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# DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF NEBIVOLOL HYDROCHLORIDE AND RAMIPRIL

[1] Patel Kavya, [2]Dobariya Nidhi, [3]Dhimmar Arpan, [4]Patel Akshit

Department of Pharmaceutical Quality Assurance Shree Naranjibhai Lalbhai Patel College of Pharmacy Umrakh, Dist-Surat, 394601

1.Dr. Ketan Shah 2. Dr.Ashok Akabari, 3. Mr. Lalit Chaudhari

Shree Naranjibhai Lalbhai Patel College of Pharmacy Umrakh, Dist-Surat, 394601

***Abstract:*** Two simple, specific, precise and accurate spectrophotometric methods have been developed for the simultaneous estimation of Nebivolol Hydrochloride (NEBI) and Ramipril (RAMI) involving first derivative Spectrophotometric method (I) and second derivative UV-Spectrophotometric Method (II). Method (I) is based on measurement of amplitude of first derivative spectrum absorbance at two wavelengths; 293 nm and 245 nm for Nebivolol Hydrochloride and Ramipril respectively. The latter (method II) depends on measurement of amplitude of the second derivative of the ratio spectrum at two wavelengths, 298 nm and 228 nm for Nebivolol Hydrochloride and Ramipril. Beer's law obeyed in concentration range of 10 - 50 µg/ mL and 10- 50 µg/ mL for Nebivolol Hydrochloride and Ramipril respectively for both methods. The proposed methods are recommended for routine analysis since they are rapid, simple and specific. The described UV methods were successfully employed for the analysis of each drug in their combined dosage form.

For method (I), the mean% recoveries were found to be 100.021±1.534 for Nebivolol Hydrochloride and 99.784±1.602 for Ramipril. For method (II), the mean% recoveries were found to be 99.757±1.467 and 99.688±1.503 for Nebivolol Hydrochloride at 298 nm and 228 nm respectively. The validation of methods was carried out utilizing ICH guidelines

**Keywords**: Nebivolol Hydrochloride, Ramipril, first derivative, second derivative, validation, UV Spectrophotometric

## 1. INTRODUCTION

Nebivolol hydrochloride and Ramipril Anti-Hypertensive Drug which is used to treat high blood pressure. An abnormal elevation in the diastolic and/or systolic pressure is known as hypertension. Although it is rarely measured in humans, hypertension is also characterized by a higher mean arterial pressure. When evaluating hypertension in the past, the diastolic number was given special attention. Still, there is a correlation between elevated systolic pressure ("systolic hypertension") and a higher risk of coronary and cerebrovascular disease (e.g., stroke). As a result, we can now see the significance of noting both the systolic and diastolic pressure numbers. The most recent U.S. national guideline. Nebivolol Hydrochloride is chemically known as 2,2'-iminobis [1-(6-fluoro-3,4-dihydro-2H-chromen-2 yl) ethanol] It has a molecular formula of $C_{22}H_{25}F_2NO_4$ with molecular weight 405.4 g/mol. The Nebivolol Hydrochloride category is Beta Blocker. It is soluble in methanol, sparingly soluble in ethanol, and very Slightly soluble in hexane. It has Melting Point between 223.0 to 228.0 °C. it is White crystalline powder and pKa is 8.1.

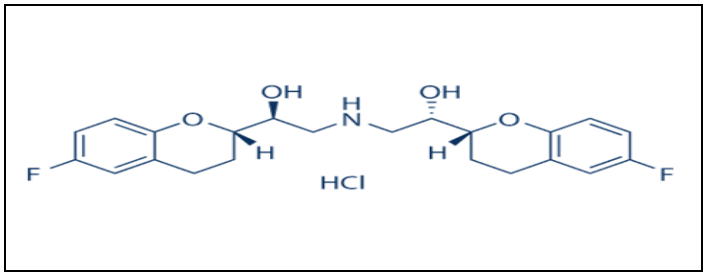


**Fig No. 1 Structure of Nebivolol Hydrochloride**

Ramipril is chemically known as 2-aza-bicyclo [3.3.0]-octane-3-carboxylic acid. It has a molecular formula of $C_{23}H_{32}N_2O$ with molecular weight 416.5 g/mol. The Ramipril category is Angiotensin-converting enzyme (ACE) inhibitors. It is soluble Poorly in water, slightly soluble in methanol and very slightly soluble in ethanol. It has Melting Point between 105°C and 112°C. it is White crystalline substance and pKa is 5.16.

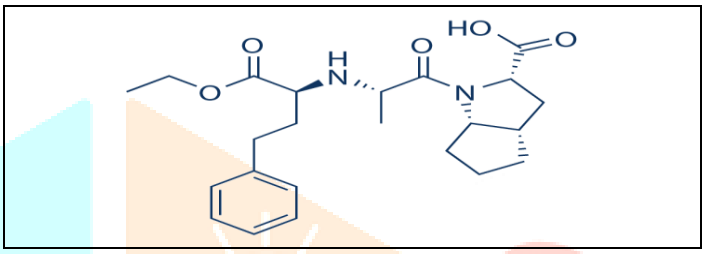


**Fig No.2 Structure of Ramipril**

### 1.1 Introduction of UV Visible Spectroscopy

All atoms and molecules are capable of absorbing energy in accordance with certain restrictions i.e., depending upon structure of substance. Energy may be furnished in the form of electromagnetic radiation ("light"). The kind and amount of radiation absorbed by the molecule depends upon the structure of the molecule and also upon the number of molecules interacting with the radiation. The study of these dependencies is called absorption spectroscopy.

The absorption of electromagnetic radiation of wavelengths between 200 and 800 nm by molecules which have π electrons or atoms possessing unshared electron pairs can be employed for both qualitative and quantitative analysis; as such, it is known as spectrophotometers. As a wide variety of pharmaceutical substances absorb radiation in the near- UV (22-380) and visible (380-800) regions of the electromagnetic spectrum, the technique is widely employed in pharmaceutical analysis.

## 2. Experimental work:

### ➢ Reagent and Materials

Nebivolol hydrochloride API, Ramipril API, Methanol.

### ➢ Preparation of Standard Stock Solution

10 mg each of Nebivolol Hydrochloride and Ramipril were weighed separately and transferred in two different 100 ml volumetric flasks. Both the drugs were dissolved in 50 ml of methanol by vigorous shaking and then volume was made upto the mark with methanol to obtain final concentration of 100 µg/ml of each drug.

### ➢ Selection of Analytical Wavelength

Using appropriate dilutions of the standard stock solution, the solutions were scanned separately in the wavelength region of 400-190 nm. The absorbance spectra, thus obtained were derivatized to remove the interference of absorbing species. The two wavelengths selected should be such that at each wavelength the absorbance difference between the components should be as large as possible. From the examination of the overlay first derivative spectra of Nebivolol Hydrochloride and Ramipril, 293 nm and 245 nm were selected as working wavelengths for the first derivative spectroscopy (Fig.14), as at 293.0 nm Ramipril is exhibited zero absorbance and at 245.0 nm Nebivolol Hydrochloride showed zero absorbance.

- **Determination of Linearity and Selection of Analytical Concentration Range**

For each drug appropriate aliquots were pipetted out from the standard stock solutions (100 µg/ml) of Nebivolol Hydrochloride and Ramipril into series of 10 ml volumetric flasks. The volume was made upto the mark with methanol to get a set of solutions having the concentration 10, 20, 30, 40, 50 µg/ml for Nebivolol Hydrochloride and 10, 20, 30, 40 ,50 µg/ml for Ramipril. The absorbance of each of these solutions were measured at the selected wavelengths (for NEBI at 293.0 nm and for RAMI at245.0 nm) and plotted against concentration. The concentration range over which the drugs obeyed Beer's law was chosen.

- **Limit of detection (LOD) and limit of quantitation (LOQ)**

The LOD and LOQ were separately determined which is based on calibration curve. The standard deviation of y- intercepts of regression lines may be used as standard deviation.

$$LOD=\frac{3.3 \times D}{S}$$

$$LOQ = \frac{10 \times D}{S}$$

Where, D = Standard deviation of the y- intercepts of regression line

S = Slope of the calibration curve

- **Procedure for Precision**

Precision of the method was determined with the synthetic mixture sample. synthetic mixture powder equivalent to 20.0 mg of NEBI and 20.0 mg of RAMI was weighed and transferred to 100 ml volumetric flask and dissolved in 50 ml methanol and the content was kept in ultra sonicator for 20 min. Finally the volume was made up to the mark with methanol. The solution was filtered through Whatman filter paper No.41.

This synthetic mixture powder solution was further diluted with methanol to obtain mixed sample Solutions in Beer Lambert's range for each drug containing 20.0 µg/ml of NEBI and 20.0 µg/ml of RAMI respectively. The concentrations were found as per the synthetic mixture analysis, given above. In intraday precision the sample was analysed six times at different time interval in the same day. Interday precision was obtained by the assay of six sample sets on different days.

- **Procedure for Analysis of Synthetic mixture**

The synthetic powder mixture equivalent to 20.0 mg of NEBI and 20.0 mg of RAMI was weighed and transferred to 100 ml volumetric flask and dissolved in 50 ml methanol and the content was kept in ultra sonicator for 20 min. Finally the volume was made up to the mark with methanol. The solution was filtered through Whatman filter paper No.41.

This synthetic mixture powder solution was further diluted to obtain 20.0 µg/ml of NEBI and 20.0 µg/ml of RAMI. These solutions were scanned in the wavelength range of 400-190 nm. The absorbance spectra was derivatized. Absorbance at 293 nm & 245nm from first derivative spectra was measured. From the standard calibration curves, the concentrations of the corresponding drugs in the sample solutions were determined.

- **Procedure for Recovery Studies**

Recovery studies were carried out by applying the method to determine drug sample present in synthetic mixture to which known amount of NEBI and RAMI corresponding to 80, 100, and 120% of label claim was added (standard addition method). In 80% recovery study amount of standard added is 4.0 mg of NEBI and 4.0 mg of RAMI (ie.. 80% addition). In 100% recovery study the amount of standard added is 5.0 mg of NEBI and 5.0 mg of RAMI (Le., 100% addition). In 120% recovery study the amount of standard added is 6.0 mg of NEBI and 6.0 mg of RAMI (i.e., 120% addition). After the addition, the contents were mixed. The mixed powder equivalent to 5.0 mg of NEBI and 5.0 mg of RAMI was weighed and transferred to 100 ml volumetric flask and dissolved in 50 ml methanol and the content was kept in ultrasonicator for 20 min. Finally the volume was made upto the mark with methanol. The solution was filtered through Whatman filter paper No.41.

The concentrations were determined as per the procedure given for the synthetic mixture. At each level, three determinations were performed and results obtained were compared with expected results.

## 3. Result and Discussion

### 3.1 Normal UV Spectra of Nebivolol Hydrochloride and Ramipril

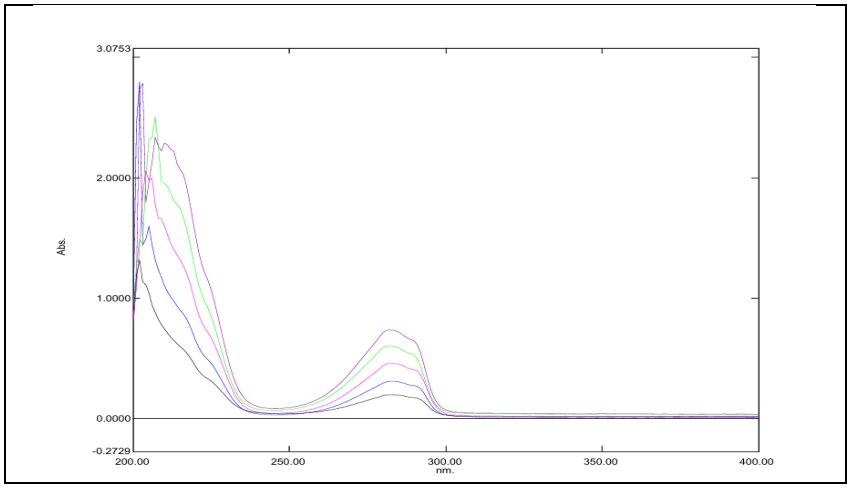


**Figure 3 Normal UV Overlay Spectra of Nebivolol Hydrochloride**

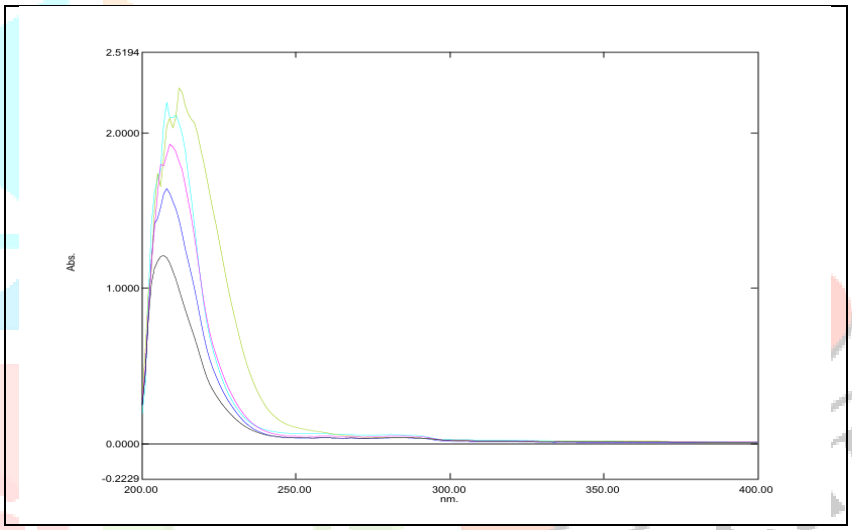


**Figure 4 Normal UV Overlay Spectra of Ramipril**

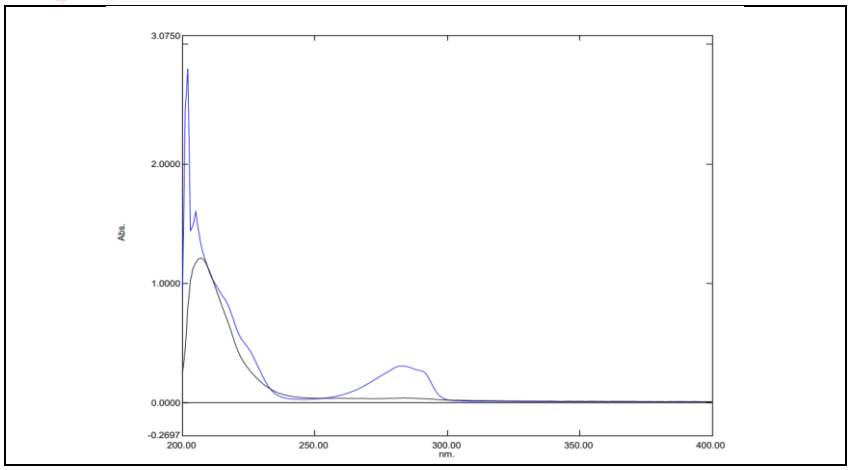


**Figure 5 Normal Overlay UV Spectra of Nebivolol Hydrochloride and Ramipril**

### 3.2 Simultaneous Spectrophotometric Determination of Nebivolol Hydrochloride and Ramipril by First Derivative Spectroscopy.

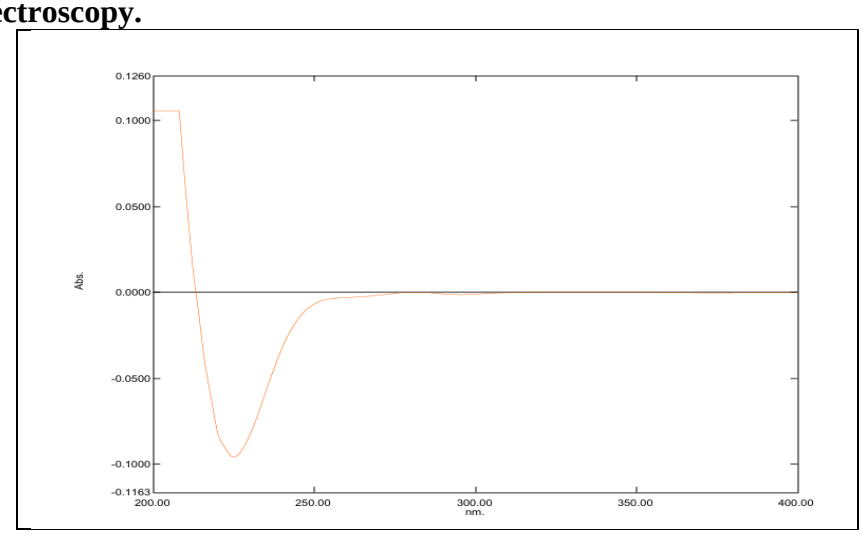


**Figure 6 1st Derivative Spectra of Ramipril**

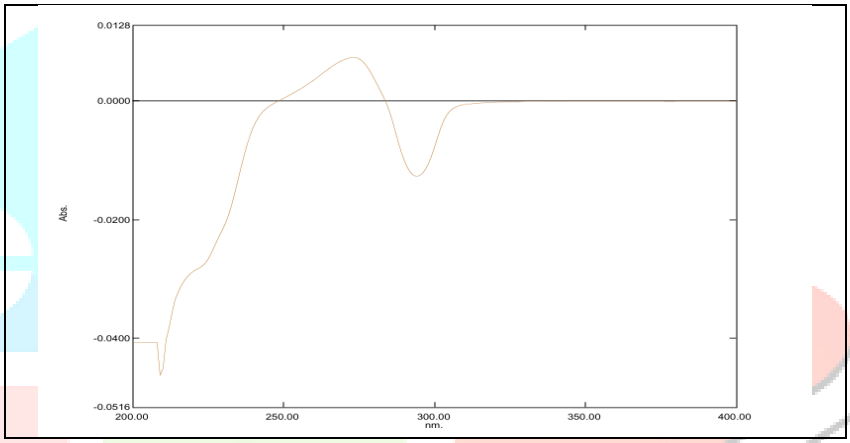


**Figure 7 1st Derivative Spectra of Nebivolol Hydrochloride**

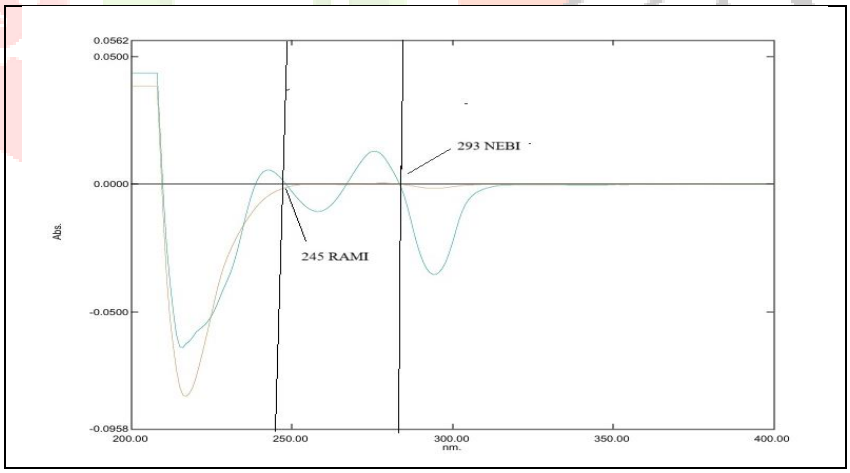


**Figure 1 First Order Derivative Overlay Spectra of Nebivolol Hydrochloride and Ramipril**

From the overlay spectra of two drug in methanol (fig. 8) the working wavelengths selected for first derivative spectroscopy method were 293nm for Nebivolol Hydrochloride and 245nm for Ramipril as at 293nm Ramipril showed zero absorbance and at 245nm Nebivolol Hydrochloride showed zero absorbance.

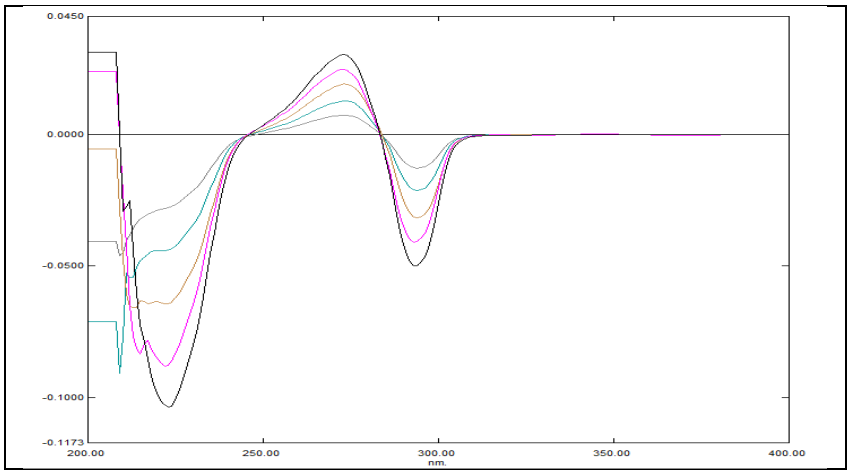


**Figure 9 First Order Derivative Spectrum of Nebivolol Hydrochloride**

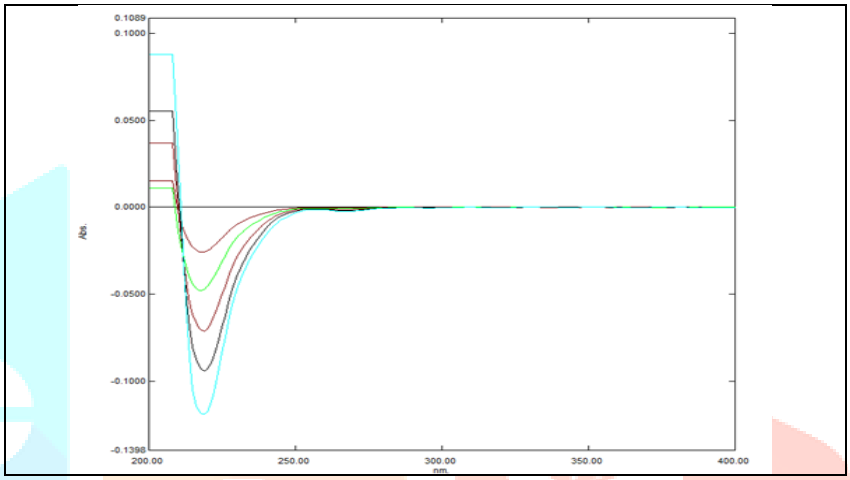


**Figure 10 Frist Order Derivative Spectrum of Ramipril**

### 3.2.1 Selectin of Analytical Concentration Range

**Table 1 Calibration Table for Nebivolol Hydrochloride and Ramipril**

| Sr. No. | For Nebivolol Hydrochloride | | For Ramipril | |
|---|---|---|---|---|
| | Concentration (µg/ ml) | Absorbance at 293nm ($\lambda_1$) ± SD | Concentration (µg/ ml) | Absorbance at 245nm ($\lambda_2$) ± SD |
| 1 | 10 | $-0.01248 \pm 3.4303\times10^{-05}$ | 10 | $-0.00125 \pm 2.429\times10^{-05}$ |
| 2 | 20 | $-0.02123 \pm 1.0328\times10^{-05}$ | 20 | $-0.00251 \pm 4.1352\times10^{-05}$ |
| 3 | 30 | $-0.03023 \pm 2.1602\times10^{-05}$ | 30 | $-0.00384 \pm 3.266\times10^{-05}$ |
| 4 | 40 | $-0.04092 \pm 1.5055\times10^{-05}$ | 40 | $-0.00538 \pm 3.2042\times10^{-05}$ |
| 5 | 50 | $-0.04935 \pm 1.4142\times10^{-05}$ | 50 | $-0.00670 \pm 2.7386\times10^{-05}$ |

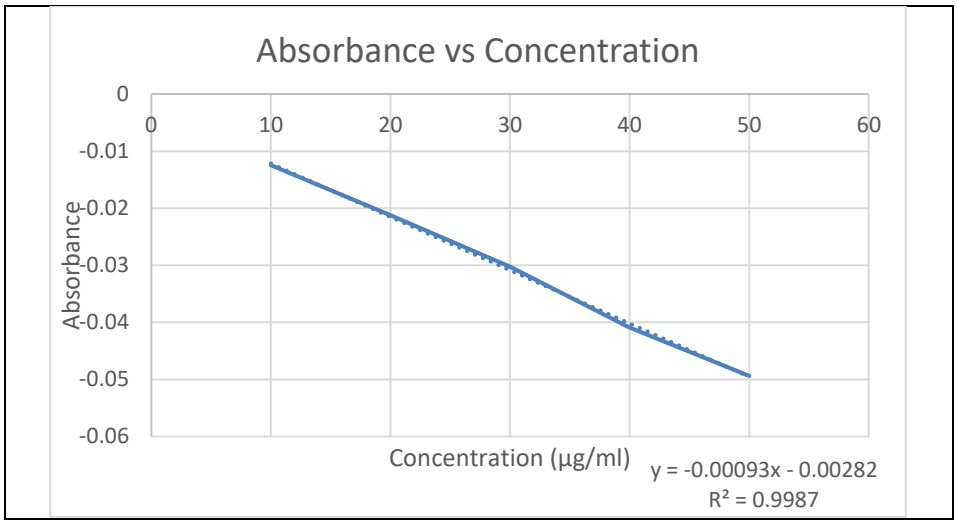


**Figure 11 First Derivative Calibration Curve for Nebivolol Hydrochloride at 293nm**

The calibration curve of Nebivolol Hydrochloride plotted at 293nm, shows that the linearity was observed in the range of 10 µg/ml to 50µg/ml. The working curve equation was found to be y =-0.00093x-0.00282 with a correlation coefficient ($R^2$) value of 0.9987 for Nebivolol Hydrochloride.

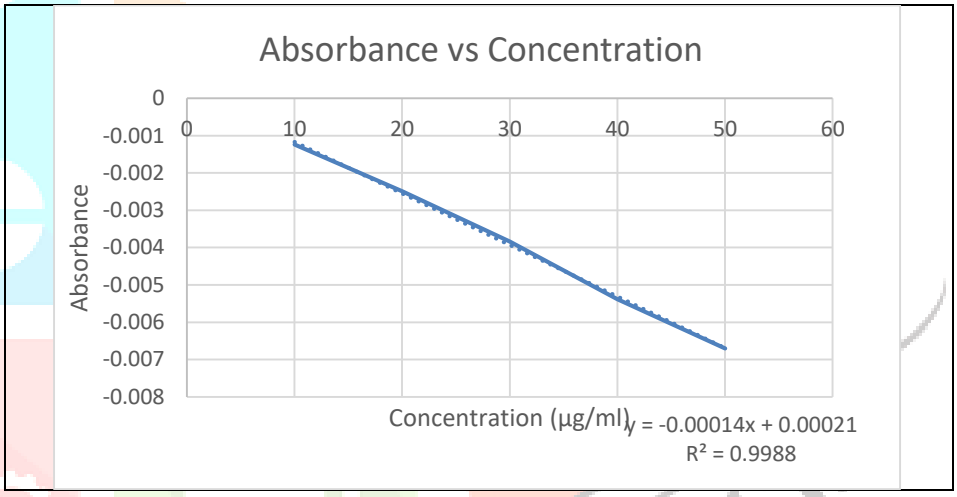


**Figure 12 First Derivative Calibration Curve for Ramipril at 245nm**

The calibration curve of Ramipril plotted at 245nm, shows that the linearity was observed in the range of 10 µg/ml to 50µg/ml. The working curve equation was found to be y =-0.00014x+0.00021 with a correlation coefficient ($R^2$) value of 0.9988 for Ramipril.

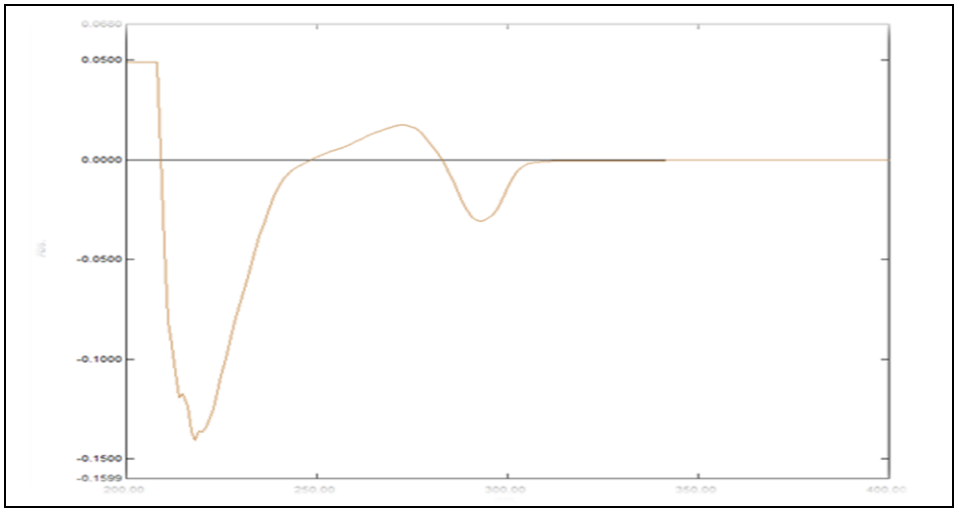


**Figure 13 First Order Derivative Spectra of Mixture**

### 3.2.2 Limit of Detection and Limit of Quantitation Using First Derivative method

**Table 2 Data for LOD and LOQ of Nebivolol hydrochloride and Ramipril**

| Drug | LOD (µg/ml) | LOQ(µg/ml) |
|---|---|---|
| **NEBI** | 0.031 | 0.091 |
| **RAMI** | 0.647 | 1.961 |

### 3.2.3 Result for Precision

**Table 3 Intra-day Precision**

| Sr. No. | Label Claim (mg/100 mg synthetic mixture (equivalent to one tablet) | | Amount Found | | % of Label Claim | |
|---|---|---|---|---|---|---|
| | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI |
| 1 | 5 | 5 | 5.012 | 4.998 | 100.24 | 99.96 |
| 2 | 5 | 5 | 4.920 | 5.089 | 98.4 | 101.78 |
| 3 | 5 | 5 | 4.856 | 5.110 | 97.12 | 102.2 |
| 4 | 5 | 5 | 5.047 | 5.134 | 100.94 | 102.68 |
| 5 | 5 | 5 | 5.089 | 4.987 | 101.78 | 99.74 |
| 6 | 5 | 5 | 4.978 | 5.118 | 99.56 | 102.36 |

**Table 4 Statistical Validation for Intra-day Precision**

| Drug | Mean | Standard Deviation | Co- efficient of Variation (%R.S.D.) | Standard Error |
|---|---|---|---|---|
| **NEBI** | 99.67 | 1.704 | 1.70 | 0.6956 |
| **RAMI** | 101.45 | 1.277 | 1.25 | 0.5214 |

**Table 5 Inter-day Precision**

| Sr. No. | Label Claim (mg/100 mg synthetic mixture (equivalent to one tablet) | | Amount Found (mg) | | % of Label Claim | |
|---|---|---|---|---|---|---|
| | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI |
| 1 | 5 | 5 | 5.112 | 4.998 | 102.24 | 99.96 |
| 2 | 5 | 5 | 4.980 | 5.034 | 99.6 | 100.68 |
| 3 | 5 | 5 | 4.966 | 5.110 | 99.32 | 102.2 |
| 4 | 5 | 5 | 5.112 | 5.124 | 102.24 | 102.48 |
| 5 | 5 | 5 | 5.189 | 4.997 | 103.78 | 99.94 |
| 6 | 5 | 5 | 4.978 | 5.128 | 99.56 | 102.56 |

**Table 6 Statistical Validation for Inter-day Precision**

| Drug | Mean | Standard Deviation | Co- efficient of Variation (%R.S.D.) | Standard Error |
|---|---|---|---|---|
| **NEBI** | 101.12 | 1.874 | 1.85 | 0.7653 |
| **RAMI** | 101.30 | 1.251 | 1.23 | 0.5105 |

### 3.2.4 Result for Analysis of Synthetic Mixture

**Table 7 Data for Synthetic Mixture**

| Sr. No. | Amount of present in (µg/ ml) | | Absorbance | | Amount found in (µg/ ml) | | Amount found in % | |
|---|---|---|---|---|---|---|---|---|
| | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI |
| 1 | 20 | 20 | -0.02114 | -0.00254 | 19.347 | 19.542 | 96.73 | 97.71 |
| 2 | 20 | 20 | -0.02126 | -0.00256 | 20.021 | 19.963 | 100.10 | 99.81 |
| 3 | 20 | 20 | -0.02138 | -0.00259 | 19.956 | 20.231 | 99.78 | 101.15 |
| 4 | 20 | 20 | -0.02148 | -0.00261 | 19.873 | 20.256 | 99.36 | 101.28 |
| 5 | 20 | 20 | -0.02156 | -0.00630 | 20.143 | 20.214 | 100.71 | 101.07 |
| 6 | 20 | 20 | -0.02169 | -0.00650 | 20.183 | 19.867 | 100.91 | 99.33 |

**Table 8 Statistical Validation for Synthetic Mixture**

| Drug | Mean (%) | Standard Deviation | Co- efficient of Variation (%R.S.D.) | Standard Error |
|---|---|---|---|---|
| **NEBI** | 99.59 | 1.518 | 1.52 | 0.6198 |
| **RAMI** | 100.05 | 1.401 | 1.40 | 0.5720 |

- The %R.S.D. is less than 2% as required by USP and ICH guidelines

### 3.2.5. Result for Recovery Studies.

**Table 9 recovery studies of NEBI**

| Sr .no | Concentration level(%)Recovery | Amt. present in formulation (mg) | Amt. present in formulation (µg/ml) | Absorbance of formulation sample (a) | Amount of drug spiked(mg) | Total absorbance of recovery sample(b) | Absorbance of Spiked drug (c=b-a) | Amount of drug recoverd( µg /ml) | Amount Recoverd (mg) | %Recovery |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 80 | 5 | 20 | -0.02142 | 4 | -0.03889 | -0.01747 | 15.75 | 3.93 | 98.43 |
| 2 | 80 | 5 | 20 | -0.02142 | 4 | -0.03911 | -0.01769 | 15.98 | 3.99 | 99.87 |
| 3 | 80 | 5 | 20 | -0.02142 | 4 | -0.03934 | -0.01792 | 16.23 | 4.05 | 101.43 |
| 4 | 100 | 5 | 20 | -0.02142 | 5 | -0.04256 | -0.02114 | 19.69 | 4.92 | 98.45 |
| 5 | 100 | 5 | 20 | -0.02142 | 5 | -0.04280 | -0.02138 | 19.95 | 4.98 | 99.75 |
| 6 | 100 | 5 | 20 | -0.02142 | 5 | -0.04311 | -0.02169 | 20.29 | 5.07 | 101.45 |
| 7 | 120 | 5 | 20 | -0.02142 | 6 | -0.04622 | -0.02480 | 23.63 | 5.90 | 98.45 |
| 8 | 120 | 5 | 20 | -0.02142 | 6 | -0.04677 | -0.02535 | 24.22 | 6.05 | 100.91 |
| 9 | 120 | 5 | 20 | -0.02142 | 6 | -0.04689 | -0.02547 | 24.35 | 6.08 | 101.45 |

**Table 10 recovery study of RAMI**

| Sr .no | Concentration level(%)Recovery | Amt. present in formulation (mg) | Amt. present in formulation | Absorbance of formulation sample (a) | Amount of drug spiked(mg) | Total absorbance of recovery sample(b) | Absorbance of Spiked drug (c=b-a) | Amount of drug recoverd( µg /ml) | Amount Recoverd (mg) | %Recovery |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 80 | 5 | 20 | -0.00385 | 4 | -0.00584 | -0.00199 | 15.71 | 3.92 | 98.18 |
| 2 | 80 | 5 | 20 | -0.00385 | 4 | -0.00588 | -0.00203 | 16.01 | 4.00 | 100.06 |
| 3 | 80 | 5 | 20 | -0.00385 | 4 | -0.00639 | -0.00206 | 16.21 | 4.05 | 101.31 |
| 4 | 100 | 5 | 20 | -0.00385 | 5 | -0.00639 | -0.00254 | 19.64 | 4.91 | 98.20 |
| 5 | 100 | 5 | 20 | -0.00385 | 5 | -0.00643 | -0.00258 | 19.92 | 4.98 | 99.60 |
| 6 | 100 | 5 | 20 | -0.00385 | 5 | -0.00648 | -0.00263 | 20.28 | 5.07 | 101.40 |
| 7 | 120 | 5 | 20 | -0.00385 | 6 | -0.00694 | -0.00309 | 23.57 | 5.89 | 98.20 |
| 8 | 120 | 5 | 20 | -0.00385 | 6 | -0.00699 | -0.00314 | 23.92 | 5.97 | 99.66 |
| 9 | 120 | 5 | 20 | -0.00385 | 6 | -0.00705 | -0.00320 | 24.35 | 6.08 | 101.45 |

**Table 11 Statistical Validation for Recovery data**

| level Of % Recovery | % Mean Recovery | | Standard derivation | | Co- efficient of Variation (%R.S.D.) | | Standard Error | |
|---|---|---|---|---|---|---|---|---|
| | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI |
| 80 | 99.910 | 99.850 | 1.500 | 1.576 | 1.507 | 1.578 | 0.866 | 0.909 |
| 100 | 99.883 | 99.733 | 1.504 | 1.604 | 1.505 | 1.608 | 0.868 | 0.926 |
| 120 | 100.270 | 99.770 | 1.599 | 1.628 | 1.594 | 1.631 | 0.923 | 0.939 |

### 3.3 Simultaneous Spectrophotometric Determination of Nebivolol Hydrochloride and Ramipril by Second Order Derivative Spectroscopy.

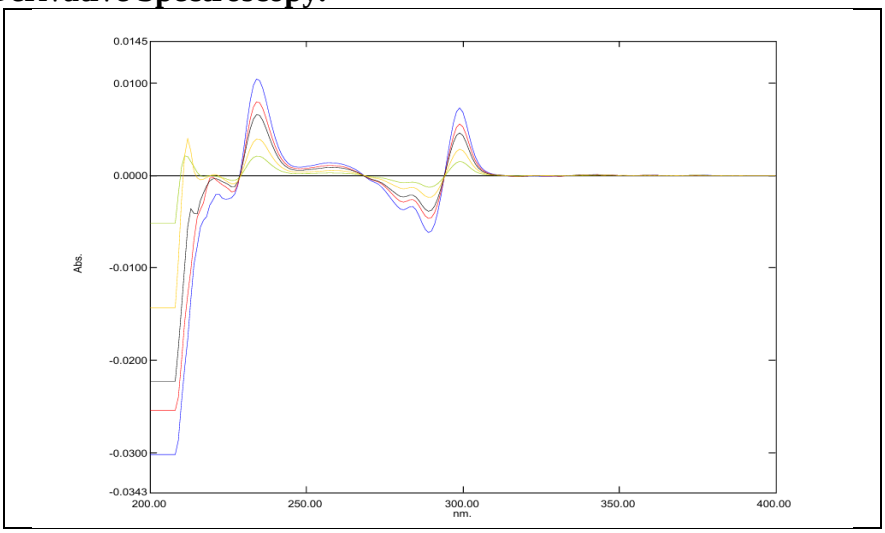


**Figure 14 Second Order Derivative Spectra of Nebivolol Hydrochloride**

From the overlay spectra of two drug in methanol (fig. 14) the working wavelengths selected for second derivative spectroscopy method were 298nm for Nebivolol Hydrochloride and 228nm for Ramipril as at 298nm Ramipril showed zero absorbance and at 228nm Nebivolol Hydrochloride showed zero absorbance.

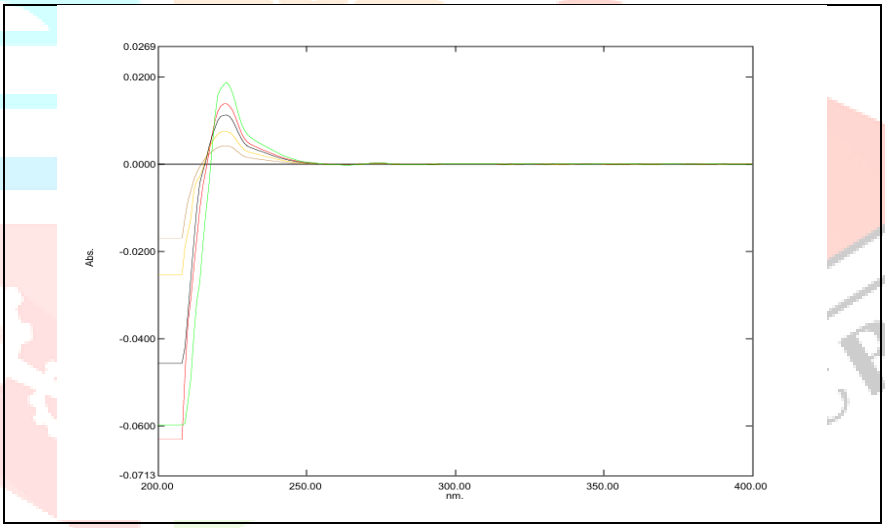


**Figure 15 Second Order Derivative Spectrum of Ramipril**

Second derivative spectrum of Nebivolol Hydrochloride in methanol is shown in fig. 15.

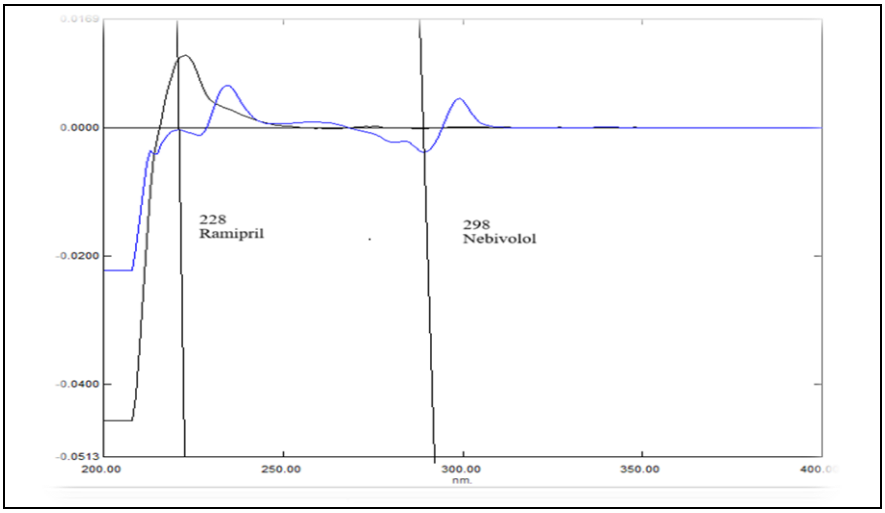


**Figure 16 Second Order Derivative Overlay Spectrum of Nebivolol Hydrochloride and Ramipril**

### 3.3.1 Selectin of Analytical Concentration Range

**Table 12 Calibration Table for Nebivolol Hydrochloride and Ramipril**

| Sr. No. | For Nebivolol Hydrochloride | | For Ramipril | |
|---|---|---|---|---|
| | Concentration (µg/ ml) | Absorbance at 298nm ($\lambda_1$) ± SD | Concentration (µg/ ml) | Absorbance at 228nm ($\lambda_2$) ± SD |
| 1 | 10 | 0.00137 ± 3.74166×10$^{-05}$ | 10 | 0.00230 ± 6.31664×10$^{-05}$ |
| 2 | 20 | 0.00235 ± 5.91326×10$^{-05}$ | 20 | 0.00381 ± 7.37564×10$^{-05}$ |
| 3 | 30 | 0.003827 ± 4.76095×10$^{-05}$ | 30 | 0.00575 ± 0.0001304×10$^{-05}$ |
| 4 | 40 | 0.00510 ± 9.71587×10$^{-05}$ | 40 | 0.00774 ± 5.1672×10$^{-05}$ |
| 5 | 50 | 0.00673± 5.49242×10$^{-05}$ | 50 | 0.00968± 2.48328×10$^{-05}$ |

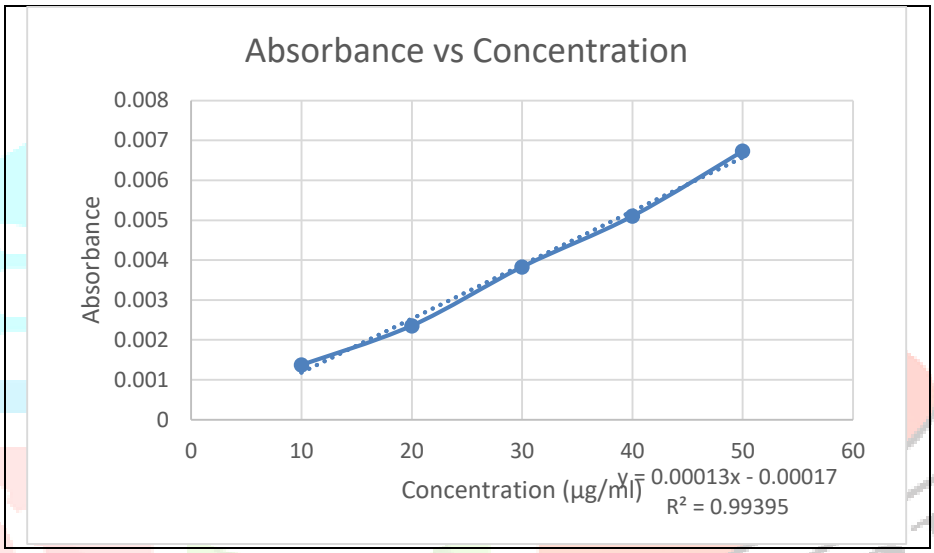


**Figure 17 Second Derivative Calibration Curve for Nebivolol Hydrochloride at 298nm**

The calibration curve of Nebivolol Hydrochloride plotted at 298nm, shows that the linearity was observed in the range of 10 µg/ml to 50µg/ml. The working curve equation was found to be y =0.00013x-0.00017 with a correlation coefficient ($R^2$) value of 0.9939for Nebivolol Hydrochloride.

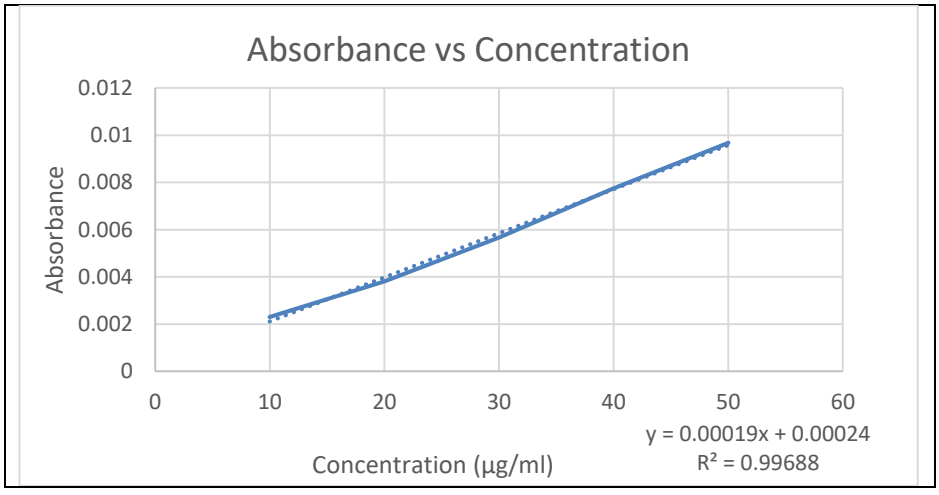


**Figure 18 Second Derivative Calibration Curve for Ramipril at 228nm**

The calibration curve of Ramipril plotted at 228nm, shows that the linearity was observed in the range of 10 µg/ml to 50µg/ml. The working curve equation was found to be y =0.00019x + 0.000024 with a correlation coefficient ($R^2$) value of 0.9968 for Ramipril.

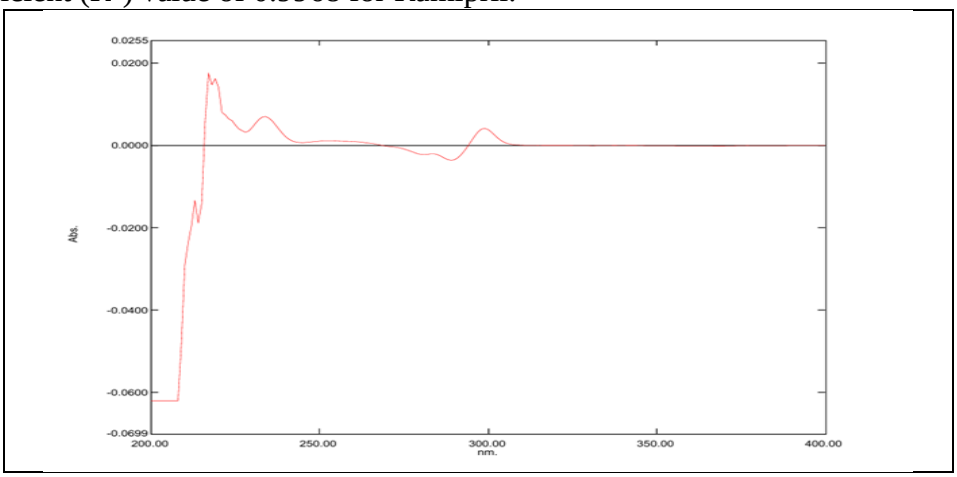


**Figure 19 Second Order Derivative Spectrum of Mixture**

### 3.3.2. Limit of Detection and Limit of Quantitation Using Second Derivative method

**Table 13 Data for LOD and LOQ of Nebivolol hydrochloride and Ramipril**

| Drug | LOD(µg/ml) | LOQ(µg/ml) |
|---|---|---|
| **NEBI** | 1.397 | 4.234 |
| **RAMI** | 0.656 | 1.987 |

### 3.3.3 Result for Precision

**Table 14 Intra-day Precision**

| Sr. No. | Label Claim (mg/100 mg synthetic mixture (equivalent to one tablet) | | Amount Found | | % of Label Claim | |
|---|---|---|---|---|---|---|
| | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI |
| 1 | 5 | 5 | 4.954 | 5.089 | 99.08 | 101.78 |
| 2 | 5 | 5 | 4.898 | 5.110 | 97.96 | 102.2 |
| 3 | 5 | 5 | 5.011 | 4.992 | 100.22 | 99.84 |
| 4 | 5 | 5 | 5.013 | 5.034 | 100.26 | 100.68 |
| 5 | 5 | 5 | 5.089 | 4.982 | 101.78 | 99.64 |
| 6 | 5 | 5 | 4.988 | 5.108 | 99.76 | 102.16 |

**Table 15 Statistical Validation for Intra-day Precision**

| Drug | Mean | Standard Deviation | Co- efficient of Variation (%R.S.D.) | Standard Error |
|---|---|---|---|---|
| **NEBI** | 99.86 | 1.280 | 1.28 | 0.5226 |
| **RAMI** | 101.05 | 1.156 | 1.14 | 0.4718 |

**Table 16 Inter-day Precision**

| Sr. No. | Label Claim (mg/100 mg synthetic mixture (equivalent to one tablet) | | Amount Found | | % of Label Claim | |
|---|---|---|---|---|---|---|
| | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI |
| 1 | 5 | 5 | 5.104 | 4.989 | 102.08 | 99.78 |
| 2 | 5 | 5 | 4.969 | 5.112 | 99.38 | 102.24 |
| 3 | 5 | 5 | 4.980 | 5.106 | 99.6 | 102.12 |
| 4 | 5 | 5 | 5.011 | 5.132 | 100.22 | 102.64 |
| 5 | 5 | 5 | 5.089 | 4.975 | 101.78 | 99.5 |
| 6 | 5 | 5 | 4.978 | 5.022 | 99.56 | 100.44 |

**Table 17 Statistical Validation for Inter-day Precision**

| Drug | Mean | Standard Deviation | Co- efficient of Variation (%R.S.D.) | Standard Error |
|---|---|---|---|---|
| **NEBI** | 100.4366 | 1.195 | 1.118 | 0.4878 |
| **RAMI** | 101.12 | 1.375 | 1.35 | 0.5612 |

### 3.3.4 Result for Analysis of Synthetic Mixture

**Table 18 Data for Synthetic Mixture**

| Sr. No. | Amount of present in (µg/ ml) | | Absorbance | | Amount found in (µg/ ml) | | Amount found in % | |
|---|---|---|---|---|---|---|---|---|
| | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI |
| 1 | 20 | 20 | 0.00389 | 0.00255 | 19.887 | 19.942 | 99.43 | 99.71 |
| 2 | 20 | 20 | 0.00391 | 0.00257 | 20.021 | 19.960 | 100.10 | 99.8 |
| 3 | 20 | 20 | 0.00393 | 0.00259 | 19.976 | 20.131 | 99.88 | 100.65 |
| 4 | 20 | 20 | 0.00395 | 0.00261 | 20.273 | 20.056 | 101.36 | 100.12 |
| 5 | 20 | 20 | 0.00397 | 0.00263 | 20.103 | 20.114 | 100.51 | 100.57 |
| 6 | 20 | 20 | 0.00400 | 0.00267 | 20.083 | 19.964 | 100.41 | 99.82 |

**Table 19 Statistical Validation for Synthetic Mixture**

| Drug | Mean (%) | Standard Deviation | Co- efficient of Variation (%R.S.D.) | Standard Error |
|---|---|---|---|---|
| **NEBI** | 100.286 | 0.6559 | 0.654 | 0.2678 |
| **RAMI** | 100.116 | 0.4107 | 0.410 | 0.1677 |

The %R.S.D. is less than 2% as required by USP and ICH guidelines

### 3.3.5. Result for Recovery Studies.

**Table 20 Recovery studies for NEBI**

| Sr .no | Concentration level(%)Recovery | Amt. present in formulation (mg) | Amt. present in formulation (µg/ml) | Absorbance of formulation sample (a) | Amount of drug spiked(mg) | Total absorbance of recovery sample(b) | Absorbance of Spiked drug (c=b-a) | Amount of drug recoverd( µg /ml) | Amount Recoverd (mg) | %Recovery |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 80 | 5 | 20 | 0.00394 | 4 | 0.00708 | 0.00314 | 15.73 | 3.93 | 98.31 |
| 2 | 80 | 5 | 20 | 0.00394 | 4 | 0.00711 | 0.00317 | 15.89 | 3.97 | 99.31 |
| 3 | 80 | 5 | 20 | 0.00394 | 4 | 0.00717 | 0.00323 | 16.21 | 4.05 | 101.31 |
| 4 | 100 | 5 | 20 | 0.00394 | 5 | 0.00783 | 0.00389 | 19.68 | 4.92 | 98.40 |
| 5 | 100 | 5 | 20 | 0.00394 | 5 | 0.00788 | 0.00394 | 19.94 | 4.98 | 99.70 |
| 6 | 100 | 5 | 20 | 0.00394 | 5 | 0.00794 | 0.00400 | 20.26 | 5.06 | 101.30 |
| 7 | 120 | 5 | 20 | 0.00394 | 6 | 0.00858 | 0.00464 | 23.63 | 5.90 | 98.45 |
| 8 | 120 | 5 | 20 | 0.00394 | 6 | 0.00864 | 0.00470 | 23.94 | 5.98 | 99.75 |
| 9 | 120 | 5 | 20 | 0.00394 | 6 | 0.00871 | 0.00477 | 24.31 | 6.07 | 101.29 |

**Table 22 Recovery Study Of RAMI**

| Sr .no | Concentration level(%)Recovery | Amt. present in formulation (mg) | Amt. present in formulation (µg/ml) | Absorbance of formulation sample (a) | Amount of drug spiked(mg) | Total absorbance of recovery sample(b) | Absorbance of Spiked drug (c=b-a) | Amount of drug recoverd( µg /ml) | Amount Recoverd (mg) | %Recovery |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 80 | 5 | 20 | 0.00260 | 4 | 0.00463 | 0.00203 | 15.69 | 3.92 | 98.06 |
| 2 | 80 | 5 | 20 | 0.00260 | 4 | 0.00466 | 0.00206 | 15.93 | 3.98 | 99.56 |
| 3 | 80 | 5 | 20 | 0.00260 | 4 | 0.00470 | 0.00210 | 16.23 | 4.05 | 101.43 |
| 4 | 100 | 5 | 20 | 0.00260 | 5 | 0.00515 | 0.00255 | 19.69 | 4.92 | 98.45 |
| 5 | 100 | 5 | 20 | 0.00260 | 5 | 0.00518 | 0.00258 | 19.92 | 4.98 | 99.60 |
| 6 | 100 | 5 | 20 | 0.00260 | 5 | 0.00522 | 0.00262 | 20.23 | 5.05 | 101.15 |
| 7 | 120 | 5 | 20 | 0.00260 | 6 | 0.00566 | 0.00306 | 23.61 | 5.90 | 98.37 |
| 8 | 120 | 5 | 20 | 0.00260 | 6 | 0.00569 | 0.00309 | 23.84 | 5.95 | 99.33 |
| 9 | 120 | 5 | 20 | 0.00260 | 6 | 0.00575 | 0.00315 | 24.30 | 6.07 | 101.25 |

**Table 22 Statistical Validation for Recovery data**

| level Of % Recovery | % Mean Recovery | | Standard derivation | | Co- efficient of Variation (%R.S.D.) | | Standard Error | |
|---|---|---|---|---|---|---|---|---|
| | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI | NEBI | RAMI |
| 80 | 99.643 | 99.683 | 1.528 | 1.688 | 1.533 | 1.693 | 0.881 | 0.974 |
| 100 | 99.800 | 99.733 | 1.453 | 1.355 | 1.455 | 1.358 | 0.838 | 0.782 |
| 120 | 99.830 | 99.650 | 1.422 | 1.466 | 1.424 | 1.471 | 0.820 | 0.846 |

## 4. Summary and Conclusion

Due to the complementary effects of β-blockers and ACE inhibitors on the interlinked pathways of the sympathetic nervous system and the renin–angiotensin–aldosterone system, both of which significantly impact cardiovascular risk and disease outcomes, the combined use of these agents is supported by a robust pharmacological and clinical rationale. Nebivolol Hydrochloride and Ramiprl in combination are available in tablet dosage form containing 5 mg and 5 mg drug respectively. The present work dealt with simultaneous estimation of Nebivolol Hydrochloride and Ramipril by different UV spectrophotometric method**.**

For first derivative method the working wavelength selected for Nebivolol Hydrochloride (293nm) and Ramipril (245nm), were apart enough to be analysed efficiently and were found to be stable in solvent methanol. The working wavelengths selected for first derivative spectroscopy method were 293nm for Nebivolol hydrochloride and 245nm for Ramipril, as at 293nm Ramipril showed zero absorbance and at 245 nm Nebivolol Hydrochloride showed zero absorbances.

For second derivative method the working wavelength selected for Nebivolol Hydrochloride (298nm) and Ramipril (228nm), were apart enough to be analysed efficiently and were found to be stable in solvent methanol. The working wavelengths selected for second derivative spectroscopy method were 298nm for Nebivolol hydrochloride and 228nm for Ramipril, as at 298 nm Ramipril showed zero absorbance and at 228 nm Nebivolol Hydrochloride showed zero absorbances.

**Table 23 Result Data for 1st Derivatives**

| Parameters | NEBI | RAMI |
|---|---|---|
| **Working λ (in methanol)** | 293nm | 245nm |
| **Berr's law range** | 10-50 µg/ml | 10-50 µg/ml |
| **Regression value** | | |
| **i) slope** | -0.00093 | -0.00014 |
| **ii) intercept** | -0.00282 | 0.00021 |
| **iii) regression coefficient ($r^2$)** | 0.99871 | 0.99887 |

**Table 24 Result Data for 2nd Derivatives**

| Parameters | NEBI | RAMI |
|---|---|---|
| **Working λ (in methanol)** | 298nm | 228nm |
| **Berr's law range** | 10-50µg/ml | 10-50 µg/ml |
| **Regression value** | | |
| **i) slope** | 0.00019 | 0.00013 |
| **ii) intercept** | 0.00015 | 0.00001 |
| **iii) regression coefficient ($r^2$)** | 0.9975 | 0.9958 |

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