of standard addition approach. To pre-analysed pattern acknowledged quantity of general Nebivolol HCl and Ramipril spiked in extraordinary concentrations. The restoration was executed in three stages 50 %, 100 % and 150 % of fashionable Nebivolol HCl and Ramipril. The results were studied in triplicate and the accuracy changed into indicated by % recovery. Accuracy was carried out by the recovery (standard addition) method and was obtained in range of 99.91%-99.98% for Nebivolol HCl and 98.66%-99.60% for Ramipril. The results of the recovery study for both drugs were showed in Table 3. The mean percentage recovery values for both drugs were found to be within the ICH-accepted range of 98-102%, with low standard deviation. These results confirm the accuracy, trueness, and reliability of the developed method and indicated that excipients present in the synthetic mixture did not interfere with the estimation of either drug.

Table 3: Recovery study

Name of Drug	% Level of recovery	Test Amount (µg/ml)	Amount of drug taken (µg/ml)	Total Std Amt (µg/ml)	Total amount Recovered (µg/ml)	% Mean Recovery ± SD(n=3)
Nebivolol HCl	50	2	1	3	2.99	99.91±0.021
	100	2	2	4	4.00	99.99±0.015
	150	2	3	5	5.00	99.98±0.014
Ramipril	50	2	1	3	2.96	98.66±0.070
	100	2	2	4	3.88	98.22±0.098
	150	2	3	5	4.98	99.60±0.263

4.2.5 Analysis of Synthetic mixture:

The developed UV spectrophotometric method was applied to the analysis of a synthetic mixture containing Nebivolol HCl and Ramipril. Percentage assay of Nebivolol HCl and Ramipril was found to be 99.95% and 99.90%, respectively. The results of the assay were stipulated in Table 4. The mean percentage assay values for both drugs were found to be close to 100%, with low standard deviation and %RSD values less than 2.0%, indicated the assay of both drugs simultaneously was passed by UV method.

Table 4: Analysis of synthetic mixture

Name of Drug	Amount in synthetic mixture (µg/ml)	Mean Amount found (µg/ml)	% Assay ± SD (n=3)	%RSD
Nebivolol HCl	2	1.999	99.95±0.0321	0.033
Ramipril	2	1.998	99.90± 0.0264	0.027

5. DISCUSSION:

The developed and validated first-order derivative UV-spectrophotometric method for the simultaneous estimation of Nebivolol Hydrochloride and Ramipril was validated in accordance with ICH Q2 (R2) guideline. The validation results demonstrate that the method is linear, precise, accurate, and sufficiently sensitive for routine quantitative analysis in a synthetic mixture. Linearity of the method was established over the concentration range of 1-5 µg/mL for both analytes at their respective analytical wavelengths of 237 nm for Nebivolol Hydrochloride and 218 nm for Ramipril. The high correlation coefficients ($R^2 \geq 0.998$) obtained from the calibration curves indicate a strong linear relationship between absorbance and concentration, confirming the suitability of the selected concentration range for quantitative estimation. The consistency of the overlain spectra further supports the absence of spectral interference within the studied range. Precision studies revealed excellent repeatability and intermediate precision of the method. The %RSD values obtained for intraday and interday precision at three concentration levels were below 2% for both drugs, satisfying the acceptance criteria recommended by ICH Q2 (R2) guideline. The observed trend of repeatability < intraday < interday precision confirms the robustness and reproducibility of the analytical procedure under normal laboratory conditions. The sensitivity of the method was confirmed by low LOD and LOQ values for both Nebivolol Hydrochloride and Ramipril. The ability to detect and quantify both drugs at sub-microgram levels highlights the suitability of the method for trace-level analysis and further supports its application in quality control laboratories. Accuracy of the proposed method was demonstrated by recovery studies using the standard addition technique. The mean percentage recovery values for both drugs were within the acceptable range of 98-102%, with low standard deviation, indicating good trueness of the method. These results also confirm that commonly used excipients present in the synthetic mixture did not interfere with the determination of either analyte. Application of the validated method to the analysis of a synthetic mixture yielded assay values close to 100% for both Nebivolol Hydrochloride and Ramipril, with low %RSD values. This confirms the practical applicability of the method for simultaneous estimation of both drugs in combined pharmaceutical formulations. Overall, the proposed UV spectrophotometric method was simple, precise, accurate, and sensitive, making it suitable for routine quality control analysis of Nebivolol Hydrochloride and Ramipril in synthetic mixture.

6. CONCLUSION:

A linear, precise, accurate first order derivative UV spectrophotometric technique was successfully developed and validated for routine measurement of Nebivolol HCl and Ramipril in laboratory-prepared synthetic mixture. The method demonstrated satisfactory linearity, precision, accuracy, and sensitivity in accordance with ICH Q2 (R2) guideline. Low values of %RSD confirmed the precision of the method, while recovery studies established its accuracy and freedom from interference by excipients. The low limits of detection and quantification further indicate the adequate sensitivity of the method for routine analytical applications. Successful application of the method to the analysis of a synthetic mixture with assay values close to 100% confirms its suitability for

simultaneous estimation of both drugs in combined dosage forms. Overall, the proposed method was economical, straightforward, and reproducible, and can be effectively employed for routine quality control analysis of Nebivolol Hydrochloride and Ramipril in a binary mixture.

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CONFLICT OF INTEREST:
The authors declare that there is no conflict of interest.

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