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Development and Validation of a Novel Simultaneous Equation UV-Spectrophotometric method for Quantitative Estimation of Nitrendipine and Atenolol in Synthetic Mixture

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ABSTRACT
This study aimed to develop and validate novel, accurate and precise UV spectrophotometric [Vierordt's (simultaneous equation)] method for the simultaneous estimation of Nitrendipine (NIT) and Atenolol (ATE) in Synthetic mixture. Methanol was used as a solvent for analysis. The λ_{max} of NIT and ATE were identified at 274 nm and 224 nm, respectively. By application of Vierordt's method, two wavelengths (274 nm and 224 nm) were used for estimation the drug amounts and derive a simultaneous equation for Nitrendipine and Atenolol. The validation followed ICH Q2 (R2) guideline, demonstrating excellent linearity (R^2 > 0.99 for both drugs), precision (%RSD < 2%), accuracy (between standard 98.5%-101.2%), and sensitivity by LOD and LOQ study were proved. The proposed method was linear for Nitrendipine in the concentration range of 1-5 µg/mL at wavelengths of 274 nm and 224 nm. Atenolol exhibited linearity over the concentration range of 2-10 µg/mL at 224 nm and 274 nm. Accuracy was carried out by the Recovery Studies and was obtained in range of 99.66%-99.80% for Nitrendipine and 99.66%-99.91% for Atenolol. LOD Values were found to be 0.138 µg/ml at 274 nm & 0.044 µg/ml at 224 nm for Nitrendipine and 0.079 µg/ml at 224 nm & 0.121 µg/ml at 274 nm Atenolol. LOQ Values were found to be 0.414 µg/ml at 274 nm & 0.132 µg/ml at 224 nm for Nitrendipine and 0.24 µg/ml at 224 nm & 0.363 µg/ml at 274 for Atenolol. Percentage assay of Nitrendipine and Atenolol was found to be 99.95% and 99.99%, respectively. The developed method was successfully applied to a synthetic mixture, showing recovery rates within acceptable limits.

1. INTRODUCTION:

Millions of people worldwide suffer with hypertension, often known as high blood pressure, a chronic illness that greatly increases the risk of cardiovascular diseases like heart attacks and strokes [1-2]. Combination medication treatments, such as Nitrendipine and Atenolol, are generally used to treat hypertension. The combination of Nitrendipine and Atenolol was studied under clinical trial [3] and it was proved that this combination is safe, effective, and well tolerable in the treatment of mild or moderate arterial hypertension [4]. The fixed-dose combination of Nitrendipine and Atenolol is a safe and effective treatment for mild to moderate hypertension. Ambulatory blood pressure monitoring demonstrated a significant reduction in 24-hour blood pressure compared with placebo, with maximal effects during daytime hours. The therapy also reduced daytime heart rate without affecting nocturnal heart rate [5]. In addition to

antihypertensive efficacy, the combination showed favourable metabolic effects, including a significant increase in high-density lipoprotein cholesterol levels and improvement in triglyceride levels in male patients. The treatment was well tolerated with good patient compliance. Preclinical studies in hypertensive rat models confirmed synergistic effects, including improved blood flow, reduced blood pressure variability, restoration of baroreflex sensitivity, and protection against cardiac hypertrophy and renal damage. Owing to complementary mechanisms of action, this combination offered superior antihypertensive and organ-protective benefits compared with monotherapy [6].

Nitrendipine (NIT) is a widely used calcium channel blocker belonging to the dihydropyridine class compounds that is being developed for the treatment of hypertension with its mild and persistent antihypertensive effect, it was preferred drug for elderly hypertensive patients. The chemical formula for Nitrendipine (NIT, Figure 1) is 5-O-ethyl 3-O-methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [7].

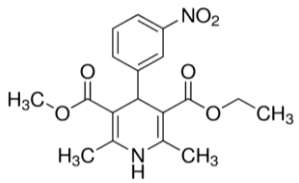

Figure 1: Nitrendipine's Chemical Structure

Atenolol is a second-generation β-1–selective adrenergic antagonist that helps lower the heart rate and blood pressure of patients while also decreasing myocardial contractility. Atenolol is approved by the U.S. Food and Drug Administration (FDA) for treating hypertension, angina pectoris, and acute myocardial infarction. Chemically, Atenolol (ATE. Figure 2) is 2-[4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl] acetamide [8].

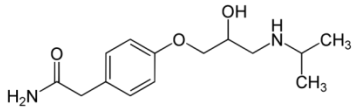

Figure 2: Atenolol's Chemical Structure

These two drugs work well together to have a synergistic effect, which makes it a worthwhile treatment option for hypertension.

To address these challenges, conventional UV spectrophotometric method as the Simultaneous Equation Method (Vierordt's method), was selected for this study. This technique offered a straightforward, cost-effective alternative to chromatographic methods [9-10], making particularly suitable for laboratories with limited resources. Unlike chromatographic procedures, UV spectrophotometric method does not require costly columns, organic solvents, or extensive sample preparation. Additionally, it provides a rapid and efficient means of quantification, especially for compounds with overlapping absorption spectra, such as Nitrendipine and Atenolol. The novelty of this work lies in the development of highly accurate, precise, and sensitive UV spectrophotometric method for the simultaneous estimation of Nitrendipine and Atenolol in synthetic mixture.

A variety of analytical approaches have been documented for the quantitative analysis of Nitrendipine and Atenolol, both individually and in combination with other drugs, such as UV spectrophotometry [11] and HPLC [12] methods have been reported for the analysis of Nitrendipine as a single component. Similarly, Atenolol has been estimated individually using UV spectroscopy [13] and HPLC [14]. For combination studies, simultaneous estimation of enalapril maleate and Nitrendipine by liquid chromatography and multivariate approaches has been described [15]. Various analytical methods have also been published for Atenolol in combination with Chlorthalidone [16], Hydrochlorothiazide [17], Trimetazidine [18], Aspirin [19], and Indapamide [20], including a stability-indicating method for Atenolol and Hydrochlorothiazide [21]. A critical review of the literature indicates that no UV spectrophotometric method has been reported for the simultaneous estimation of Nitrendipine and Atenolol in a synthetic mixture. Therefore, this drug combination was selected for the present study. The objective of the present work was to develop and validate a simple, linear, accurate, precise, sensitive, and cost-effective UV spectrophotometric method for the simultaneous estimation of Nitrendipine and Atenolol in synthetic mixtures.

2. MATERIALS AND METHODS:

2.1 Chemicals and reagents:

Nitrendipine was obtained from Anant Pharmaceutical Pvt. Ltd. Maharashtra, while Atenolol was procured from CTX Lifesciences Pvt. Ltd., Surat, Gujarat. Water and Methanol (HPLC grade) were sourced from Finar Chemicals Pvt. Ltd., India.

2.2 Instruments and apparatus:

The analysis was performed using a Shimadzu UV-1900 ultraviolet-visible spectrophotometer, with absorbances recorded using matched quartz cells of

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1 cm path length. For precise measurements Digital Analytical balance from Scale-Tec was used. For sonication, Sonicator named Digital Pro+ PS-10AS from Broleo was used. All glasswares were cleaned and meticulously cleaned with HPLC grade water, and dried before use.

2.3 Preparation of stock solution and working solution for wavelength selection:

Stock solutions of Nitrendipine and Atenolol were prepared separately by dissolving 10 mg of each drug in 100 mL methanol in a volumetric flask, resulting in a concentration of 100 µg/mL. The solutions were sonicated for 5 mins to ensure complete dissolution. From each stock solution, Nitrendipine (0.1, 0.2, 0.3, 0.4 and 0.5 ml) and Atenolol (0.2, 0.4, 0.6, 0.8 and 1.0 ml) were pipetted out in five different 10 ml volumetric flasks and made up to mark with Methanol to obtained 1, 2, 3, 4 and 5 µg/ml of Nitrendipine and 2, 4, 6, 8 and 10 µg/ml for Atenolol, respectively. These solutions were scanned individually in the ultraviolet range (400 nm to 200 nm) to determine their maximum absorbance wavelengths (λ_{max}). Overlay spectra of Nitrendipine and Atenolol were generated and then calibration curve of Absorbance v/s Concentration was plotted.

3. UV-SEPCTROPHOTOMETRIC METHOD DEVELOPMENT

Pipetted out 0.2 ml solution from stock solution of Nitrendipine (100 µg/ml) and 0.4 ml Atenolol (100 µg/ml) into different 10 ml volumetric flask and diluted upto mark with Methanol to get the 2 µg/ml of Nitrendipine and 4 µg/ml Atenolol. Every solution was scanned between 200 and 400 nm.

3.1 Vierordt's simultaneous equation

Standard Stock solutions of Nitrendipine and Atenolol in the concentration range 1-5 µg/mL and 2-10 µg/ml were made in the methanol and absorbance of these solutions was measured at 274 nm and 224 nm. Calibration curves were plotted to confirm the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. Two simultaneous equations as below were formed using these absorptivity values A (1%, 1 cm).

$$\text{At } \lambda 1 \; A1 = ax1bCx + ay1bCy \ldots\ldots\ldots\ldots\ldots\ldots (1)$$
$$\text{At } \lambda 2 A2 = ax2bCx + ay2\, bCy \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

For measurements in 1 cm cells b=1,

Rearrange eq. (2),

$$Cy = A2 - ax2Cx/\, ay2$$

Substituting for Cy in eq (1) and rearranging

$$Cx = A2ay1 - A1\, ay2/ax2\, ay1 - ax1\, ay2 \ldots\ldots\ldots\ldots\ldots\ldots (3)$$
$$Cy = A1ax2 - A2\, ax1/ax2\, ay1 - ax1\, ay2 \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where Cx and Cy are the concentration of Nitrendipine and Atenolol, respectively, A1 and A2 are absorbance at 274 nm and 224 nm respectively, ax1 and ax2 are absorptivity of Nitrendipine at 274 nm and 224 nm respectively; ay1 and ay2 are absorptivity of Atenolol at 224 nm and 274 nm respectively. By solving the two simultaneous equations, the concentrations of Nitrendipine and Atenolol in sample solutions were obtained.

4. METHOD VALIDATION

The ICH Q2 (R2) guideline [22] was adopted in the validation of the outlined processes. Linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and assay were among the validation aspects assessed [23-24].

4.1 Linearity and Range: (n=6)

Linearity was studied by preparing standard solution at 5 different concentrations. The linearity range for Nitrendipine and Atenolol were found to be 1-5 µg/ml and 2-10 µg/ml, respectively. Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

4.2 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.

1) Intraday Precision: (n=3)

Solutions containing 1, 2, 3 µg/ml of NIT and 2, 4, 6 µg/ml of ATE were analysed three times on the same day and %RSD was calculated.

2) Interday Precision: (n=3)

Solutions containing 1, 2, 3 µg/ml of NIT and 2, 4, 6 µg/ml of ATE were analysed on three different successive days and %RSD was calculated.

3) Repeatability: (n=6)

Solutions containing 2 µg/ml of NIT and 4 µg/ml of ATE were analysed for six times and %RSD was calculated. RSD was not more than 2%.

4.3 Limit of Detection (LOD)

Limit of detection can be calculated using following equation as per ICH Q2(R2) guideline.

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

where, **σ** = Standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

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4.4 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.

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

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

The accuracy of an analytical procedure expresses 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 Nitrendipine (**2 µg/ml**) and Atenolol (**4 µg/ml**). This performance was done in triplicate.

4.6 Assay as Analysis of synthetic mixture

The synthetic mixture of Nitrendipine and Atenolol was prepared in the ratio of 1:2. Accurately weighed equivalently weight of Nitrendipine (5 mg), Atenolol (10 mg) and all excipients such as Micro Crystalline Cellulose (MCC) (4 mg), Lactose Monohydrate (11 mg), Polyvinyl Pyrrolidine (13 mg), Talc (3.5 mg) and Magnesium Stearate (3.5 mg) were added in the motor pestle. After trituration, transferred this mixture in 100 ml volumetric flask and the solution was sonicated for 5 mins to ensure uniform mixing and made up to mark with Methanol. This solution was filtered through Whatmann filter paper. The filtrate was diluted to the mark with Methanol. The mixture contains 50 µg/ml of Nitrendipine and 100 µg/ml of Atenolol. For sample solution preparation, accurately 0.4 ml of the above [mixture solution of Nitrendipine (50 µg/ml) and Atenolol (100 µg/ml)] was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol. Final concentration of Nitrendipine was 2 µg/ml and Atenolol was 4 µg/ml.

5. RESULTS:

5.1 Selection of wavelength

The λ_{max} of Nitrendipine and Atenolol were identified at 274 nm and 224 nm (Figure 3), respectively. Overlain UV Spectra of Nitrendipine (2 µg/ml) and Atenolol (4 µg/ml) in Methanol (Zero order) had been showed in Figure 3.

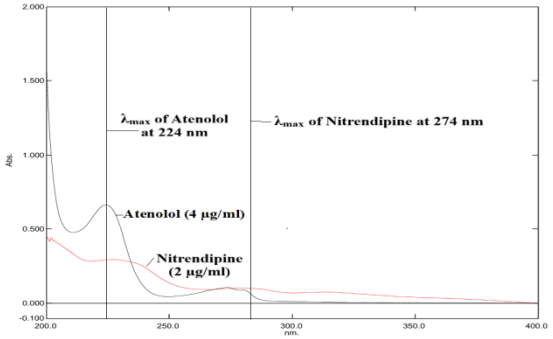

Figure 3: Overlain spectra of Nitrendipine (2 µg/ml) and Atenolol (4 µg/ml) in Methanol (Zero order)

5.2 Simultaneous equation method

UV Spectra of Nitrendipine (1-5 µg/ml) and Atenolol (2-10 µg/ml) over the linearity and range had been showed in Figure 4 and 5, respectively.

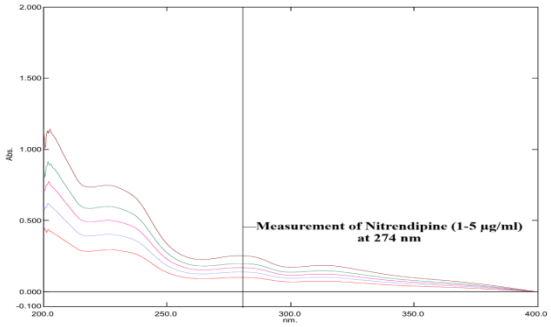

Figure 4: Overlay UV spectra of Nitrendipine (1-5 µg/ml) at 274 nm

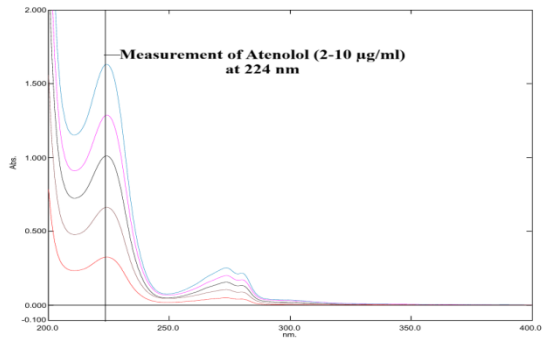

Figure 5: Overlay UV spectra of Atenolol (2-10 µg/ml) at 224 nm

5.2 Validation parameters

5.2.1 Linearity and Range:

Nitrendipine: It was demonstrated a proportional increase in absorbance with increasing concentration (linear) over the range of 1-5 µg/mL at both 274 nm and 224 nm. Correlation Coefficient for Nitrendipine 0.998 at 274 nm and 0.9979 at 224 nm were obtained. The mean absorbance values obtained from six replicate measurements showed low standard deviation, and the %RSD values at both wavelengths were below 1.5%, indicating satisfactory precision and repeatability of the proposed UV spectrophotometric method. Linearity data of Nitrendipine had been showed in Table 1.

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Table 1: Linearity data of Nitrendipine

Nitrendipine						
Conc. (µg/ml)	Mean Absorbance ± Standard Deviation (n=6)		Absorptivity		% Relative Standard Deviation	
	274 nm	224 nm	274 nm	224 nm	274 nm	224 nm
1	0.101±0.0012	0.295±0.0038	0.10	0.29	1.18	1.28
2	0.131±0.0013	0.404±0.0043	0.06	0.20	0.99	1.06
3	0.170±0.0014	0.503±0.0044	0.05	0.16	0.82	0.87
4	0.199±0.0015	0.599±0.0039	0.04	0.14	0.75	0.65
5	0.237±0.0016	0.725±0.0034	0.04	0.14	0.67	0.46

Calibration curve with Regression equation for 274 nm and 224 nm were showed in figure 6 and 7.

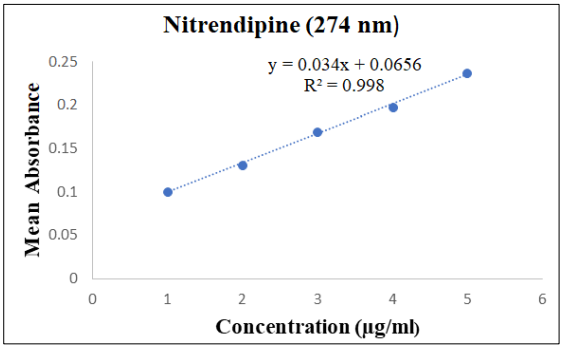

Figure 6: Calibration curve for Nitrendipine at 274 nm in Methanol

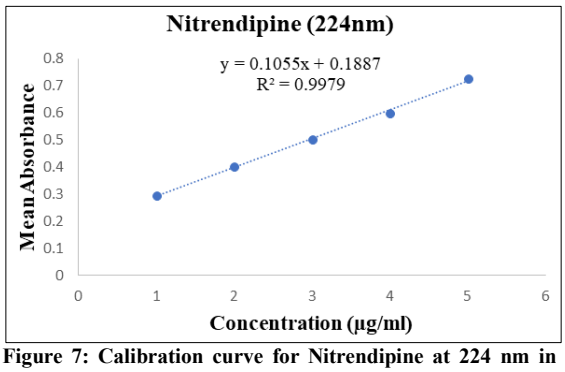

Figure 7: Calibration curve for Nitrendipine at 224 nm in Methanol

Atenolol: A linear absorbance response was observed for Atenolol in the concentration range of 2-10 µg/mL at 224 nm and 274 nm with Correlation Coefficient 0.9982 and nm and 0.999, respectively. Calibration curve of Atenolol at 224 nm and 274 nm had been showed in Figure 8 & 9. The mean absorbance values (n = 6) were associated with low standard deviation, and %RSD values less than 1.5% confirmed the precision and reproducibility of the developed analytical method. Linearity data of Atenolol showed in Table 2.

Table 2: Linearity data of Atenolol

Atenolol						
Conc. (µg/ml)	Mean Absorbance ± Standard Deviation (n=6)		Absorptivity		% Relative Standard Deviation	
	224 nm	274 nm	224 nm	274 nm	224 nm	274 nm
2	0.326 ± 0.0039	0.041 ± 0.0005	0.16	0.02	1.19	1.21
4	0.667 ± 0.0071	0.088 ± 0.0009	0.16	0.02	1.06	1.02
6	1.003 ± 0.0084	0.131 ± 0.0012	0.17	0.02	0.82	0.91
8	1.291 ± 0.0087	0.173 ± 0.0013	0.16	0.02	0.67	0.75
10	1.647 ± 0.0072	0.215 ± 0.0014	0.16	0.02	0.43	0.65

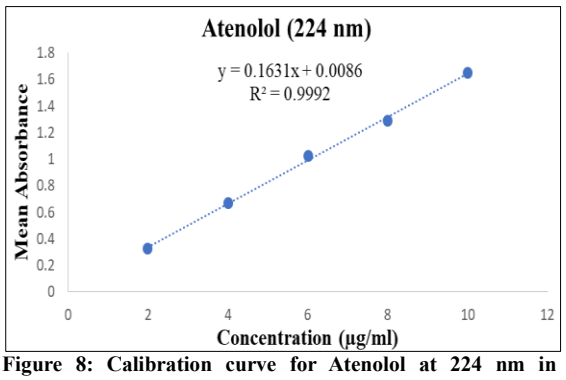

Figure 8: Calibration curve for Atenolol at 224 nm in Methanol

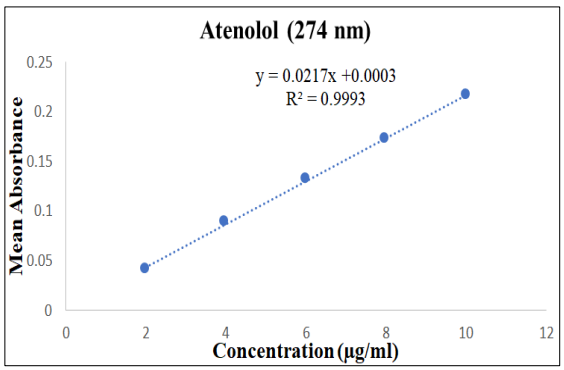

Figure 9: Calibration curve for Atenolol at 274 nm in Methanol

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5.2.1.1 Calculation for Simultaneous Equation Method for Nitrendipine and Atenolol in Synthetic Mixture.

Nitrendipine (2 µg/ml) and Atenolol (4 µg/ml) in Methanol, both the solutions were scanned over range of 200-400nm against Methanol as blank, using medium scan speed. The sampling wavelength for analysis includes 274 nm for Nitrendipine and 224 nm for Atenolol. The method employs Simultaneous Equation as per Vierodt's method and the concentrations of drugs in sample solution were determined by using the following formula:

For Nitrendipine,

$$Cx = \frac{A2\ ay1 - A1\ ay2}{a\ x2\ aY1 - a\ x1ay2}$$

Where ax_1 and ax_2 represented the absorptivity of Nitrendipine at 274 nm and 224 nm, respectively; ay_1 and ay_2 denoted the absorptivity of Atenolol at 274 nm and 224 nm, respectively; and A_1 and A_2 corresponded to the absorbance of the sample measured at 274 nm and 224 nm, respectively.

C (Nitrendipine) = (0.131) (0.166) – (0.404) (0.022)/ (0.065) (0.166) – (0.202) (0.004)
=0.0217 -0.0089/0.0108-0.000808
= 0.0128/0.00999
=1.28 µg/mL

The concentration of Nitrendipine (Cx), calculated using Vierordt's simultaneous equation method, was found to be 1.28 µg/mL.

For Atenolol,

$$Cy = \frac{A1ax2 - A2\ a\ x1}{ax2\ aY1 - a\ x1ay2}$$

where a_{x1} and a_{x2} are the absorptivity values of Nitrendipine at 224 nm and 274 nm, respectively; a_{y1} and a_{y2} represent the absorptivity of Atenolol at 224 nm and 274 nm, respectively; and A_1 and A_2 are the absorbance values of the sample measured at 224 nm and 274 nm, respectively.

C (Atenolol) = (0.667) (0.065) - (0.088) (0.202)/ (0.065) (0.166) – (0.010) (0.004)
= 0.0434 - 0.0177/ 0.0108 - 0.00004
= 0.0257/ 0.01076
= 2.38 µg/mL

The concentration of Atenolol (Cy), calculated using Vierordt's simultaneous equation method, was found to be 2.38 µg/mL.

5.2.2 Precision:

Precision expresses the closeness of agreement (degree of scatter) among a series of measurements obtained from multiple sampling of a homogeneous sample under prescribed conditions. It was evaluated and expressed as percent relative standard deviation (%RSD). Precision was assessed at three levels: repeatability, intraday precision, and interday precision.

Nitrendipine: The precision of the proposed method for Nitrendipine was evaluated by intraday, interday, and repeatability studies, and the results are summarized in Table 3. Intraday and interday precision were assessed at three concentration levels by analyzing samples three times within the same day and on three consecutive days, respectively. Repeatability was determined by multiple measurements of a single concentration. The %RSD values at both 274 nm and 224 nm were found to be less than 2.0%, indicating good precision and repeatability of the method.

Table 3: Precision study of Nitrendipine

Intraday Precision of Nitrendipine				
Conc. (µg/ml)	**Mean Absorbance ± SD (n=3)**		**% RSD**	
	274 nm	**224 nm**	**274 nm**	**224 nm**
1	0.102±0.0012	0.295±0.0038	1.17	1.28
2	0.132±0.0013	0.404±0.0043	0.98	1.06
3	0.170±0.0014	0.503±0.0044	0.82	0.87
Interday Precision of Nitrendipine				
Conc. (µg/ml)	**Mean Absorbance ± SD (n=3)**		**% RSD**	
	274 nm	**224 nm**	**274 nm**	**224 nm**
1	0.101±0.0012	0.296± 0.0039	1.18	1.31
2	0.131±0.0013	0.405±0.0044	0.99	1.08
3	0.171±0.0015	0.504±0.0045	0.87	0.89
Repeatability of Nitrendipine				
Conc. (µg/ml)	**Mean Absorbance ± SD (n=3)**		**% RSD**	
	274 nm	**224 nm**	**274 nm**	**224 nm**
2	0.101±0.0011	0.404± 0.0041	1.08	1.01

Atenolol: The precision study for Atenolol was carried out similarly, and the results are presented in Table 4**.** Intraday and interday precision were evaluated at three different concentration levels, while repeatability was assessed at a single concentration. The %RSD values obtained at 224 nm and 274 nm were below 2.0%, demonstrating satisfactory precision and consistency of the analytical method.

Table 4: Precision study of Atenolol

Intraday Precision of Atenolol				
Conc. (µg/ml)	**Mean Absorbance ± SD (n=3)**		**% RSD**	
	224 nm	**274 nm**	**224 nm**	**274 nm**
2	0.326 ± 0.0039	0.041 ± 0.0005	1.19	1.21
4	0.667 ± 0.0071	0.088 ± 0.0009	1.06	1.02
6	1.023 ± 0.0084	0.131 ± 0.0012	0.82	0.91

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Interday Precision of Atenolol				
Conc. (µg/ml)	Mean Absorbance ± SD (n=3)		% RSD	
	224 nm	274 nm	224 nm	274 nm
2	0.326 ± 0.0040	0.041 ± 0.0005	1.22	1.21
	0.667 ± 0.0072	0.089 ± 0.0010	1.07	1.12
6	1.023 ± 0.0085	0.131 ± 0.0012	0.83	0.99
Repeatability of Atenolol				
Conc. (µg/ml)	Mean Absorbance ± SD (n=3)		% RSD	
	224 nm	274 nm	224 nm	274 nm
4	0.667 ± 0.0068	0.088 ± 0.00084	1.02	0.95

5.2.3 *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 LOD and LOQ values for Nitrendipine and Atenolol showed in Table 5. 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.

Table 5: LOD and LOQ data

Parameter	Nitrendipine		Atenolol	
	274 nm	224 nm	224 nm	274 nm
LOD(µg/ml)	0.138	0.044	0.079	0.121
LOQ(µg/ml)	0.414	0.132	0.24	0.363

5.2.4 *Accuracy:*

Accuracy of the proposed method was evaluated by the standard addition method. Known amounts of Nitrendipine and Atenolol standards were added to pre-analyzed samples at three concentration levels (50%, 100%, and 150%), and the mixtures were analyzed in triplicate. The results of the recovery study for both drugs are presented in Table 6. The mean percentage recovery values for Nitrendipine and Atenolol 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 6: Recovery study

Name ofDrug	% 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)
Nitrendipine	50	2	1	3	2.99	99.66±0.0702
	100	2	2	4	3.99	99.75±0.0757
	150	2	3	5	4.99	99.80±0.0892
Atenolol	50	4	2	6	5.98	99.66±0.0404
	100	4	4	8	7.99	99.87±0.0503
	150	4	6	12	11.99	99.91±0.0611

5.2.5 *Analysis of Synthetic mixture:*

The developed UV spectrophotometric method was applied to the analysis of a synthetic mixture containing Nitrendipine and Atenolol. The results of the assay were presented in Table 7. The mean percentage assay values for Nitrendipine and Atenolol were found to be close to 100%, with low standard deviation and %RSD values less than 2.0%, indicating the accuracy and precision of the method for simultaneous estimation of both drugs.

Table 7: Analysis of synthetic mixture

Name of Drug	Amount in synthetic mixture (µg/ml)	Mean Amount found (µg/ml)	% Mean Assay ± SD (n=3)	% RSD
Nitrendipine	2	1.99	99.95 ± 0.0173	0.017
Atenolol	4	3.99	99.99 ± 0.0115	0.011

6. DISCUSSION

The present study described the development and validation of a simple, linear, sensitive, accurate, and precise UV spectrophotometric method for the simultaneous estimation of Nitrendipine and Atenolol in synthetic mixtures. The method was based on the Vierordt's simultaneous equation approach, utilizing the specific absorbance maxima of Nitrendipine at 274 nm and 224 nm and Atenolol at 224 nm and 274 nm. This approach allowed the quantification of both drugs without prior separation, demonstrated its suitability for routine analysis compliant with ICH Q2(R2) guideline.

The linearity studies for both drugs showed a proportional increase in absorbance with concentration over the selected ranges, with correlation coefficients greater than 0.99 at both wavelengths. This indicates excellent linear response, which is a prerequisite for accurate simultaneous estimation using the simultaneous equation method.

The calculated LOD and LOQ values were low for both drugs, confirming the sensitivity of the method and its capability to detect and quantify even minute concentrations.

The precision of the method, evaluated through intraday, interday, and repeatability studies, showed %RSD values below 2.0% for both Nitrendipine and Atenolol at all tested concentrations. These results demonstrate that the method is highly reproducible and reliable under the prescribed experimental conditions.

Similarly, the accuracy of the method, determined by the standard addition technique, yielded recovery values within the ICH-accepted range of 98-102%, indicating the method is free from interference by excipients and suitable for quantitative analysis of synthetic mixtures.

Application of the method to a synthetic mixture of Nitrendipine and Atenolol provided assay values of 99.95% and 99.99%, respectively, with low %RSD. This confirms the amount of the drugs and its applicability for simultaneous estimation in synthetic mixtures. The simplicity, cost-effectiveness, and reproducibility of this UV spectrophotometric method make it a practical alternative to more sophisticated techniques, such as HPLC or LC-MS, for routine quality control purposes.

Overall, the developed method fulfilled the validation parameters recommended by ICH Q2(R2), including linearity, precision, accuracy, sensitivity, and reproducibility. Its successful application to synthetic mixtures highlights its potential utility in routine pharmaceutical analysis, providing a reliable and rapid tool for quality control laboratories.

7. CONCLUSION:

A simple, rapid, and cost-effective UV spectrophotometric method was successfully developed and validated for the simultaneous estimation of Nitrendipine and Atenolol in synthetic mixtures. The method demonstrated excellent linearity, precision, accuracy, sensitivity, and reproducibility, in full compliance with ICH Q2 (R2) guideline. Recovery studies and analysis of synthetic mixtures confirmed the method's reliability and freedom from interference. Owing to its simplicity, low cost, and rapid execution, this method was well-suited for routine quality control and assay as analysis of synthetic mixture containing Nitrendipine and Atenolol. In conclusion, this UV spectrophotometric method offered a simpler, and equally reliable alternative to conventional chromatographic techniques, making it suitable for routine pharmaceutical analysis, particularly in resource-limited settings.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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