Validation Rp–Hplc Method for Simultaneous Estimation of Hydrochlorothiazide and Valsartan in Formulating Pharmaceutical form

Basim Abdullh Hussein[1], Ahmed Mahdi Saeed[2]
[1, 2]Department of Chemistry, College of Education for Pure Science, University of Diyala, Iraq.
Email: dr.ahmedalanbakey@yahoo.com

Abstract
A novel, simple and accurate, Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method for simultaneous estimation of Hydrochlorothiazide (HCT) and Valsartan (VAL) in mixture of standard and formulation tablets was validated in this research. The absorbance maximum of drugs using UV- spectroscopy was found at (318 and 250nm) for HCT and VAL respectively in deionized water: methanol mixture (60:40 V/V) as solvent. This method involves the separation of HCT and VAL on RP - HPLC Shimadzu type LC–20 - A, Japan, and Phenomenex C-18 (150 mm × 4.6 mm I.D) The elution was done using an eluent phase composed of 0.1 M ammonium acetate (AA), acetonitrile (ACN) and methanol (MOH) in the ratio of (50:25:25 V/V) with a pH adjusted at 3.0 using acetic acid). A separation was fixed for 10 min at 270 nm using a UV-Vis - detector and 1.0 mL/min flow rate. The drugs were eluted in (1.701 and 2.850 min.) for HCT and VAL respectively. The suitable conditions such as the elution phase composition, rate of flow, pH and wavelength were studied. The linearity of the method was in the range of concentration within (0.0125 – 100 and 0.1 – 100 µg/mL) for HCT and VAL respectively, while, R2 values within (0.9966 and 0.9963), and the means of recovery were found within (99.87 and 100.35) for HCT and VAL respectively. The method was applied for the estimation of gradient active of drugs in different formulating form samples. The method accuracy was validated by the mean of recovery percentages which, were found in acceptable limit.
Keywords: Estimation, RP - HPLC, Formulating, Recovery.

1. Introduction

Hydrochlorothiazide is a diuretic medication often used to treat high blood pressure and swelling due to fluid buildup. Other uses include treating diabetes insipidus and renal tubular acidosis and to decrease the risk of kidney stones in those with a high calcium level in the urine.
Valsartan, sold under the brand name Diovan among others, is a medication used to treat high blood pressure, heart failure, and diabetic kidney disease. It is a reasonable initial treatment for high blood pressure. It is taken by mouth. The combination of valsartan [an angiotensin II type 1 (AT1) receptor blocker] and hydrochlorothiazide (a thiazide diuretic), administered once daily, has been evaluated in the treatment of patients with hypertension in clinical trials ranging in duration from 8 weeks to 3 years. These studies showed that combination treatment with valsartan 80 or 160 mg and hydrochlorothiazide 12.5 or 25 mg induced significant reductions from baseline in systolic blood pressure (SBP) and diastolic BP (DBP) in patients with mild to severe hypertension. (1)
Clinical trials have demonstrated that the combination of valsartan 80 or 160 mg with hydrochlorothiazide 12.5 or 25mg is significantly more effective than either drug alone. Furthermore, valsartan plus hydrochlorothiazide was effective at reducing BP in patients unresponsive to monotherapy with either agent alone. Effective BP control with valsartan plus hydrochlorothiazide was maintained in long-term studies, with reductions observed after 3 months of treatment being similar to those seen after 1, 2 or 3 years. (2)
Various methods of analysis are announcing to determine these drugs in formulating drugs like HPLC (3,4,5,6,7,8,9,10,11), spectroscopy (12,13,14,15,16,17,18,19), The work objective is to evaluate a new accurate and easy liquid chromatography analytical method for the estimation of the drug content in formulated samples manufactured by different pharmaceutical corporation which available in the pharmaceutical market in Iraq, to tool up information about the different products, which may enforce or not enforce with the requirements of the formal method or other standard methods.

2. Materials and Methods

2.1 Chemicals and reagents

HCT and VAL standard powder was from SDI- Iraq. Methanol and acetonitrile (HPLC-grade) are from BDH. Ammonium acetate and glacial acetic acid are from BDH. Deionized water, freshly prepared was used.

2.2 Instrumentation and Conditions of chromatographic

HPLC (Shimadzu - LC – 20 - A, Japan), Germany Sartorius - balance, Karl – Kolb - Ultrasonic bath -

doi.org/10.31838/hiv22.02.469 Received: 19.04.22, Revised: 02.06.22, Accepted: 05.08.22

Germany), Shaking bath water (Taiwan) and Memmert - oven – Germany, were used in this study. HCT and VAL were separated on column type Phenomenex C-18 (150 mm × 4.6 mm I.D). Separation was utter at room temperature (~25 oC) and the run time was 10 min under Reversed Phase conditions. The elution phase was 0.1 M ammonium acetate, acetonitrile and methanol in the ratio of (50:25:25 V/V) adjusted pH with acetic acid at 3.0. The rate of flow was 1.0 mL/min, and a10 µL injector loop was used for injecting samples and detection was done at 270 nm. The eluent phase was degassing using the sonicator type - ultrasonic cleaner, power - sonic- 420, and then filtered over a 0.45 µm filter of nylon. The identity established of the compound was done through the comparing of the standard compound solution retention time with those of a sample compound solution. Chromatography was complete in temperature column that maintained at 25 ± 2 °C. The UV-spectrums of HCT and VAL selecting the detection working wavelength were taken by the Jasco - V-650 – Japan, double - beam UV-VIS - spectrophotometer has 10 mm length path quartz cells, which was used for the analytical object.

2.3 Preparation of solutions

Standard stock solution

The stock standard solution has a 1000 and 100 µg/mL of the HCT and VAL were prepared in the mixture of MOH and water using standard material of drugs. Transfer 10 mL of the stock solution 1000 µg/mL into 100 mL volumetric calibrated flask and make up to the mark with elution phase for giving a standard working solution having a100 µg/mL concentration.

Diluent

From the 1000 and 100 µg/mL stock solution, additional dilution was conducted through withdrawing a different volume (0.00125 – 10 and 0.01 – 10 mL) from standard solution of HCT, VAL into the series of 10 and 100 mL volumetric calibrated flasks and all were complete to the mark with eluent phase to prepare standard working solutions have concentrations of (0.0125 – 100 and 0.1 – 100 µg/mL).

Procedure for drugs assay in pharmaceuticals tablets:

Ten tablets of HCT and VAL drug's, formula was accurately weighed and finely powdered. An accurately quantity weighed of tablets, powder which equivalent to weight of one tablet which contain (12.5 and 80 mg) of HCT, and VAL drugs were conveyed to a (100 mL) volumetric flask and then diluted with (H2O: MOH 60: 40 V/V), the content were ultra - sonicated for 25 min. The drugs solutions volume was completed to the mark and mixed well with solvent. The solutions were filtered again using no. 1 Whatman filter paper for the removing of unwanted materials particulate. A filtered solution was appropriately further diluted with the elution phase to produce a sample solution for analysis. The amount of HCT and VAL present in the solution sample was estimated using the standard calibration graphs.

3. Results and Discussion

3.1 Estimation of detection wavelength

A drugs solution of the 10 µg/mL concentration was scanned at the range of 200 to 400 nm wavelength. It was observed that HCT and VAL solutions have shown enormous, sharp, and maximum absorbance at 318 and 250 nm wavelength respectively. Therefore, it was selected as the detection wavelength in the analysis. The spectrum study revealed that HCT and VAL solutions were indicating a well - defined λmax at 318 and 250 nm as clear in (fig. 1).

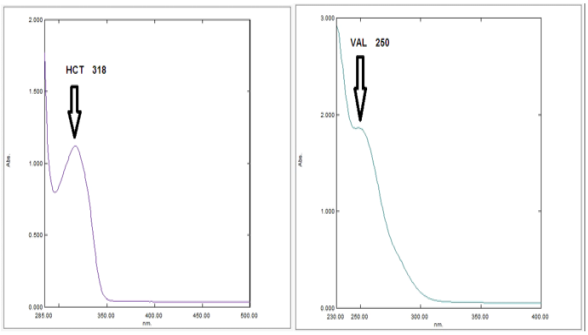

Fig. 1: UV spectra of HCT and VAL.

3.2 Method Development and System Suitability Test

Various tests were conducted to get reasonable resolution – separation of HCT and VAL using different eluent phases with different ratios of water or ammonium acetate and organic solvent. An ideal eluent phase was found to be the mixture of 0.1 M ammonium acetate, acetonitrile and methanol. This eluent phase used in ratio (50:25:25 V/V) gave a good and satisfactory resolution of HCT and VAL. The pH value (3.0) of the eluent phase, increasing or decreasing by ±0.2, did not indicate a worthy change in the analyte retention time. The time of retention using analytical column was estimated at a rate of flow with 1.0 mL/min. The volume of injection was 10 µL. The retention time of sample and standard for HCT and VAL was well pleased with high resolution in formulating sample. This labor was converging on conditions optimization for the rapid, simple, low cost, and effective analysis, involving a selection of the eluent phase to take out satisfactory results. Solvent strength, solvent type (organic solvent volume fraction in the eluent phase and pH of the mobile phase solution), the wavelength of detection and rate of flow were varied to estimation the chromatographic conditions which were given the good separation. The optimized of eluent phase conditions was conducted so there no solvent interference and excipients. The entire predicate chromatographic optimum conditions and the notice values of column efficiency, resolution and factor tailing were mentioned in table 1. The

chromatogram of HCT, VAL and mixture of drugs applied optimum condition is revealed in (fig. 2).

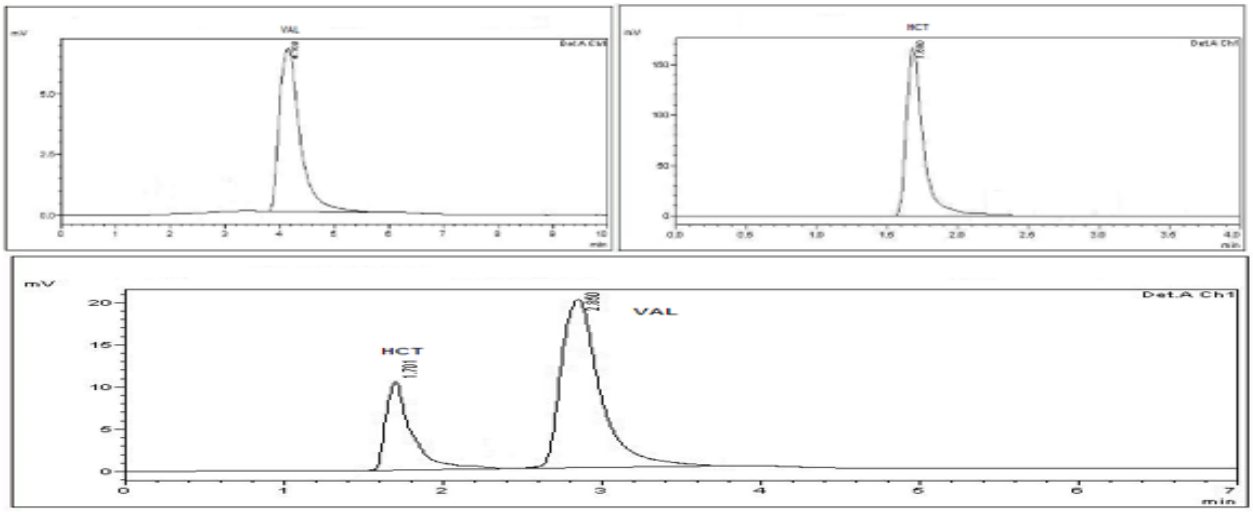

Fig. 2: HPLC chromatograms for HCT and VAL and mixture of drugs.

Table 1: The predicate optimum parameters and system suitability of HPLC method.		
Predicate optimum parameters results		
Compassion of eluent phase, 50:25:25 V/V	Column Type, Phenomenex C-18 (150 mm × 4.6 mm I.D)	
Sample Temperature, ambient		
Rate of flow, 1.0 mL/ min.	Column Temperature, 25 ± 2 °C	
Volume of injection, 10 µL	Run Time min, 10.00	
Detection wavelength, nm 270	Retention Time min, 1.701HCT, 2.850VAL	
System Suitability results		
Parameters of system Suitability	Results	Acceptance criteria
Retention time	1.701HCT, 2.850 VAL	
RSD% for area of seven injections of standard drug solution	0.401 HCT, 0.425 VAL	NMT 2.0
Peak talling factor	1.423 HCT, 1.56 VAL	NMT 2.0
Theoretical plates	3798 HCT, 3785 VAL	NLT 2000

3.3 Preparation of Calibration graph

From the standard stock solution, posterior dilutions were done with eluent phase to gain a series of standard solutions have a range of concentration with (0.0125 – 100 and 0.1 – 100 µg/mL) of drugs. The solutions were injected using injector loop of 10 µL and chromatograms were recorded. A graph were plotted by taking a concentration on X-axis and the area under the peak on Y-axis which gave a straight line. Two graphs were obtained for each one of drugs first one for low concentration and the second for high concentration as shown in (fig. 3).

3.4 Analytical method validation

Validation of progress method was conducted as per ICH Q2 R1 guideline [20]. Parameters such as linearity, precision, LOD and LOQ, accuracy, specificity, robustness and ruggedness were taken in considering testing for the analytical validation method.

3.5 Linearity and Range

The proposed RP-HPLC method was show a good linearity in the concentration range of (0.0125 - 100 and 0.1 to 100 µg/mL) for HCT, VAL respectively were represented in (fig. 3). The linear equations of the straight lines are y = 49235 x + 20236 (R^2 = 0.9966) for HCT and y = 25059 x + 73679 (R^2 = 0.9963) for VAL. The results are satisfactory, because there is a significant correlation between concentration of drugs and response factor within the concentration range.

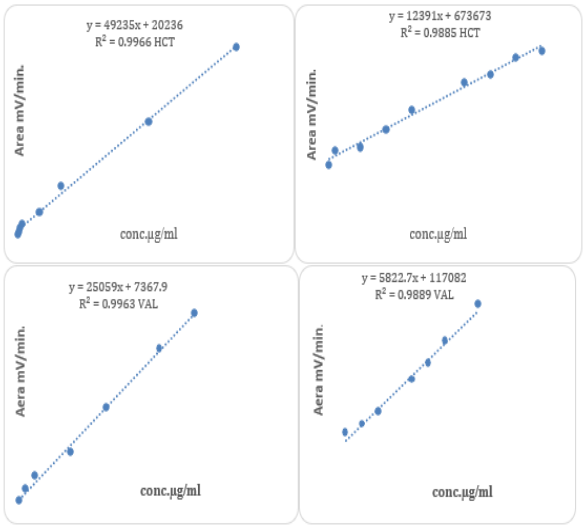

Fig. 3: Calibration graph for HCT and VAL using HPLC.

3.6 Precision

The developed method intraday precision of the was evaluated by analysing HCT and VAL samples of different concentrations three times in the same day and RSD% was estimated. The precision inter day was estimated through the samples analysing have variable concentrations of HCT and VAL in different three days and RSD% was estimated. Evaluated of

Repeatability was conducted by injecting the standard drugs solutions of (5 µg/ mL) five time in the one day and the RSD% value were calculated.

3.7 Lod and Loq

LOD and LOQ were estimated by the gradual dilution for lowest concentration, and 3.3 LOD respectively. The obtained results are tabulated in table 2.

Table 2: parameters validation summery.

Sr. No.	Validation parameters	Results	Standard values
1	Linearity Range	0.0125 – 100, 0.1 - 100 µg/mL for HCT and VAL respectively	-
2	Straight line equation	Y=49235x+20236 HCT.1 / Y=12391x+673673 HCT.2 Y=25059x+7367,9 VAL.1 / y=5822.7x+117082 VAL.2	-
3	Correlation Coefficient	R^2=0.9966 HCT.1 / R^2 =0.9885 HCT.2 R^2=0.9963 VAL.1 / R^2=0.9889 VAL.2	≥ 0.9990
4	Precision (% R.S.D.)		≤ 2.0 % R.S.D.
	Repeatability Intraday Interday	0.206 HCT, 0.305 VAL 0.411HCT, 0.423 VAL 0.656 HCT, 0.688 VAL	
5	Mean % Recovery	99.87 HCT, 100.53 VAL	95 – 105%
6	Specificity	Specific	
7	LOD (µg/mL)	0.0125, 0.05 µg/mL for HCT and VAL respectively	-
8	LOQ (µg/mL)	0.0413, 0.165 µg/mL for HCT and VAL respectively	-
9	Ruggedness	Complies	≤ 2.0 % R.S.D.
10	Robustness		≤ 2.0 % R.S.D.
	Flow rate change Wavelength change Solution pH change	Complies	

3.8 Accuracy

This study was carried out to assure the closeness of the test results obtained by the analytical method to the true value (21,22). For this method, HCT and VAL were measured at three selected different concentrations within the limits of Beer's law (5, 10, 30, 80 µg/mL). The results are tabulated in table 3, which revealed that the suggested method for detection of interesting and quite convenient with respect to the methods and parameters calculated. The recoveries of standard drugs are between (99.87 – 100.53%) for standard drugs.

Table 3: proposed method accuracy of drugs determination.

HCT µg/mL		% Recovery		%Error	R.S.D n =3
Taken	Found				
5	4.98	99.60	Mean = 99.87	- 0.40	0.14
30	29.48	98.27	S.D. = 1.434	- 1.73	0.21
80	81.40	101.75	R.S.D.=1.4359	1.75	0.17
VAL µg/mL		% Recovery			
Taken	Taken	%Error			R.S.D n =3
10	10.19	101.90	Mean = 100.53	1.90	0.13
30	29.57	98.57	SD = 1.423	1.43	0.15
80	80.90	101.13	R.S.D.=1.4155		0.09

3.9 Specificity

Specificity is the analyte ability to unequivocally assess in the presence of other components. Which may be expected to be present (23). These components might include degrades, impurities, etc. The placebo solution of eluent phase was injected. The obtained chromatogram revealed there is no inferring peaks at the drugs retention time. The obtained placebo chromatogram was compared with those obtained from the HCT standard solution. The correlation (in terms of tR and area) was good, which indicate the specificity of the method. The specificity Chromatograms and for the standard HCT were shown in (fig. 4).

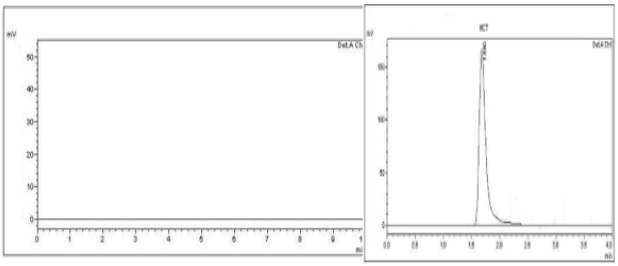

Fig. 4: Specify chromatogram for blank placebo and HCT 10 µg/ L).

3.10 Ruggedness and Robustness

The proposed method ruggedness was carried out by analysis of aliquots of sample solution (9 HCT µg/mL) by two analysts using same operational and environmental conditions. The method robustness was evaluated by changing the rate of flow by ± 0.1 mL/min. (1.1mL/min and 0.9 mL/min), changing the pH by ± 0.2 % (2.8 and 3.2%) for eluent phase and the wavelength detection changing by ± 2 nm (272nm and 268nm). The results obtained are shown in table 4.

Table 4: The ruggedness and robustness results of the proposed method

Ruggedness results			
Analyst 1		Analyst 2	
Mean % Assay*±SD	99.87±0.26	98.91±0.21	
% R.S.D.	0.313	0.261	
Robustness results			
Method Robustness Parameters	Mean*	S.D.	%R.S.D.
Flow rates change 1.0±0.1 mL/min.	99.96	0.47	0.47
Mobile phase pH changes 3.0±0.2	99.78	0.38	0.38
Detection wavelengths change 270±2 nm	100.34	0.21	0.209
*n = 3			

4. Analytical Assays

Three formulated samples were analyzed for HCT and VAL using a validated high-

performance liquid chromatography (HPLC) method with UV detection at 270 nm. A 10µL of sample were injected to HPLC analysis under the optimum separation conditions. Eluent phase 0.1 M ammonium acetate: ACN: MOH (50: 25: 25 V/V) was delivered at a flow rate of 1.0 mL/min with UV detection at 270 nm. The column was Phenomenex C-18 (150 mm × 4.6 mm I.D) and 5µm particle size.

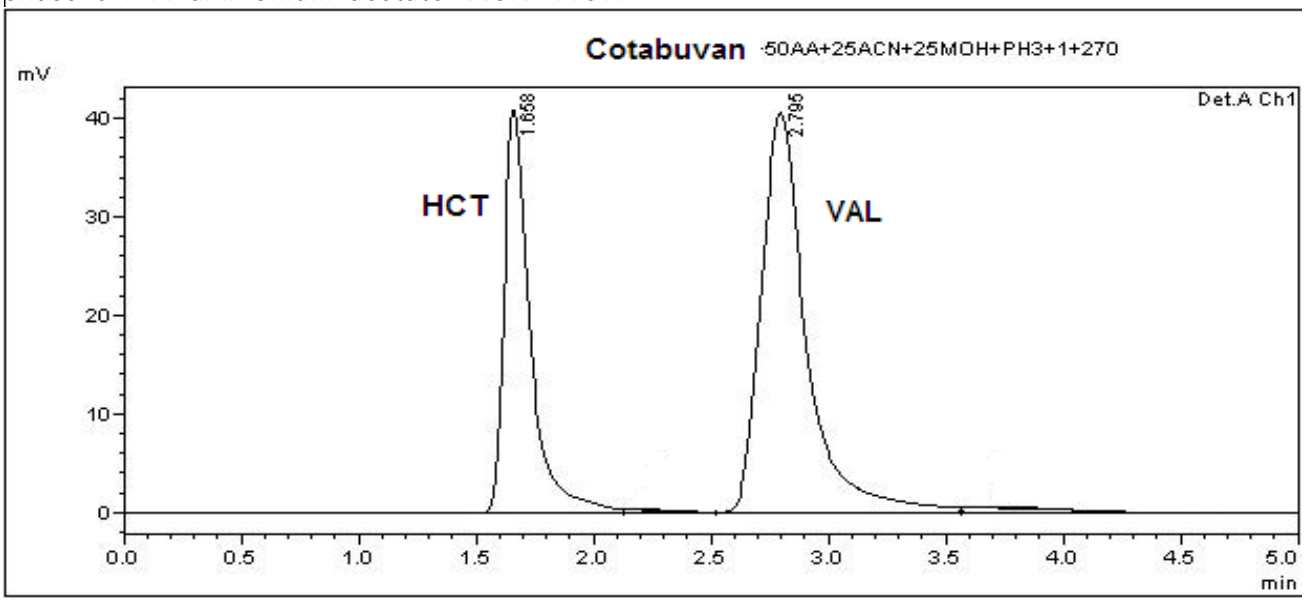

Fig. 5: Separation chromatogram of drugs in formulating sample (Cotabuvan).

Analysis was performed at room temperature (~25oC) and the total run time was 10 min. Figure 5 is shown the separation chromatograms of the drugs in formulating sample. The recoveries of drugs in samples were between 99.57 – 102.36%. The results obtained are tabulated in table 5.

Table 5: Estimated quantity of drugs in different formulating samples.

Name and Company	Drugs type Contain	Claim Label Amount mg/ tab.	Found Mean Amount mg/tab.	% Found Mean Amount	R.S.D n = 3
CO tabuvan Taboic	HCT	12.5	12.4460	99.5680	0.17
	VAL	80	81.8880	102.3600	0.21
Lastavin HTZ Ajanta	HCT	12.5	12.4460	99.5680	0.17
	VAL	80	81.8880	102.3600	0.21
CO anjinet Untied	HCT	12.5	12.4460	99.5680	0.17
	VAL	80	81.8880	102.3600	0.21

5. Conclusion

The RP – HPLC validated methods appoint here steady to be accurate, fast, simple, robust, and precise, so it can used in the routine analysis of HCT and VAL as standard and in formulating form.

6. Acknowledgement

The Authors wish to swift his grace to the Diyala University, College of Education for Pure Science, Chemistry Department for providing the facilities of research and lab for the completion of this work.

7. Contributions of Authors

The authors are all have equally contributed.

8. Interests Conflict

The author has no conflict of interest.

References

1. Carter, Barry L., et al. "Hydrochlorothiazide Versus Chlorthalidone: Evidence Supporting Their Interchangeability." Hypertension, vol. 43, no. 1, 2004, pp. 4–9, doi:10.1161/01.HYP.0000103632.19915.0E.

2. Black, Henry R., et al. "Valsartan: More than a Decade of Experience." Drugs, vol. 69, no. 17, 2009, pp. 2393–414, doi:10.2165/11319460-000000000-00000.

3. Ganthi, Hari Kishan Reddy, et al. "Stability Indicating RP-HPLC Method for Quantification of Impurities in Valsartan and Hydrochlorothiazide FDC Tablet Dosage Form." American Journal of Analytical Chemistry, vol. 07, no. 11, 2016, pp. 816–39, doi:10.4236/ajac.2016.711072.

4. Kaushik D, Antil P. "UPLC Method for Simultaneous Determination of Valsartan & Hydrochlorothiazide in Drug Products." Journal of Chromatography & Separation Techniques, vol. 04, no. 05, 2013, pp. 2–7, doi:10.4172/2157-7064.1000182.

5. Modugula, Jyothsna, et al. "Quantification of Valsartan and Hydrochlorothiazide in Bulk and Tablet Formulation by RP-HPLC Method." The Pharma Innovation Journal, vol. 8, no. 6, 2019, pp. 999–1008.

6. Rudrapal, Mithun, et al. "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Olmesartan and Hydrochlorothiazide in Tablet Dosage Form." Oriental Journal of Chemistry, vol. 31, no. 2, 2015, pp. 921–26, doi:10.13005/ojc/310236.

7. Zaid, Abdel Naser. Hplc Method for Quantification of Valsartan and Hydrochlorthiazide Combination. no. 3, 2012.
8.Elkarbane, M., et al. "Total Error-Based Validation Including the Experimental Design-Based Robustness Evaluation of a Stability-Indicating Method for the Simultaneous Quantification of Hydrochlorothiazide and Valsartan in Tablet Formulations." Acta Chromatographica, vol. 27, no. 2, 2015, pp. 195–214, doi:10.1556/AChrom.27.2015.2.1.
9. Sonali B. Gire, et al. "Development and Validation of Analytical Method for Simultaneous Estimation of Ketoconazole and Salicylic Acid in Bulk and Dosage Form." Journal of Current Pharma Research, vol. 8, no. 4, 2018, pp. 2531–48, doi:10.33786/jcpr.2018.v08i04.005.
10. Ahmed Elbashir, Abdalla, and Rami Ali Mohammed Osman. "Development and Validation of Stability Indicating HPLC Method for the Simultaneous Analysis of Amlodipine, Hydrochlorothiazide and Valsartan in Pharmaceutical Formulation." Journal of Analytical & Pharmaceutical Research, vol. 6, no. 5, 2017, doi:10.15406/japlr.2017.06.00188.
11. Patel, L. J., et al. "Hptlc Method for the Simultaneous Estimation of Bisoprolol Fumarate and Hydrochlorothiazide in Tablet Dosage Form." Indian Drugs, vol. 43, no. 8, 2006, pp. 630–35
12. Sivadas, Akhila, et al. "Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Citicoline and Piracetam in Tablet Dosage Form." Journal of Pharmacy and Bioallied Sciences, vol. 5, no. 3, 2013, pp. 202–07, doi:10.4103/0975-7406.116818.
13. Deshpande, M. M., et al. "Simultaneous Estimation of Valsartan and Hydrochlorothiazide in Fixed Dose Combination in UV Spectrophotometry." International Journal of Pharmaceutical Sciences, vol. 3, no. 1, 2012, pp. 235–40.
14. Article, Original. Simultaneous Estimation of Valsartan and Hydrochlorothiazide in Tablets by Rp-Hplc Method. no. January 2011, pp. 170–74.
15. Meyyanathan, S. N., et al. "Simultaneous Estimation of Nebivolol Hydrochloride and Valsartan and Nebivolol Hydrochloride and Hydrochlorothiazide in Pharmaceutical Formulations by Uv Spectrophotometric Methods." Indian Journal of Pharmaceutical Education and Research, vol. 44, no. 2, 2010, pp. 156–59.
16. Sivadas, Akhila, et al. "Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Citicoline and Piracetam in Tablet Dosage Form." Journal of Pharmacy and Bioallied Sciences, vol. 5, no. 3, 2013, pp. 202–07, doi:10.4103/0975-7406.116818.
17. Anandakumar, K., and M. Jayamariappan. "Absorption Correction Method for the Simultaneous Estimation of Amlodipine Besylate, Valsartan and Hydrochlorothiazide in Bulk and in Combined Tablet Dosage Form." International Journal of Pharmacy and Pharmaceutical Sciences, vol. 3, no. 1, 2011, pp. 23–27.
18. Anwekar, Himanshu, et al. I NTERNATIONAL J OURNAL OF P HARMACY & L IFE S CIENCES Liposome- as Drug Carriers. no. 7, 2011, pp. 945–51.
19. Haripriya, A., et al. Validated Eco - Friendly Derivative Spectrophotometric Method for Valsartan and Hydrochlorothiazide Combined Tablet Dosage Form. no. 8, 2012, p. 4169.
20. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1), 2005; http://www.ich.org/file admin/Public Web_ Site/ ICH_ Products/ Guidelines/ Quality/Q2_R1/ Step4/ Q2_R1_Guideline.pdf.
21. Mohammed, Omer Jasim. "Rp–Hplc Method Validation for Simultaneous Estimation of Paracetamol and Caffeine in Formulating Pharmaceutical Form." Research Journal of Pharmacy and Technology, vol. 14, no. 9, 2021, pp. 4743–48, doi:10.52711/0974-360X.2021.00825.
22. Saeed, Ahmed Mahdi. "Quantitative Assay of Aspirin and (Salicylic Acid and Heavy Metals as Impuraties) in Iraqi's Market Aspirin Tablets Using Different Analytical Methods." International Journal of Applied Pharmaceutics, vol. 10, no. 5, 2018, pp. 167–72, doi:10.22159/ijap.2018v10i5.26820.
23. Saeed, Ahmed Mahdi. "Validation of Liquid Chromatographic Analytical Method for Determination of Cephalexin and Aspirin in Pure and Pharmaceutical Preparations." International Journal of Pharmaceutical Research, vol. 12, no. 1, 2020, pp. 1625–31, doi: 10.31838/ijpr/2020.SP1.250.