Valsartan and hydrochlorothiazide in combination tablet dosage form: a simple spectrophotometric approach for simultaneous analysis

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Abstract

Background: Administration of VAL and HCT in combination has synergistic activity in hypertension management compared to each alone. Furthermore, combination products improve patient medication adherence. The combination product is recommended for further blood pressure control in patients not responding adequately to monotherapy

Objective: This research presents a UV spectrophotometric method of development and validation for simultaneous estimation of Valsartan (VAL) and Hydrochlorothiazide (HCT) combination in a tablet dosage form utilizing a cost-effective solvent.

Methods: The wavelengths selected for analysis were 248 nm and 271.5 nm for VAL and HCT, respectively. The method was linear at 4-40 µg/mL and 1-16 µg/mL for VAL and HCT, respectively, using 0.1 N NaOH as a solvent and Phosphate Buffered Saline (PBS) as the diluent.

Results: The mean % recovery was 100.61% and 100.2% for VAL and HCT, respectively, at three levels of standard addition (80, 100, and 120%.). In addition, the precision (intraday and interday) of the methods was within the limits (RSD < 2%).

Conclusion: The simultaneous estimation of valsartan and hydrochlorothiazide combination in a tablet dosage form is simple, rapid, accurate, precise, and economical. Therefore, its utilization should be considered in the quality control of pharmaceutical formulations and other routine laboratory analyses.

Keywords: *Valsartan; Hydrochlorothiazide; Spectrophotometry; Simultaneous analysis, Validation*

1. Introduction

Valsartan (VAL) is a nonpeptide tetrazole derivative that antagonizes type 1 angiotensin II receptor [1]. It is a potent orally active drug used to manage many cardiovascular conditions, including hypertension, congestive heart failure, myocardial infarction, and diabetes nephropathy[2]. It was developed by Novartis and formulated alone or with other drugs like hydrochlorothiazide[3]. Valsartan is 3-methyl-2-[pentanoyl-[[4-[2-(2*H*-tetrazolyl-5-yl)phenyl]phenyl]methyl]amino]-butanoic acid that has an empirical formula of $C_{24}H_{29}N_5O_3$, and molecular weight of 435.5 g/mol [4]. The chemical structure is shown in Fig. 1. It is available as a white, microcrystalline powder with a melting range of (105- 110) ˚C [5]. The partition coefficient of VAL is 0.033 (log P=1.499), indicating that the drug is relatively hydrophilic at physiological pH [6].

Hydrochlorothiazide is 6-chloro-1,1-dioxo-3,4-dihydro-2H-benzo[e][1,2,4]-thiadiazine-7-sulfonamide that has an empirical formula of $C_7H_8ClN_3O_4S_2$ [4]. Its chemical structure is shown in Fig. 2. It has a molecular weight of 297.7 g/mol and is available as crystals or white powder with a melting range of (273-275 °C) [7]. Hydrochlorothiazide (HCT) is a thiazide diuretic agent [8] used in combination with other agents to manage hypertension. The simultaneous equation method has been previously reported as a useful method using UV spectrophotometry to analyze combined drugs and estimate their concentrations in a mixture [9].

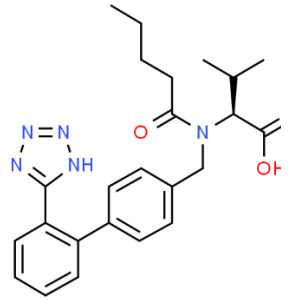

Figure 1: Structure of VAL[10]

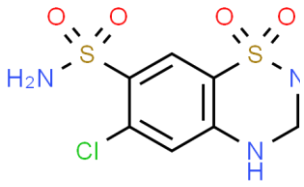

Figure 2: Structure of HCT[11]

Administration of VAL and HCT in combination has synergistic activity in hypertension management compared to each alone; furthermore, combination products improve patient medication adherence. Combination therapy is recommended for further blood pressure control in patients who do not respond to monotherapy [10]. Various analytical methods have been published for determining the amounts of valsartan and hydrochlorothiazide in tablet dosage forms using HPLC, GC, and HPTLC [11–13]. Even though the current methods are accurate, precise, and reproducible, they are quite expensive due to their expensive instrumentation, chemicals, and expertise.

Spectrophotometric UV methods are useful as they are simple, cost-effective, and suitable for simultaneous estimation of drugs for routine formulation analysis, with similar effectiveness compared to chromatographic methods [14]. The recently established analytical technique will be employed to evaluate the enhancement of permeability of valsartan and hydrochlorothiazide utilizing a permeapad membrane and a multi-station franz diffusion device. Since the concentrations of diffused APIs to the acceptor compartment

are typically relatively low, the method must be exceedingly accurate in detecting low levels of APIs. In this study, a spectrophotometric UV method was established, and validation was performed in compliance with the guidelines of the International Conference on Harmonization (ICH) and the Good Laboratory Practice directives for testing the drug content of tablets[13] . Furthermore, NaOH (0.1N) was used as a solvent in this study, and phosphate-buffered saline (PBS) was used for dilution, which is a cost-effective alternative to other organic solvents.

2. Materials and methods

2.1 Instruments

A double-beam PerkinElmer, Lambda 25. UV-Visible spectrophotometer, with wavelength range: 190-1100 nm, a spectral bandwidth of 1 nm, wavelength accuracy ±0.1 nm, wavelength reproducibility ±0.05 nm, Software UV Winlab version 5.1.2.0625, and a pair of 1 cm matched quartz cells, was used to measure the absorbance of the resulting solution.

2.2 Materials

Standard samples of Valsartan and Hydrochlorothiazide were provided by Jerusalem Pharmaceuticals Company Ltd. (JePharm). The marketed preparation Valzan HCT containing Valsartan 80 mg, Hydrochlorothiazide 12.5 mg was purchased from Pharmacare Pharmaceuticals Ltd. Sodium hydroxide (NaOH) and NaCl were purchased from Daejung Chemicals (South Korea). KCL was purchased from Fisher Scientific (USA). Na_2HPO_4 and KH_2PO_4 were purchased from Sigma-Aldrich (Germany).

2.3 Selection of an Appropriate Solvent System

From the literature, it was found that 0.1 NaOH can dissolve both VAL and HCT, it was suitable and stable [15].

2.4 Diluent

Phosphate buffer saline (PBS) with pH=7.4 was used as a diluent.

2.5 Preparation of Stock Standard Solutions

VAL (25mg) and HCT (25mg) were accurately weighed and separately transferred to 25ml volumetric flasks and dissolved in 0.1N NaOH, sonicated for three minutes, to give the standard stock solution of 1mg/ml for each. Next, 10 ml of each standard stock solution was transferred to a 100ml volumetric flask and diluted with PBS to obtain a working solution of 100 µg/ml. Aliquots were prepared by using PBS in the increasing concentration range.

2.6 Selection of Analytical Wavelengths

For the selection of analytical wavelength, stock standard solutions of VAL and HCT were scanned separately from 400 to 200 nm (Fig.3&4). The overlay spectra of both drugs were recorded (figure3 and 4). From overlay spectra, λ_{max} for VAL was 248nm (λ_1), and λ_{max} for HCT was 271.5 nm (λ_2). These wavelengths were selected to analyze both drugs using the simultaneous equation method.

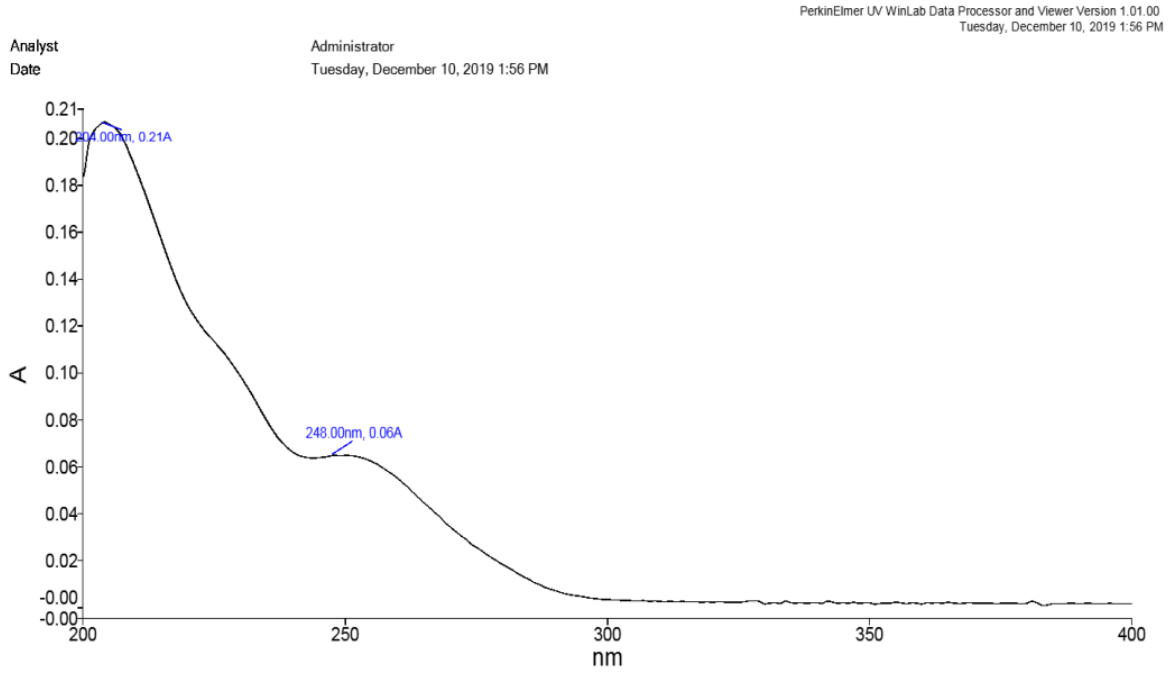

Figure 3: Overlay spectra of Valsartan

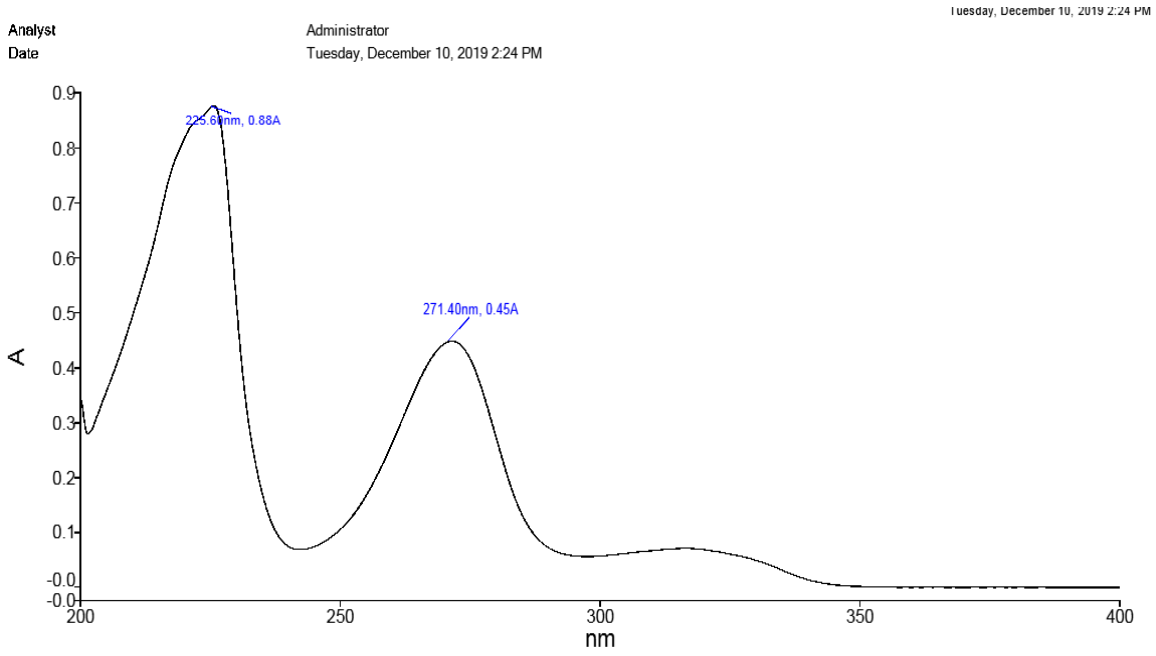

Figure 4: Overlay spectra of Hydrochlorothiazide

2.7. Linearity Study

Linearity was evaluated by analyzing different concentrations of the standard solution of VAL and HCT in the concentration range 1-40 µg/ml and 0.5-16 µg/ml for VAL and HCT, respectively. The mean of the three observations for each point was obtained. The absorbance was measured at 248 nm and 271.5 nm for VAL and HCT, respectively, and then plotted against concentration to obtain the calibration curves of VAL and HCT. Linearity was established in the concentration range 4–40 μg/mL for VAL and 1– 16 μg/mL for HCT.

Table 1. Summary of absorptivity values for VAL and HCT at 248 and 271.5.

API		Absorptivity values						mean	SD
VAL	at 248	325	313	323	328	310	321	320	7.042727
	at 271.5	169	162	155	154	165	161.54	161.09	5.76616
HCT	at 248	138	142	134	144	138	132	138	4.560702
	at 271.5	680	688	672	678	690	672	680	7.694154

2.8 Simultaneous Equation Method

The absorptivity values were calculated using the following formula:

A (1%, 1 cm) = Absorbance/Concentration (g/100 ml) Eq. 1

Where A (1%, 1 cm) is the absorptivity value.

The calculated values of absorptivity are presented in Table 1.

A simultaneous equation (formulas 2 to 4) was used to calculate the concentration of each component. As shown in table (1), the absorptivity values were substituted in the general formula (2) and (3) of the simultaneous equation method as follows [16]:

$A_1 = ax_1 C_x + ay_1 C_y$ Eq. 2

$A_2 = ax_2 C_x + ay_2 C_y$ Eq. 3

where, C_X = Concentration of VAL; C_y = Concentration of HCT; A_1 = Absorbance of mixture at 248 nm; A_2 = Absorbance of mixture at 271.5 nm; ax_1 = Absorptivity of VAL at 248 nm; ax_2 = Absorptivity of VAL at 271.5 nm; ay_1 = Absorptivity of HCT at

248 nm; ay_2 = Absorptivity of HCT at 271.5 nm.

After substitution and further calculations, the final equations were as follows:

$C_{VAL} = (A_{248} - 138\ C_{HCT})/320$ Eq. 4

$C_{HCT} = (A_{271.5} - 0.478\ A_{248})/613.93$ Eq. 5

Where C_{VAL} and C_{HCT} are the concentrations of valsartan and hydrochlorothiazide in the mixture, A248 and A271.5 absorb the mixture at λ=248 nm and λ=271.5 nm, respectively.

2.9. Application of Proposed Methods for Standard Mixture

A standard mixture of VAL and HCT was prepared by weighing 80 mg of VAL and 12.5 mg of HCT and dissolving them in 100 mL 0.1 N NaOH. Next, 1 mL of the solution was transferred to a 100 mL volumetric flask and diluted with PBS to produce 8 and 1.25 μg/mL of VAL and HCT, respectively. Then, it was analyzed. First, the absorbance was recorded at 248 nm and 271.5 nm. Then, the concentration of VAL and HCT was determined using equations (4) and (5). Results are shown in Table 2.

Table 2: Application of the proposed method for analysis of tablets.

Drug	Amount used (µg)	% Amount found (n=6)	% RSD
VAL	8	103	0.544
HCT	1.25	101	0.705

2.10. Application of Proposed Method for Analysis of Tablets

Ten tablets were weighed, the average weight determined, and finely powdered. A quantity of powder sample equivalent to 80 mg of VAL and 12.5 mg of HCT was transferred into a 100 mL volumetric flask containing 0.1 N NaOH, sonicated for 20 min; volume was adjusted to match the solvent volume and filtered through a syringe filter with 45 µm pore size. Then, 1 mL of this solution was transferred to 100 volumetric flasks and diluted with PBS to produce 8 µg/mL of VAL and 1.25 µg/mL of HCT. The final solution was analyzed against a blank sample using a UV spectrophotometer. Results are shown in Table 3.

Table 3: Application of the proposed method for analysis of tablets.

Drug	Amount used, (µg)	% Amount found (n=6)	% RSD
VAL	8	100.8	0.545
HCT	1.25	99.9	0.734

3. Validation of Proposed Methods

The method was validated in accordance with ICH and FDA requirements[17,18]. Validation parameters included linearity, accuracy, precision, specificity LOD, LOQ, ruggedness, and robustness.

3.1 Linearity

The linearity range for VAL and HCT was determined by plotting calibration curves at 248 nm and 271.5 nm, respectively. The correlation coefficients were close to unity in the 4–40 µg/ml concentration ranges for VAL and 1–16 µg/mL for HCT, demonstrating satisfactory linearity (Fig. 5&6).

3.2. Accuracy

The accuracy of the method was assessed using percentage recovery experiments performed at three levels, 80, 100, and 120%. Known amounts of standard VAL and HCT solutions were added to the pre-analyzed sample solutions, and the absorbance of each sample was recorded and reanalyzed by the simultaneous equation method. The recovery percentages were calculated using Equation (6). Results are shown in Table 4.

$$\% \text{ Recovery} = (A-B)/C * 100 \quad \text{Eq. 6}$$

Where A = total amount of drug estimated, B = amount of drug found on the pre-analyzed basis, and C = amount of bulk drug added.

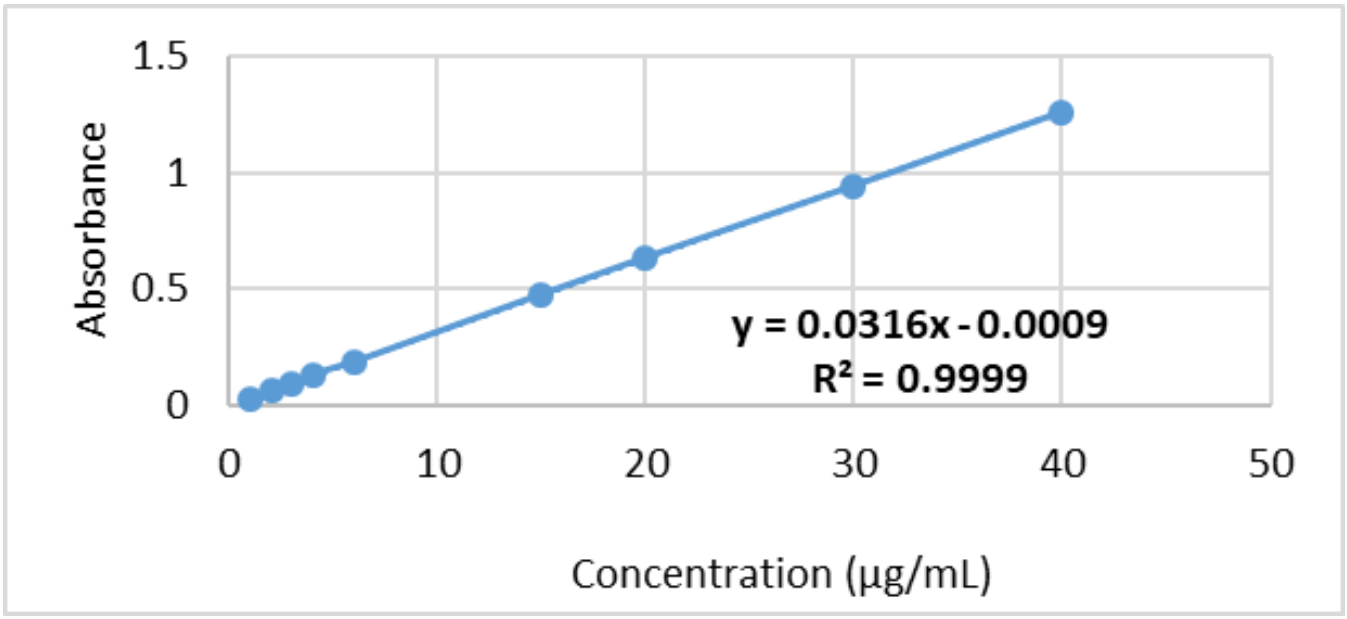

Figure 5: Calibration curve of VAL concentration (µg/mL) versus absorbance at 248

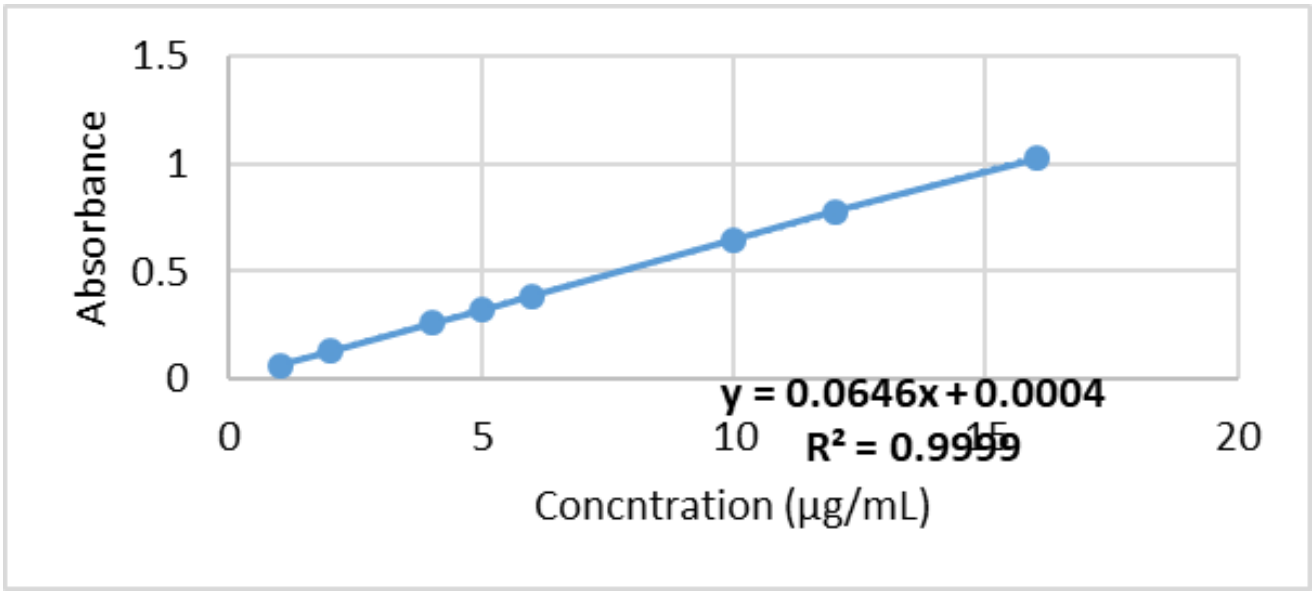

Figure 6: Calibration curve of HTC concentration (µg/mL) versus absorbance at 271.5

Table 4. Results for recovery study

Recovery level	Initial amount (µg/mL)		Concentration of drug added (µg/mL)		%, Recovery (n=3)		%RSD	
	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
80%	8	1.25	6.4	1	98.78	99.35	0.728	0.399
100%	8	1.25	8	1.25	101.95	100.83	0.358	0.138
120%	8	1.25	9.6	1.5	101.1	100.41	0.327	0.331
Mean					100.61	100.2		

3.3. Precision:

Precision is a measure of how close data values are to each other for several measurements under the same analytical conditions [19].

The intraday and interday precisions were determined by analyzing three standard solutions of VAL and HCT on the same day and three days over a week.

Intraday precision was determined by analyzing 8 μg/mL, 16 μg/mL, and 22.4 μg/mL for VAL and 1.25 μg/mL, 2.5 μg/mL, and 3.5 μg/mL HCT for three times within the same day. Interday precision was determined by analyzing the concentrations of the two drugs for three days over a week. The results are shown in Table 5.

Table 5: Summary of the precision study.

Drug	Concentration taken (µg/ml)	Intraday n=3		Interday n=3	
		% found	% RSD	% found	% RSD
VAL	8	100.1	0.76	100.8	0.555
	16	101	0.724	102	0.825
	22.4	100.3	0.631	100.3	1.724
HCT	1.25	99.7	0.736	99.1	0.278
	2.5	100.4	0.956	101.6	0.721
	3.5	99.5	0.736	100.6	1.466

3.4. Specificity:

An analytical method's specificity refers to its ability to distinguish between the analyte and its other parts of the complex mixture[20]. For specificity assessment, two excipients were used, starch and lactose. First, 80 mg VAL and 12.5mg HCT were weighed accurately and dissolved in 100ml 0.1 N NaOH. Next, 1 mL of the stock

solution was transferred to a 100 mL volumetric flask with 5 mL of 80 µg/mL starch solution and diluted with PBS. Next, 1 ml of the stock solution was transferred to another flask with 5 mL of 120 µg/mL lactose solution and diluted with PBS. Results are shown in Table 6.

Table 6: Summary of the specificity study.

Drug	Starch		Lactose	
	% found (n=3)	% RSD	%found (n=3)	% RSD
VAL	102	0.549	100.8	0.555
HCT	98.7	0.478	99.9	0.720

3.5. Ruggedness

It was proved by analyzing standard solutions of 8 μg/mL of VAL and 1.25 μg/mL of HCT by two different analysts using the same experimental and environmental conditions. Results are shown in Table 7.

Table 7. Summary of ruggedness studies.

Drugs	Starch		Lactose	
	% found (n=3)	% RSD	%found (n=3)	% RSD
VAL	100.1	0.76	100.2	0.736
HCT	99.7	0.736	99.2	0.738

3.6. Robustness

It was proved by analyzing the standard solutions 8 μg/mL of VAL and 1.25 μg/mL of HCT by two different solvent systems, methanol, and 0.1 N NaOH, using the same experimental and environmental conditions. Results are shown in Table 8.

3.7 Limit of Detection and Quantification (LOD & LOQ)

The LOD and LOQ were calculated based on the calibration curve and the standard deviation of the y-intercepts of the regression. Equations (7) and (8) were used.

$$\text{The detection lower limit (LOD)} = 3.3\ \sigma/S \quad \text{Eq. 7}$$

$$\text{The quantitation lower limit (LOQ)} = 10\ \sigma/S \quad \text{Eq. 8}$$

where,

σ = the standard deviation of the response, S = the slope of the calibration curve.

Excel 2016 and data analysis were used to obtain the standard error of intercept and regression. The LOD and LOQ values for VAL and HCT are summarized in table 9.

Table 8. Summary of the robustness study.

Drug	Methanol		0.1N NaOH	
	% found (n=3)	% RSD	%found (n=3)	% RSD
VAL	102	0.207	100.2	0.76
HCT	101.8	0.271	99.7	0.737

Table 9. Values of LOD and LOQ for VAL and HCT

API	LOD (µg/mL)	LOQ (µg/mL)
VAL	0.248	0.753
HCT	0.1946	0.589

4. Results and discussion

A simple, rapid, sensitive, and accurate UV-spectrophotometric analysis method of VAL and HCT was developed to serve as an alternative to HPLC for routine analysis. It will reduce the time, materials, and labor invested in sample preparation and analysis. An analytical method was developed for simultaneous estimation of VAL and HCT in combined pharmaceutical dosage form using a simultaneous equation. A method of analysis is often developed and validated by improving the conditions and parameters that should be followed while it is being developed and validated [21]. Various solvent systems (methanol, ethanol, acetonitrile, water, 0.1N HCl, and 0.1N NaOH) were studied to develop suitable methods of analysis based on sensitivity, availability, and toxicity. Phosphate buffer saline (PBS) pH=7.4 and 0.1 N NaOH were selected as solvent systems for the suggested method based on spectral behavior and solvent effect studies [22]. The new analytical method has the ability to detect concentrations that are significantly lower than those in previously published study[15,23]. According to the dual-wavelength approach, the absorbance difference between two points on the spectra is directly proportional to the component of interest, regardless of the interfering component [22].

As per the spectra in Fig. 3 and 4, we found that in 0.1 N NaOH, VAL and HCT showed maximum absorbance at 248 nm and 271.5 nm, respectively. Therefore, these wavelengths were selected for the determination of VAL and HCT.

We evaluated the linearity of the developed method by analyzing different concentrations of the standard solutions of VAL and HCT in triplicates. The linearity was observed in the range 4– 40 μg/mL ($R2$ = 0.999) of VAL and 1–16 μg/mL ($R2$ = 0.999) of HCT. As summarized in Table 10, the correlation coefficients were close to one, indicating good linearity.

A pharmaceutical formulation was analyzed using the proposed approach, and the % label claim of VAL and HCT was found to be 103.0 and 101.87, respectively. The drug amount determined using the proposed method corresponded well with the label claim. Recovery studies were conducted using the standard addition technique at three distinct levels (80%, 100%, and 120%.) to determine t the accuracy of the proposed procedure. As shown in table 10, the mean % recovery for VAL and HCT was found to be 100.61 and 100.2, respectively. Precision was calculated in terms of RSD percent at various levels, and repeatability was determined by analyzing three different concentrations of the pure active ingredients in triplicate.

The intraday and interday precision results revealed that the procedure was precise, which showed that the % of R.S.D. was less than 2^{24}. The results did not show any statistical difference between operators suggesting that the method developed was rugged. Furthermore, there was no statistical difference between using different solvents, indicating that the method was robust (table 8). The sensitivity of the method was assessed by determining LOD and LOQ values. For VAL, LOD and LOQ were found to be 0.248 and 0.753 μg/mL, respectively. For HCT, the LOD and LOQ were found to be 0.1946 and 0.589 μg/mL, respectively (Table 9). All the validation parameters are summarized in table (10).

Table 10. Summary of validation parameters

	VAL	HCT
λ_{max}	248	271.5
Linearity range(µg/ml)	4-40	1-16
Regression equation	y = 0.0316x - 0.0009	y = 0.0646x + 0.0004
Slope	0.0316	0.0646
Y- intercept	0.0009	0.0004
r^2	R^2 = 0.9999	R^2 = 0.9999
% Recovery (n=3)	100.61	100.2

LOD (µg/ml)	0.248	0.1946
LOQ (µg/ml)	0.753	0.589
Precision (% RSD)		
Intra- day (n=3)	0.948	0.821
Inter-day (n=3)	0.705	0.2076
Specificity (% RSD)		
Starch addition	0.549	0.478
Lactose addition	0.555	0.72
Ruggedness (% RSD)		
Analyst 1 (n=3)	0.76	0.76
Analyst 2 (n=3)	0.76	0.738
Robustness (% RSD)		
Methanol	0.2066	0.2719
0.1N NaOH	0.76	0.7367

5. Conclusion

The developed spectrophotometric method was successfully employed to determine VAL and HCT in pharmaceutical dosage forms simultaneously. The proposed methods were fully validated with satisfactory results for all the examined method validation parameters. The proposed spectrophotometric methods studied are simple, sensitive, accurate, precise, reproducible, specific, robust, and economical and can be used for the routine simultaneous estimation of VAL and HCT in pharmaceutical dosage forms.

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Author contribution

"Conceptualization, HN, WI.; methodology, HN, WI, MQ.; investigation, WI, AR; data curation, WI, HN., AA, NJ; writing—original draft preparation, WI, HN.; writing—review and editing, HN, NJ, AA, MQ, AR; supervision, HN., MQ; project administration, HN and MQ.; All authors have read and agreed to the published version of the manuscript.

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Data availability

Upon request, the corresponding author will provide the data used to support the conclusions of this study

Conflicts of interest

The authors declare that they have no conflict of interest.

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