Development and Validation of a UV-Spectroscopic Method for Simultaneous Estimation of Nitrendipine and Hydrochlorothiazide

ABSTRACT*:*

A new, simple, accurate, precise, linear, and sensitive UV-Spectroscopic method has been developed and validated for the simultaneous estimation of Nitrendipine and Hydrochlorothiazide in pharmaceutical tablet dosage form. The method was based on the absorption factor method and its use to analysis of isosbestic point present in zero order absorption spectra. For the determination of Nitrendipine and Hydrochlorothiazide, their isoabsorptive points were utilized at 282nm. The absorbance corresponding to Nitrendipine or Hydrochlorothiazide, separately, at isoabsorptive point 282 nm was calculated using absorbance factor which is the average of the absorbance of different concentrations of pure Nitrendipine using isoabsorptive point at 282 nm. The developed method was then validated for linearity, accuracy, and precision in accordance with ICH Guidelines. The linearity range at their λ max at isoabsorptive point 282nm. From all these results it can be concluded that the current research was new, accurate, efficient, precise, rapid, reproducible, simple, and sensitive. The proposed method for this research can be successfully applied to estimate the Nitrendipine and Hydrochlorothiazide in pharmaceutical tablet dosage form.

Keywords: Nitrendipine, Hydrochlorothiazide, UV-Spectroscopy, simultaneous estimation.

1. INTRODUCTION:

Both Hydrochlorothiazide and Nitrendipine acts as antihypertensive agents but both of them work with different mechanisms (Giles TD, *et. al.,* 1987). Hydrochlorothiazide is a diuretic while Nitrendipine is a dihydropyridine calcium channel blocker. Hydrochlorothiazide can also be used in swelling occurred due to fluid build-up, diabetes and renal tubular acidosis treatment (Roush GC, *et. al.,* 2021). Hydrochlorothiazide is taken by mouth and may be combined with other blood pressure medications as a single pill to increase effectiveness. Nitrendipine is used in the treatment of primary hypertension to decrease blood pressure and can reduce the cardiotoxicity of cocaine (Trouve R, *et. al.,* 1986).

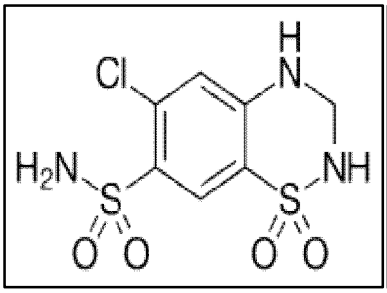

Fig. 1: Chemical structure of hydrochlorothiazide

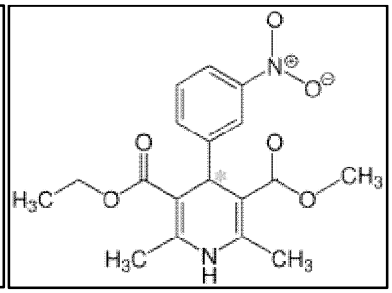

Fig. 2: Chemical structure of nitrendipine

Literature survey has revealed that the UV-Spectroscopy methods are reported for the determination of Hydrochlorothiazide and Nitrendipine individually). UV-Spectroscopy has been reported for the analysis of both these medications in pharmaceutical or pure form by UV-Spectrophotometer (Singh S, *et. al.,* 2011 and Weinstein RD, *et. al.,* 2008). The methods reported were not used for both Hydrochlorothiazide and Nitrendipine individually till no. So, our research made an attempt to develop new, simple accurate and sensitive methods to ensure the safety as well as efficacy of these medications in tablet dosage form. The method was fully validated and was successfully applied for the estimation of both these medications in tablet dosage form.

2. MATERIALS AND METHOLOGY:

2.1 Method Development:

2.1.1. UV spectrum of Nitrendipine and Hydrochlorothiazide:

The solvent utilised was methanol, which was easily soluble for both drugs. As a result, the suggested procedure's solvent was decided to be methanol.

2.1.2. Determination of Isoabsorptive Point:

An isosbestic point is a wavelength, wavenumber, or frequency that, in spectroscopy, is the wavelength, wavenumber, or frequency at which the total absorbance of a sample does not change during a chemical reaction or a physical change to the sample. Methanol was then added to each flask to achieve the desired final concentration of 5µg/ml for each drug solution. In a UV-visible spectrophotometer, the solutions were individually scanned from 200 nm to 400 nm. To calculate the isoabsorptive point, the overlaying spectrum was also collected **(**Kamboj A., *et. al.,* 2017).

2.1.3 Preparation of Standard Stock Solution:

The standard stock solutions of nitrendipine and hydrochlorothiazide were prepared by dissolving each drug separately into a 100ml volumetric flask containing methanol. An accurately measured amount of 10mg of each drug was transferred into the 100 ml volumetric flasks separately. To acquire the necessary concentration of each drug, 100µg/ml, methanol was added to the flask and it was manually shaken to complete the dissolution of drug. The flask needs to be marked, and it should be stored at room temperature (Pravin C, *et. al.,* 2018).

2.1.4 Preparation of working standard:

An accurately measured volume of a 100µg/ml stock solution of nitrendipine and hydrochlorothiazide was diluted with methanol to obtain appropriate dilutions of 1–10µg/ml and 1–12µg/ml, respectively, and was then analysed spectrophotometrically at 236 nm and 270 nm and isoabsorptive point 282 nm and 258, respectively.

2.2 Method of Validation:

The method of validation in UV-Spectroscopy was validated by following ICH-guidelines.

2.2.1 Linearity:

Reliable quantification requires the selection of an appropriate calibration model. As a result, it is necessary to look at how the concentration of an analyte in the sample and the corresponding response relate to one another. The isoabsorptive point, which is an isosbestic point present in zero order absorption spectra, can be analysed using the absorption subtraction method, which is based on the absorption factor approach and uses equal absorptivity values for the components showing this point. As a result, the linearity curve was drawn at the individual wavelengths of nitrendipine (236 nm), hydrochlorothiazide (270 nm), and the isoabsorptive point (258 nm and 282 nm) (Sharma S., *et. al.,* 2017).

2.2.2 Accuracy:

The accuracy was tested by recovery experiments. Recovery studies were carried out at 100 % level by adding a known quantity of pure drug to the Preanalyzed samples and the proposed method was followed. From the amount of drugs found, percentage recovery was calculated. 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. The recovery experiments were carried out in triplicate by spiking previously analysed samples with three different concentrations of standards (Sen A.K. *et. al.,* 2016).

2.2.3 Repeatability:

Repeated scanning and measurements of the absorbance of solutions comprising (n = 6) of nitrendipine (5µg/ml) and hydrochlorothiazide (5µg/ml) at the same time were used to test the instrument's accuracy without altering the parameters of the suggested approach.

2.2.4 Precision:

Precision is defined as the degree of agreement between quantity values acquired by repeated measurements of a quantity under predetermined conditions by the ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) and ICH. When evaluating precision, it is necessary to use the standard deviation, variance, or coefficient of variation to numerically quantify the random error or level of dispersion of a collection of individual measurements.

2.2.5 Limit of Detection:

The lowest amount of analyte in a sample that can be detected but not always quantitated as an accurate number is the Detection Limit of a specific analytical method. The expression for the detection limit (LOD) is: According to ICH recommendations, the limit of detection can be computed using the following calculation.

$LOD = 3.3 \times N/S$

Where, N is the standard deviation of the intercepts of the drug and S is the slope of the corresponding calibration curve.

2.2.6 Limit of Quantification:

The lowest amount of analyte in a sample that can be quantitatively measured with enough precision and accuracy was the quantitation limit of an analytical method. According to ICH recommendations, the limit of quantification can be computed using the following calculation.

LOQ = 10 × N/S

Where, N is the standard deviation of the intercepts of the drug and S is the slope of the corresponding calibration curve.

2.2.7 Robustness:

The impact of small, purposeful modifications to the isoabsorptive wavelength (±2 nm) on the outcomes was investigated.

2.2.8 Ruggedness:

The degree of consistency of findings produced by the successful application of the assay over different analysts is characterised as the ruggedness test of the analytical assay method. Two analysts carried out the suggested methodologies in this investigation for the determination of nitrendipine and hydrochlorothiazide.

2.2.9 Forced degradation studies:

Different ICH-recommended stress conditions (acidic, basic, oxidative, thermal, and photolytic) were used in this investigation.

3. RESULTS:

3.1 Linearity:

The standard calibration curves were constructed by plotting concentration against absorbance where each reading was an average of three determinations. Linearity of Nitrendipine and Hydrochlorothiazide at their respective wavelength and isoabsorptive point is given in Table 1.

Table 1: Standard Calibration Curve of Nitrendipine in Methanol at 236nm (n=3)

Con.(µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Mean Absorbance at 236nm	STD	RSD
1	0.025	0.024	0.024	0.024	0.001	1.373
2	0.090	0.092	0.093	0.092	0.002	1.666
3	0.179	0.18	0.178	0.179	0.001	0.559
4	0.276	0.28	0.278	0.278	0.002	0.719
5	0.376	0.375	0.379	0.377	0.002	0.553

6	0.460	0.467	0.463	0.463	0.004	0.758
7	0.54	0.543	0.542	0.542	0.002	0.282
8	0.632	0.637	0.633	0.634	0.003	0.417
10	0.797	0.8	0.81	0.802	0.007	0.848

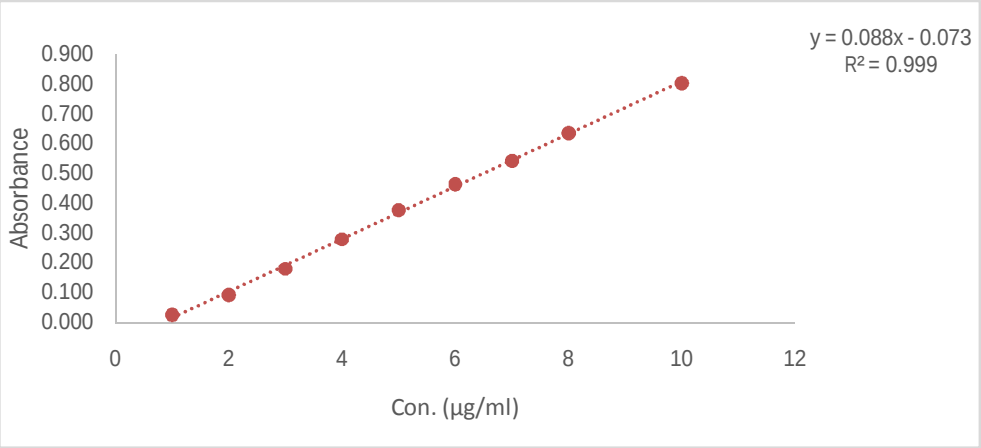

Figure 3: Standard Calibration Curve of Nitrendipine in Methanol at 236nm (n=3)

Linearity and range under the experimental conditions described, the graph of Nitrendipine in methanol obtained for UV spectrum at their λ max at 236nm. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were y = 0.0882x -0.0739(R^2 = 0.999) at 236nm for Nitrendipine and the range was found to be 1-10 µg/ml.

Table 2: Standard calibration curve of Hydrochlorothiazide in methanol at 270nm (n=3)

Con.(µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Absorbance at 270nm	STD	RSD
1	0.010	0.010	0.011	0.010	0.001	1.587
2	0.064	0.065	0.064	0.064	0.001	0.897
3	0.127	0.130	0.130	0.129	0.002	1.343
4	0.202	0.203	0.205	0.203	0.002	0.751
5	0.265	0.266	0.268	0.266	0.002	0.574
6	0.329	0.330	0.333	0.331	0.002	0.630
7	0.391	0.389	0.391	0.390	0.001	0.296
8	0.446	0.448	0.449	0.448	0.002	0.341
10	0.571	0.572	0.575	0.573	0.002	0.364
12	0.691	0.693	0.696	0.693	0.003	0.363

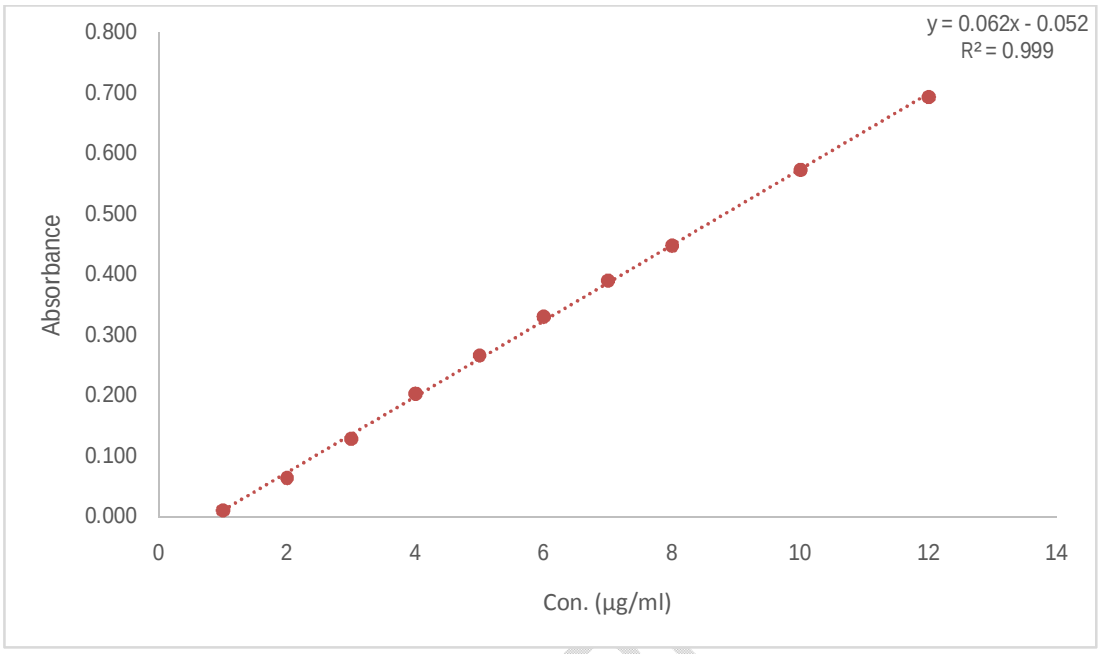

Figure 4: Standard calibration curve of Hydrochlorothiazide in methanol at 270nm (n=3)

Linearity and range under the experimental conditions described, the graph Hydrochlorothiazide in methanol obtained for UV spectrum at their λ max at 270nm. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were y = 0.0626x -0.0524 (R^2 = 0.999) at 270nm for Hydrochlorothiazide and the range was found to be 1-12 µg/ml.

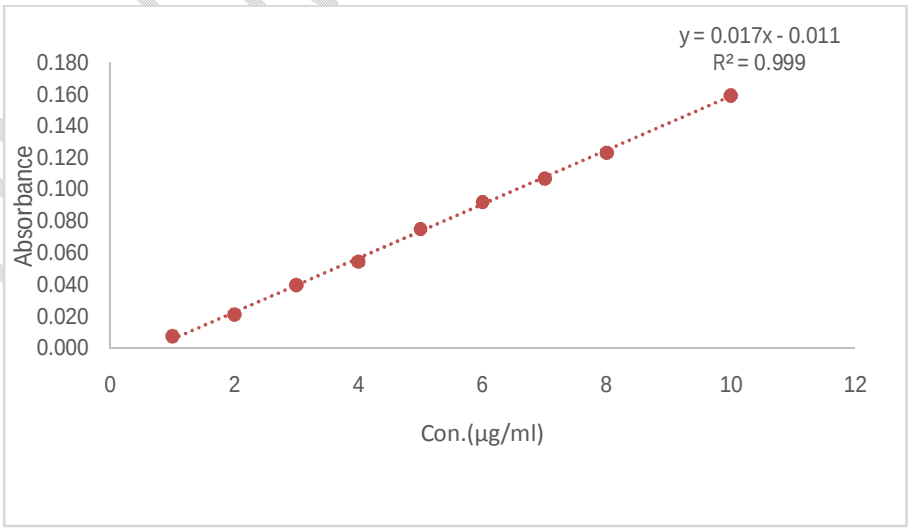

Figure 5: Standard calibration curve of Nitrendipine in methanol at isoabsorptive point 282nm (n=3)

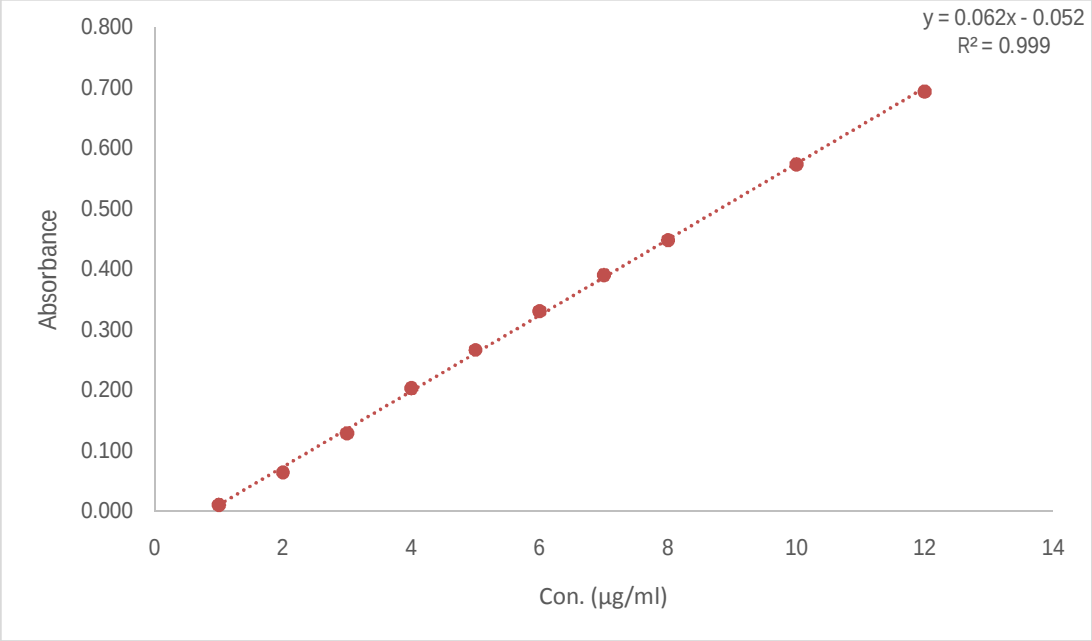

Figure 6: Standard calibration curve of Hydrochlorothiazide in methanol at 270nm (n=3)

3.2 Absorbance subtraction method:

This method is based on the absorption factor method and its use to analysis of isosbestic point present in zero order absorption spectra known as the isoabsorptive point, where the components exhibiting this point have equal absorptivity's. The only requirements of this method (AS) are the existence of isoabsorptive point of both components and the extension of the spectra of one component.

For the determination of Nitrendipine and Hydrochlorothiazide, we will utilize their isoabsorptive point at 282nm. By the analysis of the recorded absorbance at the isoabsorptive point, the absorbance corresponding to Nitrendipine or Hydrochlorothiazide, separately, at isoabsorptive point 282 nm can be calculated using absorbance factor [abs 282 / abs 236] which is the average of the absorbance of different concentrations of pure Nitrendipine using isoabsorptive point at 282 nm to that at 236 nm which shows no contribution of Hydrochlorothiazide and then the absorbance of Nitrendipine and can be obtained after subtraction.

Absorbance of Nitrendipine in the mixture at ʎ282 = abs282 / abs236 (absorption factor) × abs ʎ236 (Nitrendipine + Hydrochlorothiazide).

Absorbance of Hydrochlorothiazide in the mixture at ʎ282= abs ʎ282((Nitrendipine + Hydrochlorothiazide)– (abs282/ abs236 × abs ʎ 236 (Nitrendipine + Hydrochlorothiazide))).

Where, abs ʎ Nitrendipine + Hydrochlorothiazide is the absorbance of the binary mixture at 236 nm and abs282/ abs236 is the absorbance factor of pure Nitrendipine at 282 nm to 236 nm and it was calculated and found to be 0.218.

The calculated absorbance value corresponding to Nitrendipine and Hydrochlorothiazide can be separately used to identify each of their concentration using the unified regression equations using isoabsorptive point 282 nm.

The advantage of the absorbance subtraction method (AS) over the conventional isoabsorptive point is that there is no need for another complementary spectrophotometric method to measure the concentration of one of the two components to get the second by subtraction.

Absorption Factor is calculated by the formula mentioned below:

$$\text{Absorption Factor} = \frac{\text{Absorbance at Isoabsorptive Point}}{\text{Absorbance at absorbance maxima of drug}}$$

Absorbance factor of Nitrendipine and Hydrochlorothiazide was as given below in Table. 3.

Table 3: Absorbance factor of Nitrendipine

Con.(µg/ml)	Absorbance at 282nm	Absorbance at 236 nm	Absorption factor
1	0.008	0.024	0.315
2	0.021	0.092	0.233
3	0.040	0.179	0.223
4	0.055	0.278	0.197
5	0.075	0.377	0.200
6	0.092	0.463	0.199
7	0.107	0.542	0.198
8	0.124	0.634	0.195
10	0.160	0.802	0.199
		Average	**0.218**

Table 4: Absorbance factor of Hydrochlorothiazide

Con. (µg/ml)	Absorbance at 282nm	Absorbance at 270 nm	Absorption factor
1	0.009	0.010	0.871
2	0.031	0.064	0.487
3	0.057	0.129	0.444

4	0.092	0.203	0.451
5	0.119	0.266	0.448
6	0.150	0.331	0.455
7	0.195	0.390	0.500
8	0.218	0.448	0.486
10	0.280	0.573	0.488
12	0.326	0.693	0.514
		Average	**0.514**

Absorption factor for Nitrendipine and Hydrochlorothiazide was found to be 0.218 and 0.514.

3.3 Accuracy of the method:

This parameter is performed to determine the closeness of test results with that of the true value which is expressed as % recovery. These studies were performed at three different levels (80%, 100%, and 120%) and the % recovery of Nitrendipine and Hydrochlorothiazide was calculated. The mean % recoveries were between 99.628-100.012% and 98.483-100.299 for Nitrendipine and Hydrochlorothiazide respectively as shown in Table 5.

Table 5: Accuracy results of Nitrendipine and Hydrochlorothiazide

Preanalyzed mixture of Nitrendipine and Hydrochlorothiazide con. (µg/ml)	**Con. (µg/ml) added**	**Percentage recovery of Nitrendipine**	**Percentage recovery of Hydrochlorothiazide**
5+5	4 (µg/ml)	99.869	99.687
5+5	4 (µg/ml)	100.154	100.128
5+5	4 (µg/ml)	100.012	101.081
	Mean	**100.012**	**100.299**
	Std	**0.142**	**0.713**
	%RSD	**0.142**	**0.711**
5+5	5 (µg/ml)	99.500	100.035
5+5	5 (µg/ml)	99.628	99.177
5+5	5 (µg/ml)	99.756	99.728

	Mean	**99.628**	**99.647**
	Std	**0.128**	**0.435**
	%RSD	**0.129**	**0.436**
5+5	6 (µg/ml)	99.781	100.262
5+5	6 (µg/ml)	100.130	99.204
5+5	6 (µg/ml)	99.781	98.982
	Mean	**99.897**	**99.483**
	Std	**0.202**	**0.684**
	%RSD	**0.202**	**0.688**

3.4 Repeatability:

The precision (system, method) of the proposed method was evaluated by carrying out six independent assays of the test sample. RSD (%) of six assay values obtained was calculated. The intermediate precision was carried out by analysing the sample on different days. The % RSD and % assay for repeatability and interday precision was found to be 0.193, 0.753, 0.230%, 0.777% and 99.960, 99.626, 100.144%, 100.116% for Nitrendipine and Hydrochlorothiazide respectively.

Table 6: Repeatability data of Nitrendipine and Hydrochlorothiazide

Concentration (µg/ml)	**Percentage recovery of Nitrendipine**	**Concentration (µg/ml)**	**Percentage recovery of Hydrochlorothiazide**
5	99.746	5	100.586
	100.002		98.870
	100.002		98.870
	99.746		99.177
	100.259		99.972
	100.002		100.279
Mean	**99.960**	Mean	**99.626**
Std	**0.193**	Std	**0.750**
%RSD	**0.193**	%RSD	**0.753**

Table 7: Interday Precision data of Nitrendipine and Hydrochlorothiazide

Concentrati	**Da**	**Percentage**	**Concentrati**	**Da**	**Percentage recovery of**

on (µg/ml)	y	recovery of Nitrendipine	on (µg/ml)	y	Hydrochlorothiazide
5	1	100.144	5	1	100.586
	2	99.886		2	99.485
	3	100.401		3	100.279
	4	100.144		4	100.586
	5	100.401		5	98.870
	6	99.886		6	100.893
	Mean	**100.144**		Mean	**100.116**
	Std	**0.230**		Std	**0.777**
	%RSD	**0.230**		%RSD	**0.777**

3.5 Limit of Detection and Limit of Quantitation:

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated by using the equations LOD = 3.3 x N / S and LOQ = 10 x N /S, where N is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.049 µg/ml and 0.150µg/ml for Nitrendipine and 0.026µg/ml and 0.078µg/ml for Hydrochlorothiazide respectively.

3.6 Ruggedness:

To evaluate the ruggedness of the proposed UV method, the analysis was performed by different analysts. The results presented in Table 8 indicated that the selected method has a percentage RSD less than 2%, indicating the developed method was unaffected and hence rugged.

Table 8: Ruggedness data of Nitrendipine and Hydrochlorothiazide

Analyst 1			
Concentration (µg/ml)	**Percentage recovery of Nitrendipine**	**Concentration (µg/ml)**	**Percentage recovery of Hydrochlorothiazide**
5	99.746	5	99.177
	99.746		100.586
	99.746		99.177
	100.002		100.279
	99.489		99.485
	99.489		100.893
Mean	99.703	Mean	**99.933**

Std	**0.193**	Std	**0.750**
%RSD	**0.194**	%RSD	**0.750**
Analyst 2			
Concentration (µg/ml)	**Percentage recovery of Nitrendipine**	**Concentration (µg/ml)**	**Percentage recovery of Hydrochlorothiazide**
5	99.489	5	100.893
	100.002		98.870
	99.746		100.586
	100.002		100.279
	99.489		99.485
	99.489		100.893
Mean	**99.703**	Mean	**100.168**
Std	**0.252**	Std	**0.823**
%RSD	**0.253**	%RSD	**0.822**

3.7 Robustness

To evaluate the robustness of the method, the optimized method parameters like isoabsorptive point were varied at different levels. The results presented in Table 9 indicated that the developed method was unaffected by small variations in the isoabsorptive point in the optimized method parameters.

Table 9: Robustness data of Nitrendipine and Hydrochlorothiazide

Wavelength (nm)	**Concentration (µg/ml)**	**Percentage recovery of Nitrendipine**	**Concentration (µg/ml)**	**Percentage recovery of Hydrochlorothiazide**
281	5	100.002	5	98.870
281	5	100.002	5	100.279
281	5	99.746	5	100.586
	Mean	**99.917**	Mean	**99.912**
	Std	**0.148**	Std	**0.915**
	%RSD	**0.148**	%RSD	**0.916**
282	5	99.746	5	99.177
282	5	100.259	5	99.972
282	5	100.002	5	100.279
	Mean	**100.002**	Mean	**99.809**
	Std	**0.256**	Std	**0.568**

	%RSD	**0.256**	%RSD	**0.569**
283	5	100.002	5	100.279
283	5	99.489	5	100.893
283	5	100.002	5	100.279
	Mean	**99.831**	Mean	**100.484**
	Std	**0.296**	Std	**0.355**
	%RSD	**0.297**	%RSD	**0.353**

3.8 Forced Degradation study:

Forced degradation studies were performed to demonstrate the stability of the sample. Degradation studies were carried out under conditions of acid, base, thermal, oxidation, and UV light. The forced degradation profile of Nitrendipine and Hydrochlorothiazide is given below in Table 10.

Table 10: Degradation profile of Nitrendipine and Hydrochlorothiazide in various stress conditions

Acid degradation								
Time interval (Hr)	**Percentage recovery**	**Percentage degradation**	**Percentage degradation**	**STD**	**Percentage recovery**	**Percentage degradation**	**Percentage degradation**	**STD**
1hr	96.412	3.588	2.990	0.533	98.944	1.056	2.242	1.333
	97.181	2.819			98.023	1.977		
	97.438	2.562			96.307	3.693		
3 hr	91.282	8.718	8.974	0.678	98.042	1.958	2.590	0.842
	90.256	9.744			96.454	3.546		
	91.539	8.461			97.735	2.265		
Basic degradation								
1hr	96.925	3.075	3.845	0.769	95.513	4.487	2.627	1.675
	95.386	4.614			98.763	1.237		
	96.155	3.845			97.842	2.158		
3 hr	95.386	4.614	5.213	0.533	95.946	4.054	3.337	1.399
	94.616	5.384			98.276	1.724		
	94.360	5.640			95.766	4.234		
Oxidative degradation								
1hr	97.694	2.306	3.246	0.8	98.817	1.183	1.466	0.6
	96.412	3.588			98.944	1.056		

	96.155	3.845		24	97.842	2.158		02
3 hr	90.256	9.744	10.684	0.900	90.820	9.180	8.524	1.296
	89.231	10.769			90.639	9.361		
	88.461	11.539			92.969	7.031		
Photolytic degradation								
1hr	98.720	1.280	0.853	0.529	98.997	1.003	1.984	1.441
	98.976	1.024			98.690	1.310		
	99.746	0.254			96.361	3.639		
3 hr	97.951	2.049	1.964	0.391	97.101	2.899	2.531	1.207
	97.694	2.306			98.817	1.183		
	98.464	1.536			96.487	3.513		
Thermal degradation								
1hr	99.489	0.511	0.853	0.296	99.485	0.515	1.515	1.115
	98.976	1.024			98.690	1.310		
	98.976	1.024			97.282	2.718		
3 hr	97.694	2.306	2.477	0.527	97.408	2.592	1.917	0.590
	97.951	2.049			98.510	1.490		
	96.925	3.075			98.330	1.670		

A forced degradation study of the combination of nitrendipine and hydrochlorothiazide was performed by exposing the sample for 1 and 3hr for each stress condition. In Acid degradation, the percentage degradation of the nitrendipine was found to be in a range of 2-9%, and for hydrochlorothiazide, it was found to be 2-3%. Similarly in the basic condition the percentage degradation was found to be 3-5% for nitrendipine and 2-4% for hydrochlorothiazide. In oxidation conditions, it was found to be 3-10% for nitrendipine and 1-9% for hydrochlorothiazide. Furthermore, in other stress conditions like UV and thermal stress conditions, the percentage degradation of the nitrendipine was found to be in a range of 1-3% and 1-3% for hydrochlorothiazide (Shakya A.K. *et. al.,* 2016; Tipre DN *et. al.,*2001).

Conclusion:

The developed UV-Spectroscopy Method for the estimation of two anti-hypertensive medications that are Nitrendipine and Hydrochlorothiazide using Shimadzu® UV-Spectrophotometer is new, simple, accurate, precise, linear, and sensitive for the simultaneous estimation of Nitrendipine and Hydrochlorothiazide in pharmaceutical tablet dosage form. So it can be employed for a routine analysis as well as control analyses for the pharmaceutical tablet dosage form of both medications.

Consent and Ethical Approval:

It is not applicable.

References:

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