SIMPLE AND ECONOMICAL UV-SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF CHLORTHALIDONE AND NEBIVOLOL IN COMBINED TABLET DOSAGE FORM: AN ALTERNATIVE APPROACH TO THE HPLC METHOD

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Three simple and affordable UV spectrophotometric methods have been proposed for the simultaneous determination of chlorthalidone and nebivolol in a synthetic mixture, as well as a combined dosage form. Method I use the simultaneous equation methodology and has a linearity range of 5–25 μg/mL for chlorthalidone at 233 nm and 5–90 μg/mL for nebivolol at 280 nm respectively. The linearity ranges for chlorthalidone at 228–238 nm and nebivolol at 275–285 nm were found to be 5–60 and 5–100 μg/mL respectively, using method II, the area under the curve method. The linearity range for method III, the first derivative method, is 10–35 μg/mL for chlorthalidone at 227 nm and 10–35 μg/mL for nebivolol at 275 nm. The two diagnostic plot residuals normal probability plot and residuals versus expected values plot are utilized for the verification of outcome data and found to be optimal for three methods. The method has been validated for accuracy, precision, recovery studies, linearity, specificity, and stability studies according to the International Council of Harmonisation guideline Q2R1. These developed methods have been utilized in routine analysis for the simultaneous determination of chlorthalidone and nebivolol without pre-extraction.

Keywords: *chlorthalidone, nebivolol, UV spectrophotometry, equation method, area under the curve, first derivative method, validation, pre-extraction.*

УФ-СПЕКТРОФОТОМЕТРИЧЕСКИЙ МЕТОД СОВМЕСТНОГО ОПРЕДЕЛЕНИЯ ХЛОРТАЛИДОНА И НЕБИВОЛОЛА В ТАБЛЕТИРОВАННОЙ ЛЕКАРСТВЕННОЙ ФОРМЕ КАК АЛЬТЕРНАТИВА ВЭЖХ-АНАЛИЗУ

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Предложены простые и доступные УФ-спектрофотометрические методы для одновременного определения хлорталидона и небиволола в синтетической смеси, а также комбинированной лекарственной форме. Метод I (метод линейного уравнения) показал диапазоны линейности 5–25 мкг/мл для хлорталидона при длине волны 233 нм и 5–90 мкг/мл, для небиволола при длине волны 280 нм; метод II (метод площади под кривой) — диапазоны линейности 5–60 мкг/мл для хлорталидона при 228–238 нм и 5–100 мкг/мл для небиволола при 275–285 нм соответственно; метод III (метод первой производной) — 10–35 мкг/мл для хлорталидона при 227 нм и 10–35 мкг/мл для небиволола при 275 нм. Для проверки данных использованы два диагностических графика остатков (нормальной вероятности и остатков) в зависимости от ожидаемых значений. Методы проверены на точность, прецизионность, линейность, специфичность и стабильность в соответствии с рекомендациями Международного совета по гармонизации Q2R1, использованы в рутинном анализе для одновременного определения хлорталидона и небиволола без предварительной экстракции.

Ключевые слова: *хлорталидон, небиволол, УФ-спектрофотометрия, метод уравнений, площадь под кривой, метод первой производной, валидация, предэкстракция.*

Introduction. Hypertension is a chronic pathological disease of the cardiovascular system characterized by diverse sequelae such as hypertrophic cardiomyopathy, hypertensive encephalopathy, and malignant retinopathy. In 2014, the global prevalence of hypertension in adults was 30% and is rising rapidly [1]. Nebivolol (nebi) is a highly selective, long-acting beta-blocker. It possesses vasodilatory effects that are mediated by nitric oxide (NO) through activation of the beta-3 receptor. Nebi is used to treat high blood pressure, either alone or in combination with other antihypertensive medicines that lower blood pressure [2]. In an experimental myocardial infarction model, nebivolol also showed decreased left ventricular dysfunction [3]. It is safe and effective in geriatric patients with heart failure [4].

It significantly lowers blood pressure in patients with hypertension, minimizing the risk of cardiovascular problems. Chlorthalidone (chlor) may be preferable over thiazide diuretics in the treatment of primary hypertension owing to the difference in pharmacokinetics and pharmacodynamics [4]. Currently, combination therapy using two or more antihypertensive drugs is widely recognized owing to its superior efficacy and reduced cardiovascular risk. Combination therapy reduces unwanted adverse effects and drug-induced tolerance by decreasing the concentration of the individual drug [5]. A combination of nebi (5 mg) + chlor (25 mg) tablets is available on the market to treat hypertension [6].

Chemically, chlor is 2-chloro-5-(1-hydroxy-3-oxo-2*H*-isoindoline-1-yl) benzene sulfonamide. The molecular weight is 338.8 g/mol and its chemical formula is $C_{14}H_{11}ClN_2O_4S$ [7]. Chemically nebi is (1R) 1-[(2R)-6-fluoro-3,4-dihydro-2H-1-benzopyran-2-yl] 2[(2R)-2-[(2S)-6-fluoro-3,4-dihydro-2H-1-benzopyran-2-yl][2-hydroxyethyl]amino}ethan-1-ol hydrochloride. The molecular weight of nebi is 405.435 g/mol and the chemical formula is $C_{22}H_{26}ClF_2NO_4$ [8].

Many researchers have reported the methods for the determination of nebi and chlor individually in active pharmaceutical ingredient (API) or in different matrices with other drugs using spectrophotometric [9–22], HPLC [23–47], and LCMS [48–59] methods. However, all the methods described in the literature have the disadvantage that the methods are complicated and time-consuming, require expensive chemicals and solvents for the separation process, and also require skilled technical personnel to operate the instrument. The preparation of the sample solution takes time. From the literature review, it is additionally concluded that there is no simultaneous determination of chlor and nebi using the spectroscopic method. Therefore, in accordance with the International Council of Harmonisation (ICH) Q2R1 guideline, we have developed a fast, inexpensive, and accurate method for the simultaneous measurement of chlor and nebi in a synthetic combination or experimental formulation. This newly established method can be adopted for routine analysis and quality control of the above drugs alone or in combination without prior separation.

Experimental. Reference standard chlor and nebi had been purchased from Yarrow Chem Pvt Ltd., Mumbai, 421201, India, and Triveni Interchem Pvt Ltd., Imran Nagar, Vapi Dist., Valsad, Gujarat, 396195, India respectively. Spectroscopic grade methanol was procured from SD Fine-Chem Ltd., Mumbai, 400013, India. Type I water used in the preparation of the solution was obtained from the Milli-Q apparatus, Model: VOE-WPS-ECO.

Shimadzu UV-Vis spectrophotometer model (S/N): UV-1700 (A11024403486) with quartz cells (1 cm) and a computer connection. UV Probe, Version: 2.43 software was used for data processing and interpretation.

Preparation of chlor and nebi stock solution. Fifty milligrams of chlor and nebi (reference standards) each were dissolved in 50% methanol and made up to the volume of 50 mL to achieve a concentration of 1000 µg/mL. Additionally, the same solvent system was used for the preparation of appropriate dilutions.

Different aliquots of stock solution (1000 µg/mL) were used to prepare different concentrations ranging from 5 to 35 and 5 to 75 µg/mL for chlor and nebi respectively. The absorption spectra of these solutions were recorded using a prepared solvent as a blank. Thereafter, the absorbance of chlor and nebi was measured at a maximum wavelength (λ_{max}).

The tablets are manufactured in-house and the average of investigational table is 102 mg only (nebi 5 mg and chlor 25 mg). The excipients of the formulated tablets include hydroxypropyl methyl cellulose, hydroxypropyl cellulose, tartaric acid, talc, and xanthan gum as bulking agents. The equivalent weight of a powder tablet was dissolved in 100 mL of 50% methanol and sonicated for 10 min at room temperature. The same solvent system was used to make additional dilutions, which were then filtered through Whatman 41 filter paper.

Method I: equation method. A calibration curve for chlor and nebi was plotted at an absorbance of 233 and 280 nm respectively against corresponding concentrations, followed by the determination of the regression equation for each drug [60].

Method II: area under the curve. The calibration curve was made by measuring and plotting the area under the curve (AUC) spectra of chlor and nebi within the wavelength range of 228 to 238 nm and 275 to 285 nm respectively, against the corresponding concentrations. The regression equation was also derived [61].

Method III: first derivatives method. To divide the spectra produced from the chlor and nebi amplitude at each wavelength, the absorption spectra of 25 μg/mL chlor and 55 μg/mL nebi were utilized as the corresponding divisors. The first-order (9 nm) derivative spectra of both the drugs were collected. Regression equations were created by plotting the absolute values of the 1D signals at 227 nm (for chlor) and 275 nm (for nebi) against the corresponding concentrations [62].

The proposed analytical technique has been verified as per the ICH criteria for various parameters, including linearity, the limit of detection (LOD) and limit of quantification (LOQ), accuracy, precision, specificity, and stability [63].

Result and discussion. *Equation method.* Two wavelengths, 233 and 280 nm, were selected from superimposed spectra for chlor and nebi, respectively (Fig. 1). The absorbance values were used to derive simultaneous equations. At specific wavelengths, the absorbances of the sample solution A_1 and A_2 were measured, and the following equation was used to determine the concentrations of the two drugs in the sample:

$$A_1 = 0.04682C_x + 0.0108C_y \text{ at 233 nm}, \quad (1)$$

$$A_2 = 0.00284C_x + 0.00146C_y \text{ at 280 nm}, \quad (2)$$

where A_1 and A_2 are the absorbances of the combination at 233 and 288 nm, respectively; 0.04682 and 0.00284 are the absorptivity of chlor at 233 and 280 nm, respectively; 0.0108 and 0.00146 are the absorptivity of nebi at 233 and 280 nm, respectively. The experimental data for the equation technique were fitted using slope equations for responses, as given in Table 1. Statistical analysis has been performed for variable interactions and pertinent effects using ANOVA. Chlor and nebi had P values of 0.0048 and 0.0009, respectively, demonstrating statistical significance at a 95% level of confidence. The ambit of fit of the polynomial model equation is illustrated by the R^2, coefficient of determination, as demonstrated in the result

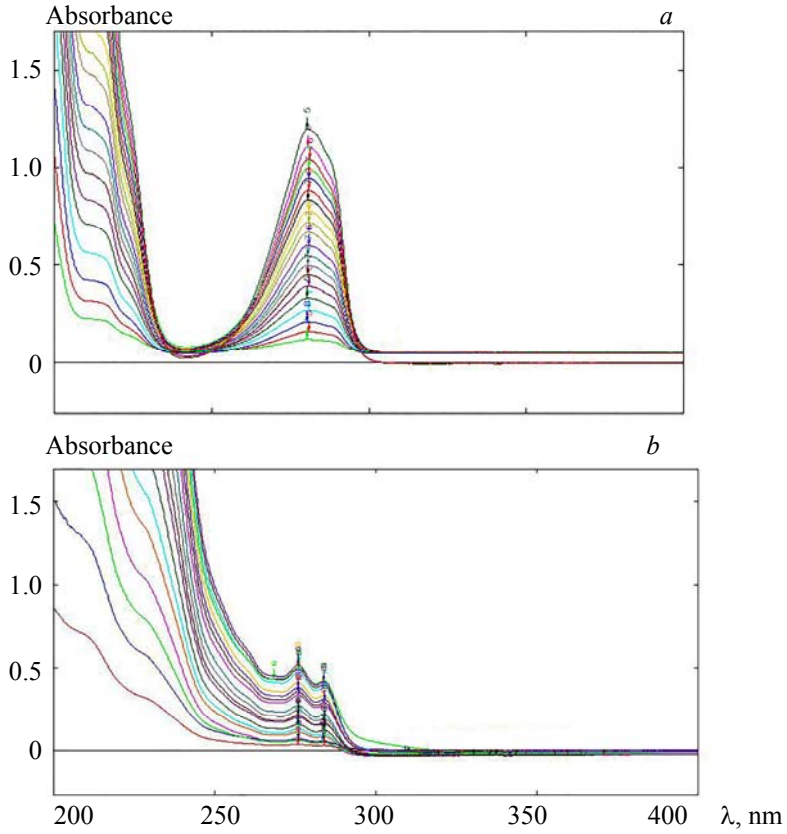

Fig. 1. UV overlain spectra of chlor (a) and nebi (b) for the simultaneous equation method.

as 0.998 and 0.999 for the respective values of chlor and nebi, whereas 0.9974 and 0.999 reflect the same for the modified R^2 values. The strong correlation between the experimental data and the fitted model is indicated by the high fitted R^2 values >0.80 [64, 65]. The response of chlor and nebi are obtained by studying a diagnostic plot such as a residuals normal probability plot and residuals versus expected values plot. A rigorous examination (Fig. 2) reveals that the residuals lie on a straight line, demonstrating that the errors are normally distributed and the model precisely fits the data [66]. In the residual versus expected response, no apparent pattern has been observed (Fig. 2). The plot displays almost equal variance above and below the *x*-axis, proving the applicability of the suggested model and upholding the independence and constant variance assumptions. The fitted model for the response of chlor and nebi can be accepted, as the constant variance and normality assumptions of the residuals were confirmed to be accurate [67].

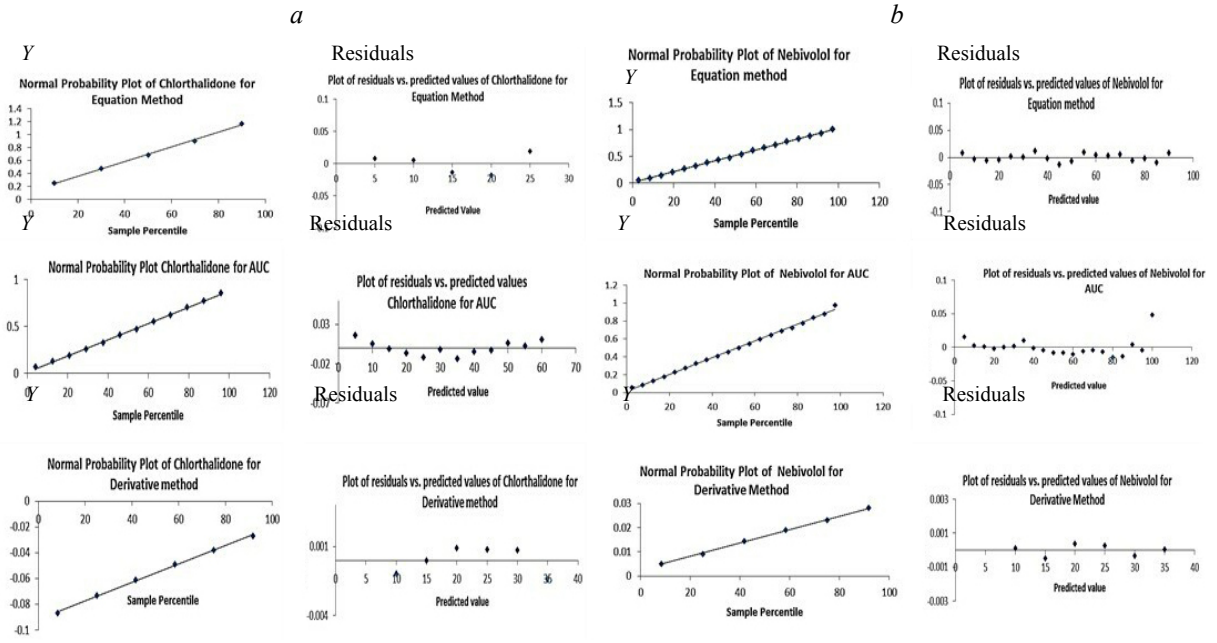

Fig. 2. Diagnostic plots of chlor (a) and nebi (b).

TABLE 1. Regression Table

Parameters	Method I		Method II		Method III	
	Chlor	Nebi	Chlor	Nebi	Chlor	Nebi
λ, nm	233	280	228–238	275–285	227	275
Range, μg/mL	5–25	5–90	5–60	5–100	10–35	10–35
Intercept (*a*)	0.0151	0.0143	0.0201	0.0085	0.0022	0.0043
Slope (*b*)	0.0453	0.0113	0.0144	0.0094	0.0024	0.0009
Correlation coefficient, *r*	0.998	0.999	0.9989	0.997	0.998	0.998
Adjusted correlation coefficient, *r*	0.9974	0.999	0.998	0.997	0.997	0.997
Standard error of intercept	0.019	0.003	0.005	0.006	0.001	0.0004
Standard deviation of intercept, Sa	0.042	0.015	0.019	0.029	0.003	0.001
Standard deviation of slope, Sb	0.040	0.030	0.031	0.062	0.002	0.0009
Limit of detection	1.96	1.93	1.70	1.82	3.65	3.49
Limit of quantitation	5.88	5.84	5.15	5.51	11.06	10.58
t-test	0.791	−4.035	−3.545	−1.308	−1.778	−9.717
P-value	0.0048	0.0009	0.0053	0.0020	0.0014	0.0006

Area under curve (AUC) method. In the presence of broad spectra and the absence of strong peaks, the AUC approach is used. This approach is based on calculating the integrated absorbance value between the two wavelengths of interest. This wavelength range was chosen after several experiments to get a good linear relationship between the AUC and the concentration. Calibration curves were recorded for the estimation of chlor and nebi using the AUC technique in their respective wavelength ranges 228–238 and 275–285 nm. For the tablet, the AUC is shifted to the range 260–270 and 295–305 nm for chlor and nebi, respectively. The region for both substances was then merged (Fig. 3). Two concurrent Eqs. (3) and (4), which were generated and solved, were used to determine the amounts of chlor and nebi:

$$A_1 = 0.0174C_x + 0.0560C_y \text{ at } 228–238 \text{ nm}, \quad (3)$$

$$A_2 = 0.004C_x + 0.0600C_y \text{ at } 275–285 \text{ nm}, \quad (4)$$

where A_1 and A_2 are the respective areas of the combination at 228–238 and 275–285 nm. The absorptivities of chlor at 228–238 and 275–285 nm are 0.0174 and 0.004, respectively. The absorptivities of nebi at 228–238 and 275–285 nm are 0.056 and 0.060, respectively. The experimental data obtained from the AUC equation method were fitted using slope equations for the responses and shown in Table 1. The most relevant effects and the variable interactions examined by ANOVA has been proved to be statistically significant, with P values of 0.0053 and 0.0020 for chlor and nebi, respectively, with a confidence level of 95%. The degree of accuracy of the polynomial model can be determined by calculating the R^2 coefficient of determination. In this case, the values of chlor and nebi have R^2 coefficients of 0.998 and 0.9975, respectively. Additionally, the modified R^2 values for chlor and nebi are 0.9987 and 0.9974 for the modified R^2 values [64, 65]. The fitted model and the empirical data have a strong correlation, as seen by the high fitted R^2 values >0.80. The response of chlor and nebi is tested using the diagnostic plots, including a residuals standard probability plot and a residuals vs expected values plot. The study (Fig. 2) reveals that the residuals are linear, indicating that the errors are typically distributed, and that the model fits the data well. There are no obvious trends in residuals vs projected values. The graph (Fig. 2) illustrates almost equivalent variability above and below the x-axis, suggesting the adequacy of the proposed model and not violating the speculations of independence or constant variance. Consequently, it was determined that the hypotheses of normality and constant variance of the residuals were adequate, and that the fitted framework for the response of chlor and nebi can be accepted [66, 67].

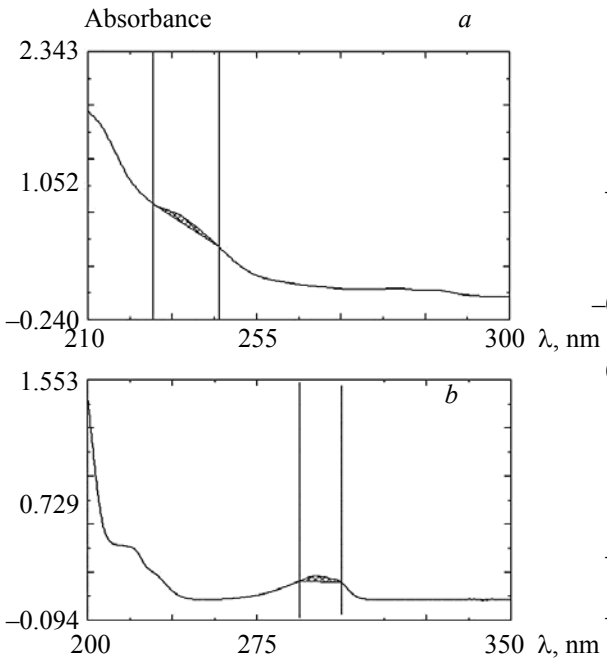

Fig. 3. UV overlain spectra of chlor (a) and nebi (b) for the AUC method.

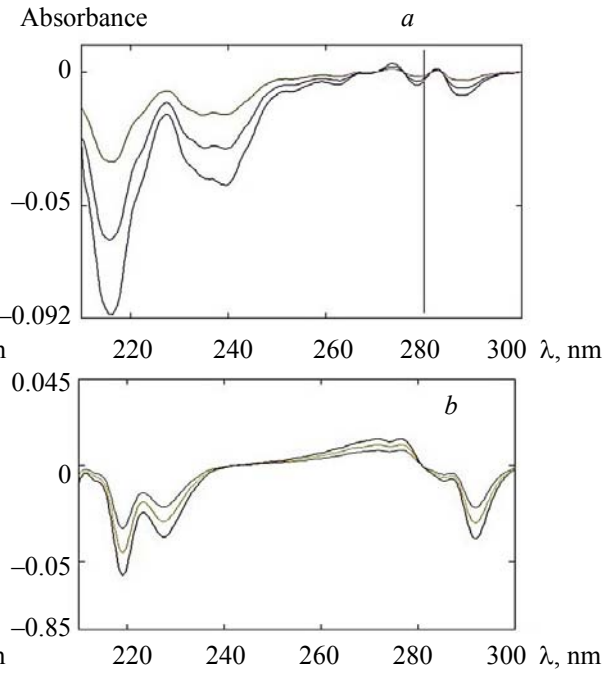

Fig. 4. UV overlain spectra of chlor (a) and nebi (b) for the 1D method.

First-derivative method (1D). In order to address the issue of overlap and simultaneous evaluation of the two medications, first-derivative spectrophotometry was investigated. For the purpose of determining the proper wavelength interval, the influence of the first derivative curves was investigated. The value influences the peak shapes, locations, and zero crossing points of the mixture's chemical components. Chlor could be detected at 227 nm using the initial (1D) derivative signals, even when the coincident values of nebi were zero. Similar to this, 1D spectra made it easier to investigate nebi at 275 nm, whereas chlor had no effect (Fig. 4). Equation technique empirical data were fitted utilizing slope equations for responses, as listed in Table. 1 The most pertinent effects and the variable interactions are examined using ANOVA. The *P* values for chlor and nebi are 0.00149 and 0.0006, showing the statistical value of an effect at the 95% confidence level. The level of fit of the polynomial model equation is illustrated by the R^2 coefficient of determination, as explained in the result as 0.9981 and 0.9983 for the respective values of chlor and nebi, whereas 0.9976 and 0.9979 do the same with the modified R^2 reflect values. The high fitted R^2 values >0.80 show a solid link between the experimental data and the fitted model [63, 64]. We investigate the responses of chlor and nebi using the diagnostic plots, including a normal probability plot of residuals and a plot of residuals vs actual values. Careful examination (Fig. 2) shows that the residuals lie on a straight line, showing that the errors are normally distributed, thus confirming the fact that the model fits the data satisfactorily. There is no obvious pattern followed in the residual vs expected response (Fig. 2). The graph shows almost equal variability above and below the *x*-axis, demonstrating the acceptability of the proposed model and not violating the assumptions of independence or constant variance. The fitted concept for the response of chlor and nebi may be accepted as the hypotheses of normality and constant variance of the residuals were found to be met [66, 67].

Linearity and range. Chlor and nebi response and corresponding concentrations were discovered to be linearly related. Calculations were made for the regression equation of each drug, $y = bc + a$ (Table 1). In these investigations, chlor or nebi was employed in at least six different concentrations. The high value of the correlation coefficients (*r*) of the regression equation and the low value of the percentage relative error served as evidence that the calibration curves were linear (Table 1). A slope and intercept standard deviation (Sb, Sa) as well as the analytical data for the calibration curves are given in Table 1. The linearity of the calibration curves is demonstrated by these statistics [68].

Limit of detection and limit of quantification. The LOQ and LOD were established in accordance with ICH recommendations. LOQ was the minimal concentration with a standard deviation-to-slope ratio of at least 10 ($\sigma/S \sim 10$), whereas the LOD was the lowest concentration with a standard deviation-to-slope ratio of at least 3 ($\sigma/S \sim 3$). Table 1 presents the findings.

Accuracy and precision. Using triplicate measurements for each concentration within a day, accuracy and intraday precision (reproducibility) for the suggested procedures were assessed at three concentration levels within the linearity ranges of each medication. Similar to this, accuracy and precision over a 3-day period (intermediate precision) were assessed using duplicate measurements of the same three concentrations. When the accompanying regression equations were used to determine the concentrations recovered, they were found to be reasonable. The outcomes of this experiment are provided in Tables 2 and 3. Recovery studies shown in Table 4 show properly recovered concentrations, together with low percentage relative standard deviation (percentage RSD) and percentage relative error (Er percent) values (less than 2.2%), which support the high precision and accuracy of the methodologies created to assess both drugs in their active pharmaceutical component form [68].

TABLE 2. Accuracy of the Drug Substance ($n = 6$)

Drug	Method	Nominal conc., µg/mL	Found conc., µg/mL±SD	Average error, %	% RSD
Chlor	I	10	10.01±0.088	0.193	0.881
		15	14.65±0.1011	−2.353	0.690
		20	19.28±0.263	−3.725	1.365
	II	25	22.12±0.280	−13.018	1.268
		30	26.79±0.347	−11.958	1.295
		35	31.72±0.367	−10.318	1.158
	III	20	19.38±0.240	−3.162	1.24
		25	24.59±0.318	−1.648	1.293
		30	28.97±0.240	−3.552	0.830

Continue Table 2

Drug	Method	Nominal conc., µg/mL	Found conc., µg/mL±SD	Average error, %	% RSD
Nebi	I	45	40.74±0.487	−4.259	1.196
		50	46.64±0.335	−3.359	0.718
		55	53.42±0.610	−2.951	1.142
	II	45	44.23±0.429	−1.729	0.971
		50	47.99±0.437	−4.182	0.910
		55	52.89±0.429	−3.993	0.812
	III	20	20.44±0.22	3.344	1.086
		25	25.96±0.231	3.703	0.890
		30	31.11±0.293	3.565	0.944

TABLE 3. Precision Data of Chlor and Nebi (n = 6)

Drug	Method	Nominal conc., µg/ml	Found conc., µg/ml±SD	Average error, %	% RSD
Chlor	I	15	14.309±0.439	−4.906	3.069
	II	30	28.129±0.242	−6.655	0.861
	III	30	29.361±0.340	−2.187	1.1586
Nebi	I	50	47.320±0.606	−5.677	1.282
	II	50	48.475±0.509	−3.155	1.051
	III	25	24.87±0.226	−0.528	0.911

Specificity. By keeping an eye out for interference from usual tablet excipients, the specificity of the method was examined, and it was shown that the signals were produced only by the analytes. Hydroxypropyl methyl cellulose, hydroxypropyl cellulose, tartaric acid, talc, and xanthan gum are the active ingredients of the formulation. It was discovered that the excipients had no bearing on the results (Fig. 5). It was found that chlor and nebi may both be assessed concurrently using the spectrophotometric method, whether they are co-formulated tablets or laboratory-prepared mixes.

TABLE 4. Recovery Studies of Chlor and Nebi (n = 3)

Drug	Method	Powdered tablet taken, mg	Add API, mg	Nominal conc., mg	Found conc., mg	Average conc. found, mg±SD	% Recovery	% RSD
Chlor (80% level)	I	114.80	20	45	43.5	42.93±0.513	96.66	1.195
		113.20	20	45	42.8		95.11	
		115.30	20	45	42.5		94.44	
	II	114.20	20	45	43.4	43.133±0.251	96.44	0.583
		115.10	20	45	43.1		95.77	
		115.40	20	45	42.9		95.33	
	III	115.00	20	45	43.3	43.06±0.251	96.22	0.584
		115.10	20	45	42.8		95.11	
		115.20	20	45	43.1		95.77	
Chlor (100% level)	I	115.00	25	50	48.8	48.26±0.472	97.62	0.979
		115.10	25	50	47.9		95.81	
		115.010	25	50	48.1		96.28	
	II	115.20	25	50	47.8	48.26±0.450	95.63	0.934
		115.50	25	50	48.3		96.64	
		115.10	25	50	48.7		97.44	
	III	115.40	25	50	47.7	47.90±0.435	95.48	0.909
		115.20	25	50	47.6		95.28	
		115.30	25	50	48.4		96.87	
Chlor (120% level)	I	115.10	30	55	54	53.43±0.550	98.18	1.030
		115.11	30	55	53.4		97.09	
		115.20	30	55	52.9		96.18	

Continue Table 4

Drug	Method	Powdered tablet taken, mg	Add API, mg	Nominal conc., mg	Found conc., mg	Average conc. found, mg±SD	% Recovery	% RSD
Chlor (120% level)	II	115.10	30	55	53.9	53.46±0.585	98.01	1.095
		115.30	30	55	52.8		96.22	
		115.02	30	55	53.7		97.63	
	III	115.20	30	55	53.1	53.36±0.378	96.54	0.709
		115.10	30	55	53.2		96.72	
		115.02	30	55	53.8		97.81	
Nebi (80% level)	I	115.50	4	9	8.6	8.60±0.100	95.55	1.162
		115.11	4	9	8.5		94.41	
		115.01	4	9	8.7		96.66	
	II	115.31	4	9	8.52	8.57±0.049	94.67	0.575
		115.11	4	9	8.6		95.56	
		115.21	4	9	8.61		95.67	
	III	115.30	4	9	8.69	8.64±0.104	96.55	1.208
		115.20	4	9	8.52		94.63	
		115.10	4	9	8.71		96.75	
Nebi (100% level)	I	115.01	5	10	9.62	9.61±0.090	96.22	0.938
		115.11	5	10	9.52		95.23	
		115.15	5	10	9.7		97.47	
	II	115.06	5	10	9.52	9.61±0.100	95.25	1.047
		115.10	5	10	9.72		97.29	
		115.01	5	10	9.6		96.33	
	III	115.15	5	10	9.5	9.58±0.127	95.32	1.329
		115.10	5	10	9.52		95.24	
		115.02	5	10	9.73		97.36	
Nebi (120% level)	I	115.10	6	11	10.8	10.59±0.176	98.18	1.662
		115.01	6	11	10.5		95.45	
		115.05	6	11	10.49		95.36	
	II	115.15	6	11	10.7	10.71±0.090	97.27	0.846
		115.02	6	11	10.63		96.63	
		115.05	6	11	10.81		98.27	
	III	115.02	6	11	10.6	10.70±0.096	96.36	0.897
		115.07	6.02	11	10.72		97.45	
		115.15	6	11	10.79		98.09	

Application of the developed methods. As the commercial dosage form was not available locally, the laboratory-prepared solution was assessed using the suggested spectrophotometric methods. The suggested methods were applied right away to the dosage form without extensive sample preparation procedures or extraction. None of the inactive substances interfered in any way. Additionally, statistical comparisons between the outcomes of the suggested spectrophotometric methods for the two medications were performed using ANOVA. The *P* values obtained within the threshold range show that there were no appreciable differences among the three suggested options. These findings made it abundantly evident that all the suggested techniques offer excellent and comparable analytical performance when used for both drugs in their combined formulation.

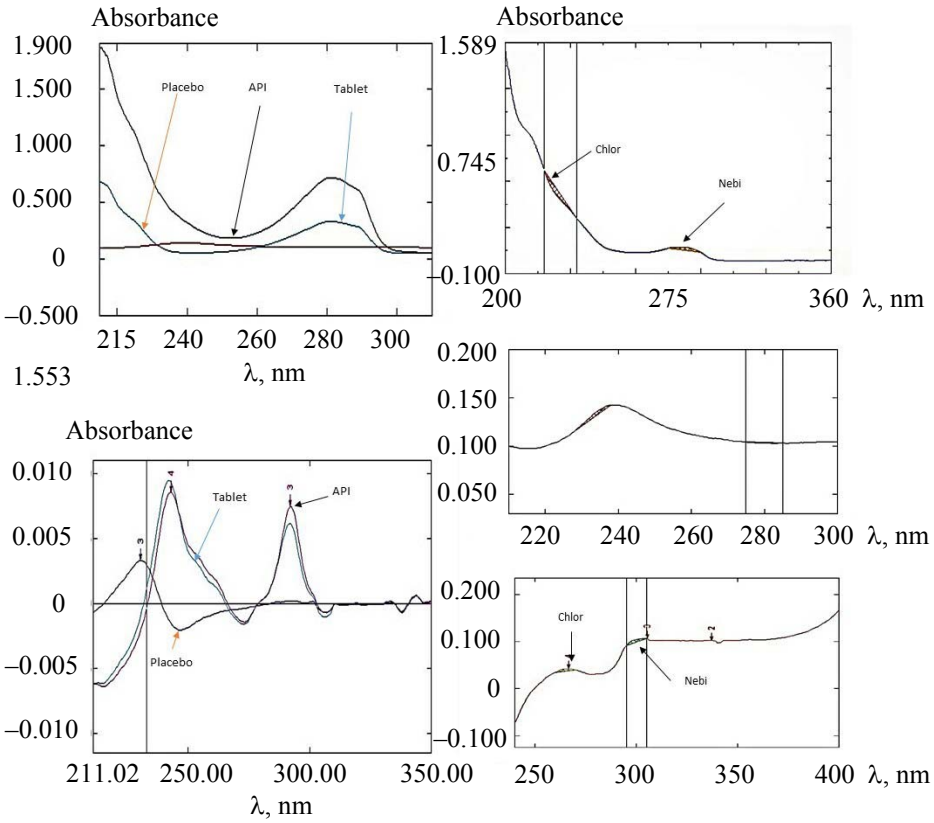

Fig. 5. UV spectra of both APIs (chlor and nebi), placebo, and tablet for the specificity study.

Conclusions. For the simultaneous measurement of chlorthalidone and nebivolol in pure form, synthetic combinations, and laboratory-produced dosage forms with different ratios of both active drugs, three novel, accurate, and reliable spectrophotometric procedures have been devised. In comparison with the organic solvents often utilized in chromatographic procedures, the 50% aqueous medium used for the spectrophotometric determination of the two drugs is superior, inexpensive, and ecologically benign. Furthermore, no major chemometric or mathematical modification of the absorbance data is necessary for the current spectrophotometric approaches. These techniques also have the benefit of being straightforward and not requiring costly or sophisticated equipment. Although a minute amount of impurity is present in both APIs, there is no significant impact on the estimation of the two drugs by UV spectroscopy. Finally, the suggested approaches were validated and successfully employed to distinguish chlorthalidone and nebivolol in pharmaceutical formulations and in pure form without any interference from other related excipients or matrix, and even without pre-separation.

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