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Development and Validation of Stability Indicating HPTLC Methods for the Estimation of Antihypertensive Drugs (Nebivolol and Hydrochlorothiazide)

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ABSTRACT

The devised method passed the ICH Q2 (R1) validation test, and the findings imply it might be utilised for both the regular monitoring of pharmaceutical formulation and raw material quality. Results for Nebivolol and Hydrochlorothiazide were consistently linear across concentration ranges of 4-24 and 10-60 µg/mL, respectively, according to the linearity study. The proposed approach was determined to be appropriate based on precision data and minimal relative standard deviation (RSD). Our lab confirmed the LOD and LOQ values for cilnidipine and lisinopril dihydrate. The method's accuracy within the given range is demonstrated by the low relative standard deviation (RSD) values. We found that the suggested method was linear, sensitive, accurate, and exact when it came to estimating hydrochlorothiazide and nebivolol in bulk and in pharmaceutical formulations. This conclusion was reached when the validation inquiry was finished and the results were discovered.

Keywords- ICH, Hypertension, Blood pressure, HPLC.

I. INTRODUCTION

In addition, despite efforts to reduce its prevalence, hypertension remains a substantial risk factor for cardiovascular disease and mortality [1, 2, 3, 4]. In 2015, approximately 1.5 billion individuals across the globe had systolic blood pressure readings of 140 mm Hg or diastolic readings of 90 mm Hg recorded at work [5]. A recent study found that the number of patients diagnosed with hypertension between the ages of 30 and 79 had more than doubled [6]. The number of people with a history of hypertension in 1990 was 331 million for women and 317 million for men. In 2019, 626 million women and 652 million men have hypertension in their family history. Seventy percent of the world's mortality and morbidity burden is attributable to systolic blood pressure of 140 mm Hg or more, according to research [7,8,9]. The World Health Organisation reports that cardiovascular diseases are the top killers and causes of disability worldwide. Despite significant strides in lowering blood pressure over the last several decades, hypertension (HTN) remains a key cause for worry as the leading preventable cause of death and disability from cardiovascular disease. The number of persons with hypertension in the age group of 30–79 has doubled by 2019 compared to the year 2010, even if the worldwide

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age-standardized prevalence has not changed [1]. In 2017, guidelines released by the American College of Cardiology and the American Heart Association narrowed the definition of hypertension to a systolic blood pressure of 130 mmHg or higher and/or a diastolic blood pressure of 80 mmHg or higher. The prevalence of hypertension rose from 18.9% to 43.5%, and this shift might have played a role in that [2,3,4]. The criteria (≥140/≥90 mmHg) did not change in either the 2018 European Guidelines or the most current 2020 International Society of Hypertension recommendations, regardless of whether this were true or not [3-5]. Despite differences in definition, a worldwide review of hypertension prevalence, treatment, and control revealed a substantial increase in the number of patients diagnosed with the condition between 1990 and 2019 [1]. Research has demonstrated that the heart's ventricular and atrial myocardium, together with the epicardial and intramural coronary arteries, can develop morphological, functional, and neurohumoral abnormalities as a result of long-term hypertension. This is the verdict that several studies have come to. Often shortened to "HD," hypertensive heart disease describes this condition. There is currently no widely accepted description or classification of HHD, despite the proposal of various terms to describe these morpho-functional changes, such as "hypertensive heart disease" [7,8,9], "hypertensive cardiomyopathy" [10], and "hypertensive heart failure" [11]. Approximately one-third of all fatalities in developed nations are attributable to cardiovascular disorders (CVD). High blood pressure, or hypertension, is a major risk factor for cardiovascular disease and one of the most pervasive risk factors overall [3, 4]. Nearly 40% of the population was affected by hypertension in 2008, when the disease was classified as affecting individuals aged 25 and up [3]. Heart disease and stroke account for at least 45% and 51% of all fatalities, respectively, on a global scale. High blood pressure. Approximately $329 billion in direct and indirect medical costs were attributed to cardiovascular illnesses in the US in 2013 and 2014 [5]. So, programmes that may effectively treat or prevent hypertension and are implemented on a large basis are urgently needed if we wish to alter this trend. Every day, more and more extensive evaluations of new and promising interventions are needed to drive evidence-based policy and clinical practice. Several randomised clinical trials (RCTs) have been carried out with the aim of preventing and managing hypertension [6–11]. It is not only impractical, but also inappropriate or unethical, to conduct randomised controlled trials (RCTs) on all treatments. This is so even though randomised controlled trials (RCTs) are the gold standard for deciding how well health interventions work (i.e., how they would perform in a perfect world). External validity is poor since interventions are typically conducted under more stringent circumstances. Moreover, the intended people or settings may not be the ones to which the results of randomised controlled trials (RCTs) are most relevant [11]. To find out how an intervention works in the real world and to get more communities to use evidence-based health interventions, researchers have come up with various experimental designs. Examples of this include experiments that occur in nature and quasi-experiments. The terms "quasi-experiments" and "natural experiments" are often thought to mean the same thing. This is something that many people do. These two ideas will be distinguished during the course of the inquiry, as has been done previously [12].

II. MATERIALS & METHODS

Nebivolol & Hydrochlorothiazide:

Preliminary Study

The physical and chemical properties of Nebivolol and Hydrochlorothiazide were researched, and this chapter presents a summary of the findings, which includes a description, a measurement of the melting point, and an identification using infrared spectroscopy.

Simultaneous Estimation of Nebivolol and Hydrochlorothiazide:

In Pharmaceutical Formulation by Dual Wavelength Spectroscopy Method Apparatus and Material

Both the UV Spectrophotometer and the Sartorius CP224S analytical balance were produced through the manufacturing process in Gottingen, Germany. Class 'A' volumetric glassware was utilised that was utilised in this process. In addition to methanol, the pharmaceutical company Torrent Pharmaceutical Ltd. was responsible for the production of both nebivolol and hydrochlorothiazide respectively.

Preparation of Stock Solutions

Following the evaluation of the powder's weight, ten milligrammes of were transferred to a volumetric flask that had a capacity of one hundred millilitres. Following that, the solution was diluted with methanol until it reached a volume of one hundred millilitres, which resulted in a concentration of one hundred micrograms per millilitre. To attain a concentration of 100 µg/ml, a quantity of 10 mg of was measured and then diluted with methanol until it reached a volume of 100 ml. Through this process, the concentration was achieved.

Preparation of Calibration Curve for Nebivolol and Hydrochlorothiazide:

In the following, you will find an explanation of the technique that was utilised in the creation of Series A, B, and C by making use of the standard stock solutions of nebivolol and hydrochlorothiazide. During the manufacturing process of Series A, Nebivolol and Hydrochlorothiazide, it was successfully achieved to achieve a concentration range of 4–24 µg/ml. It was essential to follow out this method in order to acquire the degree of concentration that required the most effort. For the purpose of manufacturing cilnidipine with a concentration range of 10–60 µg/ml, a sufficiently large quantity of the standard stock solution of cilnidipine was extensively diluted with methanol. In order to

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successfully create the concentration of 10–60 µg/ml, which was utilised for the manufacturing of Series B medication, this specific technique was carried out at the appropriate time. The Series C formulation contains two components: nebivolol and hydrochlorothiazide. These are the two components that contain the medicine. When it comes to the concentrations of hydrochlorothiazide, the range of concentrations is from 4 to 24 µg/ml, whereas the values of Nebivolol range from 10 to 60 µg/ml.

Preparation of Sample Solution

After pulverising two hundred tablets, each of which contained five milligrammes of Nebivolol and Hydrochlorothiazide, the pills were then placed into a volumetric flask that had a capacity of one hundred millilitres. Both tablets carried the same amount of medication. Subsequent to the incorporation of fifty millilitres of methanol into the mixture, it was subjected to sonication for a duration of twenty minutes. Following this, the solution was filtered through Whatman filter paper, and then methanol was applied to bring the volume up to the mark. The objective was to determine the ultimate concentration of Hydrochlorothiazide (25 µg/ml) and Nebivolol (10 µg/ml). To do this, two millilitres were extracted from his solution and transferred into a volumetric flask that had a capacity of ten millilitres by volume. After that, the volume of the flask was marked, which was the next step.

Method Validation

In order to validate the method, a number of validation parameters were utilised. These parameters included linearity, precision, recovery, limit of detection and limit of quantification, as well as the assay of nebivolol and hydrochlorothiazide. In accordance with the requirements of ICH Q2 (R1), each and every one of the validation parameters was accurately computed, and the results were presented.

III. RESULTS & DISCUSSION

Preliminary Study

Table 1: Physical Properties of Nebivolol and Hydrochlorothiazide

Physical Property	Nebivolol		Hydrochlorothiazide	
	Observed	Standard	Observed	Standard
Appearance	Crystalline Powder	Fine Crystalline Powder	White fine Powder	White fine Powder

Melting Point Determination

After taking the melting points of both API and comparing them to the melting points that were recorded, it was discovered that the melting points of both API fell within the range of the melting values that were reported.

Table 2 Melting Point Study of Drug Sample

Drug Sample	Observed Melting Point	Reported Melting Point
Nebivolol	222-230 C	220-240 C
Hydrochlorothiazide	272-274 C	273-275 C

IR Identification

IR Spectral Analysis of Nebivolol

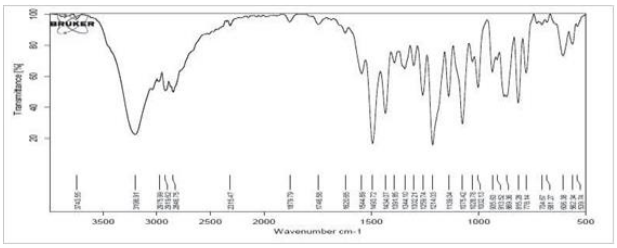

Figure 1: IR Spectra of Nebivolol

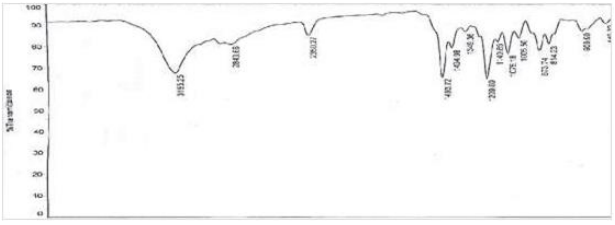

Figure 2: IR Spectra of Nebivolol Standard

A comparison was made between the KBr pellets technique and the reference spectrum, and it was discovered that the transmittance peaks in the API spectra were identical. The IR spectrum of Nebivolol was obtained using this technique.

Table 3: IR Spectral Interpretation of Nebivolol

Sr. No.	Observed Frequency (cm^{-1})	Mode of Vibration	Frequency Range (cm^{-1})
1.	873.74	Aromatic	800-900
2.	1493.72	N-N Stretching	1350-1500
3.	2843.66	C-H Stretching	2800-2900
4.	3195.25	OH Stretching	3300-3500

IR Spectral Analysis of Hydrochlorothiazide

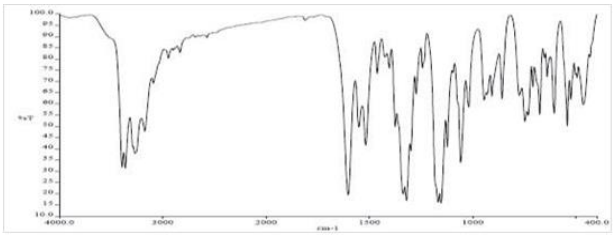

Figure 3 IR Spectra of Hydrochlorothiazide

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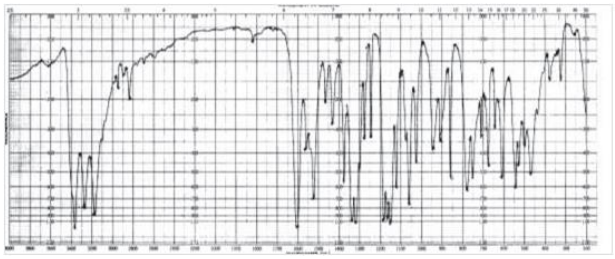

Figure 4: IR Spectra of Hydrochlorothiazide Standard

After using the KBr pellets approach to obtain the infrared spectrum of hydrochlorothiazide and comparing it to the reference spectrum, it was discovered that the transmittance peaks in the API spectra were identical.

Table 4 IR Spectral Interpretation of Hydrochlorothiazide

Sr. No.	Observed Frequency (cm^{-1})	Mode of Vibration	Frequency Range (cm^{-1})
1.	3471	N-H amine (ring)	3200-3500
2.	1486, 1591	C=C (aromatic)	1405-1550
3.	1339.88	C-N Stretching	1250-1280
4.	2843.66	C-H Stretching	2800-2900
5.	3195.25	OH Stretching	3300-3500
6.	3436	N-H (free)	3350-3500

The Dual Wavelength Spectroscopy Method for the Estimation of NEB and HTZ in Pharmaceutical Formulation at the Same Time Second wavelength λ_2 (286.0 nm) was selected where the absorbance of HTZ was same as at λ_1, and NEB was also given absorbance at this selected wavelength.

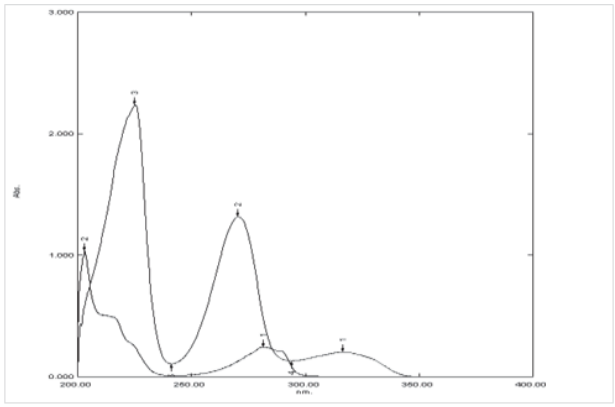

Figure 5 Overlain Spectra of Nebivolol and Hydrochlorothiazide

The difference in absorbance between these two wavelengths (A286.0–A315.5) is sufficient to balance out the contribution of the absorbance of HTZ that is present in the combination.

Table 5: Determination of NEB alone and NEB in the presence of HTZ

SERIES A				SERIES C			
Concentration of mixture (µg/ml)		Absorbance at 286.0 nm ± S.D (n = 5)	% RSD	Concentration of mixture (µg/ml)		Absorbance at 286.0 nm – 315.5 nm ± S.D (n = 5)	% RSD
NEB	HTZ			NEB	HTZ		
4	0	0.057 ± 0.001	1.75	4	10	0.057 ± 0.001	1.75
8	0	0.109 ± 0.001	0.91	8	20	0.108 ± 0.002	1.92
12	0	0.162 ± 0.003	1.85	12	30	0.163 ± 0.002	1.22
16	0	0.218 ± 0.001	0.45	16	40	0.218 ± 0.003	1.60
20	0	0.259 ± 0.004	1.73	20	50	0.264 ± 0.003	1.32
24	0	0.314 ± 0.005	0.18	24	60	0.323 ± 0.003	1.08

Table 6 Determination of HTZ alone and HTZ in the presence of NEB

SERIES B				SERIES C			
Concentration of mixture (µg/ml)		Absorbance at 315.5 nm ± S.D (n = 5)	% RSD	Concentration of mixture (µg/ml)		Absorbance at 315.5 nm ± S.D (n = 5)	% RSD
NEB	HTZ			NEB	HTZ		
0	10	0.105 ± 0.001	0.95	4	10	0.097 ± 0.0005	0.59
0	20	0.204 ± 0.0015	1.74	8	20	0.211 ± 0.003	1.44
0	30	0.298 ± 0.0005	0.19	12	30	0.313 ± 0.005	1.75
0	40	0.393 ± 0.003	0.77	16	40	0.404 ± 0.0005	0.14
0	50	0.494 ± 0.004	0.88	20	50	0.496 ± 0.005	1.00
0	60	0.603 ± 0.002	0.33	24	60	0.617 ± 0.003	0.48

Method Validation

Linearity

Both Nebivolol and Hydrochlorothiazide were found to have a linearity range that was confirmed to be between 4 and 24 µg/mL and 10 to 60 µg/mL, respectively. Nebivolol and hydrochlorothiazide were used as the standard and sample, respectively, in order to determine the specificity of the procedure. Both the normal NEB and the HTZ obtained a correlation value of 0.9987, while the HTZ had a coefficient of 0.9981.

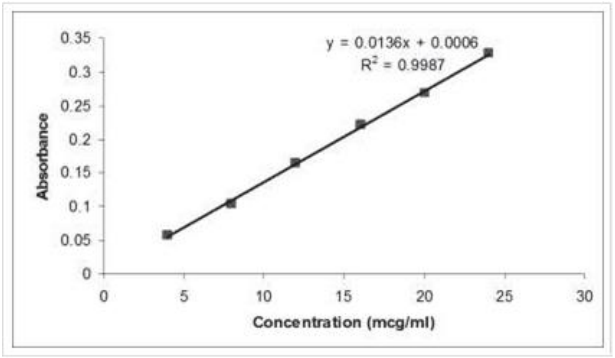

Figure 6 Calibration curve of Nebivolol

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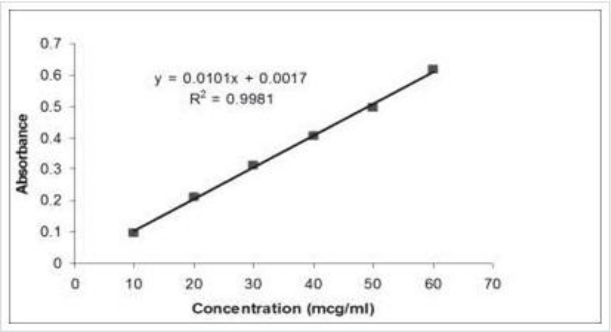

Figure7 Calibration curve of Hydrochlorothiazide

Precision

The DW Spectroscopic method was shown to have a relative standard deviation (RSD) of less than 2%, which implies that it is repeatable.

Table 7: Precision Data for Nebivolol and Hydrochlorothiazide

NEB and HTZ (10 : 25 µg/ml)	Absorbance at 286.0 nm	Absorbance at 315.5 nm	Absorbance at 286.0–315.5 nm
1	0.390	0.255	0.135
2	0.392	0.256	0.136
3	0.390	0.257	0.133
4	0.393	0.255	0.134
5	0.390	0.256	0.138
6	0.388	0.251	0.137
MEAN	0.390	0.255	0.135
S.D.	0.001	0.002	0.001
% RSD	0.450	0.820	1.380

Table 8: Regression Analysis Data for DW Spectroscopic method

Parameters	NEB estimation at 286.0 nm	HTZ estimation At 315.5 nm
Wavelength for measurement	286.0 nm	315.5 nm
Linearity	4–24 µg/ml	10–60 µg/ml
Regression line equation (Y)	Y=0.0136X + 0.0006	Y=0.0101X + 0.0017
Slop (b)	0.0136	0.0101
Intercept (a)	0.0006	0.0017
Correlation coefficient (r)	0.9987	0.9981
%Recovery (n = 5)	98.82%	100.24%

Recovery Study

The RSD values confirmed that the developed DW Spectroscopic method is accurate.

Table 9 Recovery data for the DW Spectroscopic Method

Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (µg/mL)	Amount of recovered (µg/mL)	% Recovery ± %RSD
NEB	I	10	7.5	17.23	98.48 ± 0.041
	II	10	10	19.66	98.30 ± 0.077
	III	10	12.5	22.08	98.16 ± 0.005
HTZ	I	25	18.75	43.89	100.32 ± 0.025
	II	25	25	50.32	100.65 ± 0.028
	III	25	31.25	56.56	100.55 ± 0.028

Analysis of Pharmaceutical Formulation

Nebivolol and hydrochlorothiazide were both determined to be present in tablet dosage forms by the utilisation of the DW spectroscopic approach. It was noticed that the excipient did not cause any interference; hence, the approach that was described is suitable for the routine assessment of nebivolol and hydrochlorothiazide in pharmaceutical formulations.

Table 10 Estimation of NEB and HTZ in Tablet Dosage Form

Formulations (Tablet)	Amount taken (µg/mL)		Amount found (µg/mL)		%Assay ± %RSD	
	NEB	HTZ	NEB	HTZ	NEB	HTZ
Set-1	10	25	9.88	25.07	98.82 ± 0.045	100.31 ± 0.025
Set-2	10	25	9.88	25.27	98.82 ± 0.045	101.10 ± 0.018

IV. CONCLUSION

The Dual Wavelength Spectroscopic method that was presented was successfully utilised in order to determine the levels of nebivolol and hydrochlorothiazide that were present in the sample. Based on the findings, it was decided that the current approach has the potential to be utilised for the routine testing of raw materials as well as in the formulation of pharmaceuticals. The method that was produced was verified in accordance with ICH Q2 (R1) without any variations, and the verification was carried out without any deviations. Based on the results obtained from the linearity investigation, it was determined that the proposed method was capable of producing linear outcomes across the entire concentration range of 4–24 and 10–60 µg/mL for Nebivolol and hydrochlorothiazide, respectively.

The findings of the precision data and the low levels of the relative standard deviation (RSD) led to the conclusion that the proposed approach was accurate. This conclusion was reached based on the previous sentence. The limits of detection (LOD) and limits of quantification (LOQ) for nebivolol and hydrochlorothiazide were established and determined. After conducting the experiment, it was found that the recovery rate for lisinopril dihydrate was 98.82%, while the recovery rate for cilnidipine was 100.24%.

Due to the fact that the relative standard deviation (RSD) figures were quite low, it may be concluded that the technique was accurate within the range that was specified. When it came to the simultaneous estimation of nebivolol and hydrochlorothiazide in bulk and pharmaceutical formulations, the approach that was provided was found to be linear, sensitive, exact, and accurate. As a result of the validation research being successfully completed and the discoveries that were uncovered, this conclusion was arrived at as a consequence.

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Volume-3 Issue-3 || June 2024 || PP. 158-164

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