Research Article

Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Valsartan and Hydrochlorothiazide in Tablet Dosage Form

Monika L. Jadhav,[1] Manoj V. Girase,[1] Shripad K. Tidme,[2] and Manish S. Junagade[2]

[1] *R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dhule District 425405, India*
[2] *MGV's College of Pharmacy, Panchavati, Nashik 422003, India*

Correspondence should be addressed to Monika L. Jadhav; monika.jadhav@yahoo.co.in

Two UV-spectrophotometric methods have been developed and validated for simultaneous estimation of valsartan and hydrochlorothiazide in a tablet dosage form. The first method employed solving of simultaneous equations based on the measurement of absorbance at two wavelengths, 249.4 nm and 272.6 nm, $\lambda_{\max}$ for valsartan and hydrochlorothiazide, respectively. The second method was absorbance ratio method, which involves formation of Q-absorbance equation at 258.4 nm (isoabsorptive point) and also at 272.6 nm ($\lambda_{\max}$ of hydrochlorothiazide). The methods were found to be linear between the range of 5–30 μg/mL for valsartan and 4–24 μg/mL for hydrochlorothiazide using 0.1 N NaOH as solvent. The mean percentage recovery was found to be 100.20% and 100.19% for the simultaneous equation method and 98.56% and 97.96% for the absorbance ratio method, for valsartan and hydrochlorothiazide, respectively, at three different levels of standard additions. The precision (intraday, interday) of methods was found within limits (RSD $<$ 2%). It could be concluded from the results obtained in the present investigation that the two methods for simultaneous estimation of valsartan and hydrochlorothiazide in tablet dosage form are simple, rapid, accurate, precise and economical and can be used, successfully, in the quality control of pharmaceutical formulations and other routine laboratory analysis.

1. Introduction

Valsartan (VAL) (Figure 1), a nonpeptide, is N-(1-oxopentyl)-N-[[2′-(1H-tetrazol-5-yl) [1,1′-biphenyl]-4-yl] methyl]-L-valine [1]. It is a potent, highly selective, orally active, specific angiotensin II receptor antagonist used as a hypotensive drug. Hydrochlorothiazide (HCT) (Figure 2) is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphon-amide,1,1-dioxide, which is a diuretic drug [2]. In patients with moderate hypertension, first-line therapy with the fixed-dose of valsartan/hydrochlorothiazide combination leads to BP normalization with high response rates. The rationale behind this drug combination is that in treatment of hypertension in patients whose blood pressure is not adequately controlled by monotherapy, oral administration of valsartan with

hydrochlorothiazide has been found more effective than the use of either drug alone [3, 4]. Very few methods for determination of valsartan and hydrochlorothiazide in tablet dosage form have appeared in the literature. A liquid chromatography/tandem mass spectrometry method includes protein precipitation using acetonitrile and the analytes were separated on a Zorbax SB-Aq C18 column using acetonitrile −10 mM ammonium acetate (60 : 40, v/v, pH 4.5) as mobile phase [5]. Another literature stated tablet formulation and development of validated stability indicating HPLC method [6] and HPLC method for simultaneous determination of amlodipine, valsartan, and hydrochlorothiazide in dosage form and spiked human plasma [7]. There are some methods for simultaneous estimation of both drugs using HPTLC [8, 9] and ion pair
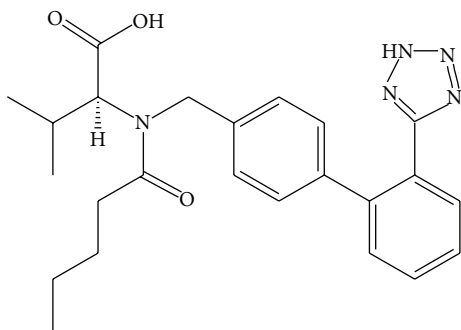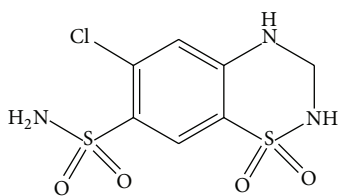
Figure 1: Structure of VAL.

Figure 2: Structure of HCT.

Figure 3: Overlay spectra of VAL and HCT.

chromatography [10]. Some UV spectroscopic techniques are also reported in which simultaneous method, AUC method, and first derivative method were developed [11–16].

Simultaneous estimation of drug combination is generally done by separation using chromatographic methods like HPLC, GC and HPTLC, and so forth. These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent, and expertise. Hence it is worthwhile to develop simpler and cost effective method for simultaneous estimation of drugs for routine analysis of formulation. Spectrophotometric analysis fulfils such requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods [17]. Another advantage of proposed methods is that 0.1 N sodium hydroxide was used as a solvent which is very economical as compared to methanol.

The purpose of this research was to establish such a method and, after validation in accordance with International Conference on Harmonization (ICH) guidelines [18] and the directives for good laboratory practice, to use the method for analysis of the drug content of tablets. The present work describes two spectrophotometric methods for estimation of valsartan with hydrochlorothiazide in combination which are simultaneous equation method (method 1) and absorbance ratio method (method 2).

2. Materials and Methods

2.1. Instrument. A double-beam Shimadzu UV-Visible spectrophotometer, with spectral bandwidth of 1 nm, wavelength accuracy ±0.5 nm, Model-UV 2450 PC (Japan), Software-UV-Probe, and a pair of 1 cm matched quartz cells, was used to measure absorbance of the resulting solution.
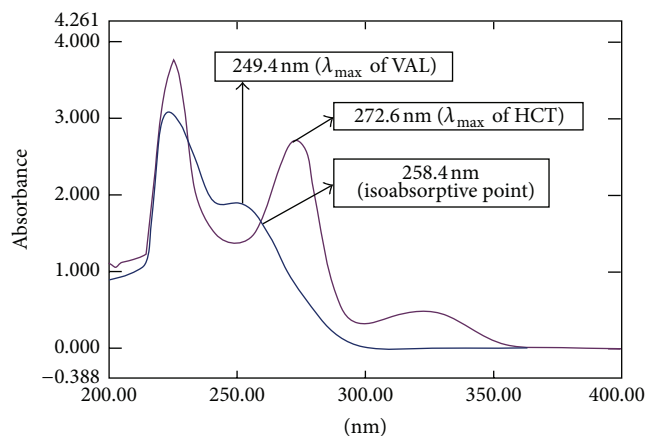
2.2. Materials. Standard gift sample of Valsartan was provided by Lupin Pharmaceuticals Ltd, Pune, and Hydrochlorothiazide by Jubilant Organosys Ltd., (Mysore), India. The marketed preparation VALENT-H containing Valsartan 80 mg, Hydrochlorothiazide 12.5 mg manufactured by Lupin Pharmaceuticals Pvt Ltd. (J & K), India, was used for analysis. Sodium hydroxide was procured from S.D Fine chem. Ltd, India, which was used as a solvent for both methods.

2.3. Selection of an Appropriate Solvent System. Various solvent systems like distilled water, 0.1 N HCl, 0.1 N NaOH, and methanol were tried to select an appropriate solvent with good suitability and stability. A solvent system, 0.1 N NaOH, was selected for the determination of valsartan and hydrochlorothiazide, since both drugs were soluble in 0.1 N NaOH.

2.4. Preparation of Stock Standard Solutions. Stock standard solution of valsartan (1000 μg/mL) was prepared by dissolving 10 mg valsartan in 10 mL of 0.1 N NaOH in 10 mL volumetric flask with vigorous shaking. From this stock standard solution, 1 mL was withdrawn and diluted to 10 mL using solvent to get working solution of 100 μg/mL. Similarly, stock standard solution of hydrochlorothiazide (1000 μg/mL) was prepared by dissolving 10 mg hydrochlorothiazide in 10 mL of 0.1 N NaOH in 10 mL volumetric flask with vigorous shaking. This solution was further diluted to get working solution of 100 μg/mL.

2.5. Selection of Analytical Wavelengths. For selection of analytical wavelengths, working solutions of both the drugs were scanned separately between 400 nm and 200 nm. The overlay spectra of both drugs were recorded (Figure 3). From overlay spectra, wavelengths 249.4 nm ($\lambda_{\max}$ of VAL) and 272.6 nm ($\lambda_{\max}$ of HCT) were selected for analysis of both drugs using simultaneous equation method. Also, wavelengths 258.4 nm (isoabsorptivity point) and 272.6 nm ($\lambda_{\max}$ of HCT) were selected for analysis of both drugs using absorbance ratio method.

TABLE 1: Application of proposed method for standard mixture.

Name of drug	Amount taken (mg)	% Amount found ($n = 6$)		% RSD	
		Method 1	Method 2	Method 1	Method 2
VAL	80	100.84	99.68	0.29	0.96
HCT	12.5	101.42	100.88	0.30	0.42

2.6. Linearity Study. The linear absorbances were obtained in the concentration range 5–30 μg/mL for VAL and 4–24 μg/mL for HCT. Absorbances of these solutions were measured at 249.4 nm and 272.6 nm, for method 1, and at 258.4 nm and 272.6 nm, for method 2. Calibration curve was constructed by plotting absorbance versus concentration. But the tablet ratio was 80 : 12.5, that is, 6.4 : 1 for VAL and HCT. Thus, for method development the concentration range selected for VAL was 12.8–76.8 μg/mL and for HCT was 2–12 μg/mL according to tablet ratio for both methods.

2.7. Method 1: Simultaneous Equation Method

2.7.1. Determination of A (1%, 1 cm) Values of Drugs at Selected Wavelengths. A (1%, 1 cm) values of drugs were calculated using the following formula:

$$A\,(1\%,\,1\,\text{cm}) = \frac{\text{Absorbance}}{\text{Concentration} \ (\text{g}/100\,\text{mL})}. \tag{1}$$

A set of two simultaneous equations were framed using these A (1%, 1 cm) values which are given below:

$$C_{\text{VAL}} = A_2 a y_1 - \frac{A_1 a y_2}{a x_2 a y_1} - a x_1 a y_2,$$

$$C_{\text{HCT}} = A_1 a x_2 - \frac{A_2 a x_1}{a x_2 a y_1} - a x_1 a y_2, \tag{2}$$

where A_1 and A_2 are absorbance of mixture at 249.4 nm and 272.6 nm; ax_1 and ax_2, A (1%, 1 cm) of VAL at 249.4 nm and 272.6 nm, respectively; and ay_1 and ay_2, A (1%, 1 cm) of HCT at 249.4 nm and 272.6 nm, respectively. C_{VAL} and C_{HCT} are concentrations of VAL and HCT in mixture.

By rearranging (2), concentration C_{VAL} and C_{HCT} can be obtained as follows:

$$C_{\text{VAL}} = \frac{A_2 \times 297.3 - A_1 \times 588.7}{-150753.36},$$

$$C_{\text{HCT}} = \frac{A_1 \times 144 - A_2 \times 328.8}{-150753.36}. \tag{3}$$

2.8. Method 2: Absorbance Ratio Method. A set of equations for absorbance ratio method were framed using these A (1%, 1 cm) values which are given below:

$$Q_x = \frac{ax_2}{ax_1}, \qquad Q_Y = \frac{ay_2}{ay_1}, \qquad Q_M = \frac{A_2}{A_1}, \tag{4}$$

where A_1 and A_2 are absorbance of mixture at 258.4 nm and 272.6 nm; ax_1 and ax_2, A (1%, 1 cm) of VAL at 258.4 nm and 272.6 nm, respectively; and ay_1 and ay_2, A (1%, 1 cm) of HCT at 258.4 nm and 272.6 nm, respectively. C_{VAL} and C_{HCT} are concentrations of VAL and HCT in mixture.

Concentration C_{VAL} and C_{HCT} can be obtained as follows:

$$C_{\text{VAL}} = \frac{Q_M - Q_Y}{Q_X - Q_Y} \cdot \frac{A_1}{ax_1},$$

$$C_{\text{HCT}} = \frac{A_1}{ax_1} - C_{\text{VAL}}. \tag{5}$$

2.9. Application of Proposed Methods for Standard Mixture. Standard mixture of VAL and HCT was prepared by weighing 80 mg of VAL and 12.5 mg of HCT in 10 mL of volumetric flask. 5 mL of 0.1 N NaOH was added to the volumetric flask and both drugs were dissolved completely. Finally, volume was adjusted up to the mark with 0.1 N NaOH to get the concentration of 8000 μg/mL of VAL and 1250 μg/mL of HCT. Appropriate volume 0.1 mL was transferred to 10 mL of volumetric flask and diluted up to mark with the same solvent to obtain strength 80 and 12.5 μg/mL of VAL and HCT, respectively; the solutions were scanned in the range 400–200 nm, and absorbances of the sample solutions were recorded at 249.4 nm and 272.6 nm, that is, A_1 and A_2, respectively, for method 1. The concentrations of the two drugs in sample solution (C_{VAL} and C_{HCT}) were determined, by using (3). For method 2, absorbances of the sample solutions were recorded at 258.4 nm and 272.6 nm, that is, A_1 and A_2, respectively. The concentrations of the two drugs in sample solution (C_{VAL} and C_{HCT}) were determined, by using (5). Results are shown in Table 1.

2.10. Application of Proposed Method for Analysis of Tablets. Twenty tablets were weighed, 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 10 mL volumetric flask containing 0.1 N NaOH, sonicated for 20 min; volume was adjusted to mark with same solvent and filtered through Whatmann filter paper no. 41. The resulting solution was further diluted to get concentration 80 μg/mL of VAL and 12.5 μg/mL of HCT. Prepared solution was scanned within 400 to 200 nm, and absorbance of sample solution at selected wavelengths was recorded against blank. For method 1, the concentrations of the two drugs in sample solutions (C_{VAL} and C_{HCT}) were determined, using (3). For method 2, the concentrations of the two drugs in sample solutions (C_{VAL} and C_{HCT}) were determined, using (5). The results of the same are reported in Table 2.

TABLE 2: Application of proposed method for analysis of tablets.

Tablet sample	Label claim (mg/tab)	% Label claim ($n = 6$)		% RSD	
		Method 1	Method 2	Method 1	Method 2
VAL	80	98.75	98.29	1.20	0.54
HCT	12.5	101.50	99.12	0.29	0.68

TABLE 3: Results of recovery studies.

Recovery level	Initial amount (μg/mL)		Concentration of std drug added (μg/mL)		% Recovery ($n = 3$)			
					Method 1		Method 2	
	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
80%	20	2.5	16	1.6	98.89	100.55	99.51	99.63
100%	20	2.5	20	2.0	100.98	99.83	100.8	99.76
120%	20	2.5	24	2.4	100.73	100.19	101.0	100.3
	Mean				**100.20**	**100.19**	**100.40**	**100.20**

3. Validation of Proposed Methods

The method was validated in terms of linearity, accuracy, precision, specificity LOD, LOQ, ruggedness, and robustness.

3.1. Accuracy. Accuracy of the method was assessed by percentage recovery experiments performed at three different levels, that is, 80, 100, and 120%. Known amounts of standard VAL and HCT solutions were added to the preanalyzed sample solutions; absorbances were recorded and reanalyzed by proposed method. The % recovery was calculated by using

$$\% \text{ Recovery} = \frac{A - B}{C} \times 100, \qquad (6)$$

where A = total amount of drug estimated, B = amount of drug found on preanalyzed basis, and C = amount of bulk drug added.

Results of recovery studies are shown in Table 3.

3.2. Precision. Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

3.2.1. Intraday and Interday Precision. Intraday and interday variations were determined by analyzing three different solutions of VAL and HCT within the same day and three different days over a period of week.

Intraday precision was determined by analyzing 38.4 μg/mL, 51.2 μg/mL, and 64 μg/mL for VAL and 6 μg/mL, 8 μg/mL, and 10 μg/mL HCT for three times within the same day.

Interday precision was determined by analyzing the above mentioned concentrations of both the drugs for three different days over a period of week. The results are shown in Table 4.

3.3. Specificity. The interference of other excipients was evaluated. 10 μg/mL of microcrystalline cellulose and starch were added separately to standard solutions 64 μg/mL of VAL and 10 μg/mL of HCT using the same experimental and environmental conditions.

3.4. Ruggedness. Ruggedness of the method was proved by analyzing the standard solutions 64 μg/mL of VAL and 10 μg/mL of HCT by two different analysts using the same experimental and environmental conditions.

3.5. Robustness. Robustness of the method was proved by analyzing the standard solutions 64 μg/mL of VAL and 10 μg/mL of HCT by two different solvents using the same experimental and environmental conditions.

4. Results and Discussions

4.1. Method 1. Analytical method has been developed for simultaneous estimation of VAL and HCT in combined pharmaceutical dosage form using simultaneous equation. In 0.1 N NaOH, VAL showed maximum absorbance at 249.4 nm and HCT at 272.6 nm. Linearity was observed in the range 5–30 μg/mL ($R^2 = 0.998$) of VAL and 2–16 μg/mL ($R^2 = 0.998$) of HCT. The proposed method was applied for pharmaceutical formulation, and % label claim of VAL and HCT was found to be 98.75 and 101.50, respectively. The amount of drug estimated by proposed method was in good agreement with the label claim. Accuracy of the method was checked by the recovery studies at three different levels, that is, 80%, 100%, and 120%. The mean % recovery for VAL and HCT was found to be 100.20 and 100.19, respectively. The method was found to be precise as indicated by the interday and intraday analysis, showing that % R.S.D. is less than 2. The results did not show any statistical difference between operators suggesting that method developed was rugged. Also, there was no any statistical difference between various strengths of solvents suggesting that method was robust. The sensitivity of method was assessed by determining LOD and LOQ. For VAL, LOD and LOQ were found to be 1.60 and 4.87 μg/mL, respectively. For HCT, the LOD and LOQ were found to be 0.37 and 1.12 μg/mL, respectively.

TABLE 4: Results of intraday and interday precision.

Drug	Amount taken (μg/mL)	Intra-day ($n = 3$) % RSD		Inter-day ($n = 3$) % RSD	
		Method 1	Method 2	Method 1	Method 2
VAL	38.4	0.368	1.589	1.122	1.055
	51.2	0.358	0.760	0.879	0.908
	64	0.276	1.076	0.793	0.799
	Mean	**0.334**	**1.143**	**1.143**	**0.920**
HCT	6	1.439	1.085	1.722	1.376
	8	1.674	1.059	1.227	1.360
	10	0.970	0.987	1.256	0.895
	Mean	**1.361**	**1.044**	**1.044**	**1.210**

TABLE 5: Summary of validation parameters.

Methods	Method 1		Method 2	
	VAL	HCT	VAL	HCT
$\lambda_{\max}$	249.4 nm	272.6 nm	258.4 nm	272.6 nm
Linearity range (μg/mL)	12.8–76.8	2–12	12.8–76.8	2–12
Regression equation	$Y = 0.150x + 0.081$	$Y = 0.181x + 0.079$	$Y = 0.0269X + 0.064$	$Y = 0.0542X + 0.013$
Slope (m)	0.150	0.181	0.0269	0.0542
Y – intercept (c)	0.081	0.079	0.064	0.013
Correlation coefficient (r^2)	0.998	0.998	0.999	0.999
% recovery ($n = 3$)	100.20	100.19	100.40	100.20
LOD (μg/mL)	1.60	0.37	1.60	0.32
LOQ (μg/mL)	4.87	1.12	4.86	0.97
Molar absorptivity (lit/mole/cm)	14894.78	16613.89	12242.19	16643.66
Sandell's sensitivity (μg/sqcm/0.001)	0.02924	0.01793	0.03558	0.01789
Standard error	1.196×10^{-3}	2.468×10^{-3}	0.8381×10^{-3}	2.8324×10^{-3}
Precision (% RSD)				
Intra- day ($n = 3$)	0.334	1.361	1.143	1.044
Inter-day ($n = 3$)	0.931	1.402	0.920	1.210
Specificity (% RSD)				
Addition of MCC	1.6	1.8	0.9	1.2
Addition of starch	0.5	0.8	0.3	0.7
Ruggedness (% RSD)				
Analyst I ($n = 3$)	0.5	0.6	0.2	0.5
Analyst II ($n = 3$)	1.2	1.1	1.1	0.9
Robustness (% RSD)				
0.2 N NaOH ($n = 3$)	0.8	1.2	1.6	1.5
0.5 N NaOH ($n = 3$)	0.5	1.3	0.8	1.2

4.2. Method 2. Analytical method has been developed for simultaneous estimation of VAL and HCT in combined pharmaceutical dosage form using absorbance ratio method. In 0.1 N NaOH, wavelengths 258.4 nm (isoabsorptivity point) and 272.6 nm ($\lambda_{\max}$ of HCT) were selected for analysis of both drugs using absorbance ratio method. Linearity was observed in the range 5–30 μg/mL ($R^2 = 0.999$) of VAL and 2–16 μg/mL ($R^2 = 0.999$) of HCT. The proposed method was applied for pharmaceutical formulation, and % label claim of

VAL and HCT was found to be 98.29 and 99.12, respectively. The amount of drug estimated by proposed method was in good agreement with the label claim. Accuracy of the method was checked by the recovery studies at three different levels, that is, 80%, 100%, and 120%. The mean % recovery for VAL and HCT was found to be 100.40 and 100.20, respectively. The method was found to be precise as indicated by the interday and intraday analysis, showing that % R.S.D. is less than 2. The results did not show any statistical difference between

operators suggesting that method developed was rugged. Also, there was not any statistical difference between various strengths of solvents suggesting that method was robust. The sensitivity of method was assessed by determining LOD, and LOQ. For VAL, LOD and LOQ was found to be 1.60 and 4.86 μg/mL, respectively. For HCT, the LOD and LOQ was found to be 0.32 and 0.97 μg/mL, respectively.

The summary of validation parameters is presented in Table 5.

5. Conclusion

The proposed spectrophotometric methods were found to be simple, sensitive, accurate, precise, reproducible, specific, robust, and economical and can be used for the routine simultaneous estimation of VAL and HCT in pharmaceutical formulations.
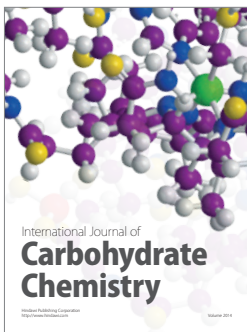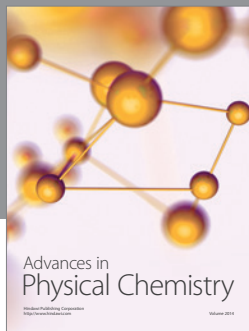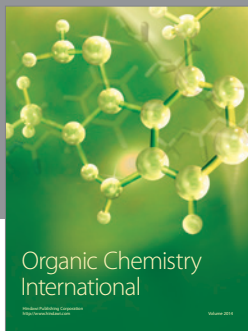
Conflict of Interests

There are no competing interests amongst the authors. The authors declare that there is no conflict of interests.

Acknowledgment

References

[1] Indian Pharmacopoeia, *Government of India, Ministry of Health and Family Welfare*, vol. 3, The Indian Pharmacopoeia Commission, Ghaziabad, India, 6th edition, 2010.

[2] Indian Pharmacopoeia, *Government of India, Ministry of Health and Family Welfare*, vol. 2, The Indian Pharmacopoeia Commission, Ghaziabad, India, 6th edition, 2010.

[3] K. Wellington and D. M. Faulds, "Valsartan/hydrochlorothiazide: a review of its pharmacology, therapeutic efficacy and place in the management of hypertension," *Drugs*, vol. 62, no. 13, pp. 1983–2005, 2002.

[4] R. Kondrack and S. Mohiuddin, "Valsartan/hydrochlorothiazide: pharmacology and clinical efficacy," *Expert Opinion on Drug Metabolism and Toxicology*, vol. 5, no. 9, pp. 1125–1134, 2009.

[5] H. Li, Y. Wang, Y. Jiang et al., "A liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of valsartan and hydrochlorothiazide in human plasma," *Journal of Chromatography B*, vol. 852, no. 1-2, pp. 436–442, 2007.

[6] M. Kharoaf, N. Malkieh, M. Abualhasan, R. Shubitah, N. Jaradat, and A. N. Zaid, "Tablet formulation and development of a validated stability indicating HPLC method for quantification of valsartan and hydrochlorthiazide combination," *International Journal of Pharmacy & Pharmaceutical Sciences*, vol. 4, no. 3, pp. 284–290, 2012.

[7] S. M. El-Gizawy, O. H. Abdelmageed, M. A. Omar, S. M. Deryea, and A. M. Abdel-Megied, "Development and validation of HPLC method for simultaneous determination of amlodipine, valsartan, hydrochlorothiazide in dosage form and ppiked human plasma," *American Journal Analytical Chemistry*, vol. 3, pp. 422–430, 2012.

[8] B. R. Kadam and S. B. Bari, "Quantitative analysis of valsartan and hydrochlorothiazide in tablets by high performance thin-layer chromatography with ultraviolet absorption densitometry," *Acta Chromatographica*, no. 18, pp. 260–269, 2007.

[9] N. J. Shah, B. N. Suhagia, R. R. Shah, and N. M. Patel, "HPTLC method for the simultaneous estimation of valsartan and hydrochlorothiazide in tablet dosage form," *Indian Journal of Pharmaceutical Sciences*, vol. 71, no. 1, pp. 72–74, 2009.

[10] N. M. Bhatia, M. S. Bhatia, P. B. Choudhari, and K. B. Ingale, "Development and validation of spectrophotometric and ion pair chromatographic technique for estimation of valsartan and hydrochlorothiazide," *Journal of Pharmaceutical Research and Health Care*, vol. 2, no. 1, pp. 2–14, 2010.

[11] M. M. Deshpande, M. P. Ahajan, and S. D. Sawant, "Simultaneous estimation of valsartan and hydrochlorothiazide in fixed dose combination in UV spectrophotometry," *International Journal of Pharmaceutical Sciences*, vol. 3, no. 1, pp. 235–240, 2012.

[12] A. B. Chaudhary, R. K. Patel, S. A. Chaudhary, and K. V. Gadhvi, "Estimation of valsartan and hydrochlorothiazide in Pharmaceutical dosage forms by absorption ratio method," *International Journal of Applied Biology and Pharmaceutical Technology*, vol. 1, no. 2, pp. 455–464, 2010.

[13] E. atana, A. Altınay, N. Gunden, S. A. Ozkan, and Z. Enturk, "Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first-derivative UV spectrophotometry and LC," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 25, no. 5-6, pp. 1009–1013, 2001.

[14] N. Erk, "Spectrophotometric analysis of valsartan and hydrochlorothiazide," *Analytical Letters*, vol. 35, no. 2, pp. 283–302, 2002.

[15] R. G. Varsha, K. G. Baheti, S. Indraksha, and M. H. Dehghan, "Estimation of amlodipine besylate, valsartan and hydrochlorothiazide in bulk mixture and tablet by UV spectrophotometry," *Indian Journal of Pharmaceutical Sciences*, vol. 74, no. 1, pp. 18–23, 2012.

[16] V. K. Redasani, P. V. Patel, and S. J. Surana, "Spectrophotometric method for simultaneous estimation of valsartan and hydrochlorothiazide in combined tablet dosage form," *Der Pharmacia Sinica*, vol. 2, no. 3, pp. 123–130, 2011.

[17] R. B. Patel and M. R. Patel, "An introduction to analytical method development for pharmaceutical formulations," *Pharmaceutical Information*, vol. 6, no. 4, 2008.

[18] "ICH: Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1)," 2005.