Eurasian Journal of Analytical Chemistry, 2016, **11**(5), 245-253

Simultaneous Estimation of Cilnidipine and Valsartan by RP-HPLC in Tablet Formulation

Ramanlal N. Kachave
Jawaharlal Nehru Technological University, INDIA
Mayura Kale
Government College of Pharmacy Aurangabad, INDIA
Rajendra D. Wagh
ARA College of Pharmacy, INDIA

•*Received 10 September 2015* •*Revised 12 May 2015* •*Accepted 17 May 2016*

A new high performance liquid chromatography method was developed and validated for the quantitation of Cilnidipine and Valsartan in pharmaceutical formulations. Determination was performed using an ODS C18, 250mm x 4.6mm, 5µm column, a mobile phase containing Methanol: Water (85:15) pH 3 adjusts with ortho-phosphoric acid in isocratic flow rate 1.0 mLmin-1. The method was validated with respect to linearity, precision, robustness, and accuracy. The calibration graphs ranged from 1-5 µg/mL in Cilnidipine and 8-40 µg/mL Valsartan Intra- and interday relative standard deviation values for the standard solutions were 0.5%, 1.64% and 0.22%, 1.62 %. Robustness of relative standard deviation values was 0.334, 0.101 respectively. Total recoveries of Cilnidipine and Valsartan from the laboratory prepared mixtures were 98.94 % and 99.04 % respectively.

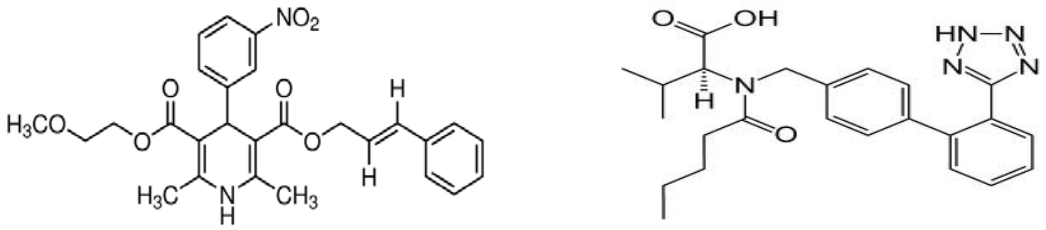
Keywords: cilnidipine, valsartan, ODS, RP-HPLC

INTRODUCTION

Genetically Cilnidipine (CLD) (Fig.01A) chemically, 1,4Dihydrogen -2, 6dimethyl1-4(3nitrophenyl-3,5pyridinecarboxylic acid 2-methoxyethyl (2E)-3-phenyl ester is a dual blocker of L-type of Voltage-gated calcium channel in vascular smooth muscle and N-type of calcium channels in sympathetic nerve terminals that supply blood vessels**.** Valsartan (VAL) (Fig.01B) is chemically know as (2S)-3-methyl-2-[N-({4-[2H-1234-tetrazol-5-yl) phenyl] phenyl} methyl) pentanamido] butanoic acid. Valsartan is ARB (Angiotensin receptor blocker) that selectively inhibits the binding of Angiotensin II. To AT1 (Angiotensin Receptor), this is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone one-secreting effective of angiotensine2 and result in a decrease in vascular resistance and blood pressure. [1-7]

Correspondence: Ramanlal N. Kachave,
Jawaharlal Nehru Technological University Kakinada -Andhra Pradesh India -500 003,
Amrutvahini College of Pharmacy, Amrutnagar, P.O. Sangamner (S.K.) INDIA-422608
E-mail: ramanlalkachave26@gmail.com
doi: 10.12973/ejac.2016.137a

ISSN: 1306-3057



[A] **[B]**

Figure 1 Chemical Structure of Fig. [1A] Cilnidipine and Fig. [1B] Valsartan

Tablet dosage forms containing Cilnidipine and Valsartan in ratio of 10 mg: 80mg of various brands are available in the market. Cilinidipine has been reported to be determined by HPLC [8-12] from formulations and in biological fluids. Ratio spectra derivative Spectrophotometry, Valsartan determination has been done by HPLC and the stability indicating assay method. [13-26] However, there is no method available for the simultaneous determination of these two drugs. Therefore, an attempt was made to develop a new, rapid, and sensitive method for the simultaneous determination of Cilnidipine and Valsartan. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH norm, which is mandatory also [27-29].

EXPERIMENTAL

Materials

Cilnidipine was obtained from J.B.Chemicals and Pharmaceutical Valsartan was kindly gifted by Lupin Laboratories Ltd. Methanol and Acetonitrile HPLC grade was form Merck Laboratories .Water used AR grade. All other reagents used in AR grade.

Instrumentation

The pH of the mobile phase was checked on a microprocessor water proof pH tester (pH tester 20, eutech instruments, oakton, USA). The overall illumination at the point of placement of samples was 6000 lux, which was tested using a calibrated lux meter (Lutron LX-102 digital light meter, Marcucci S.P.A, vignate, Milan). HPLC (Water 600 controller) instrument equipped with a model code 6CE In Line Degasser Af , Reciprocating pump ,Rheodyne 7725i manual injector with a 20µl fixed loop and HPLC syringe of 100µI and with UV-Vis detector. Separation and quantitation were made on ODS C18, and 250 mm x 4.6mm, 5µm column data analyzed by using Data Ace software

Chromatographic Conditions

In Initially to estimate Cilnidipine and Valsartan simultaneously number of mobile phases in different ratios were tried, taking into consideration the system suitability parameters like RT, tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Water (pH3): Methanol in the ratio of (15:85 pH- 3 with Orthophosphoric acid). The mobile phase was filtered through 0.45µm Nylon membrane filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1 mLmin-1. Considering the overlay spectra of these two drugs, 245 nm seems to be the most

suitable detection wavelength. As at 245 nm is isobestic point of CLD and VAL responses of both drugs fairly well and satisfactorily result (Figure 2).

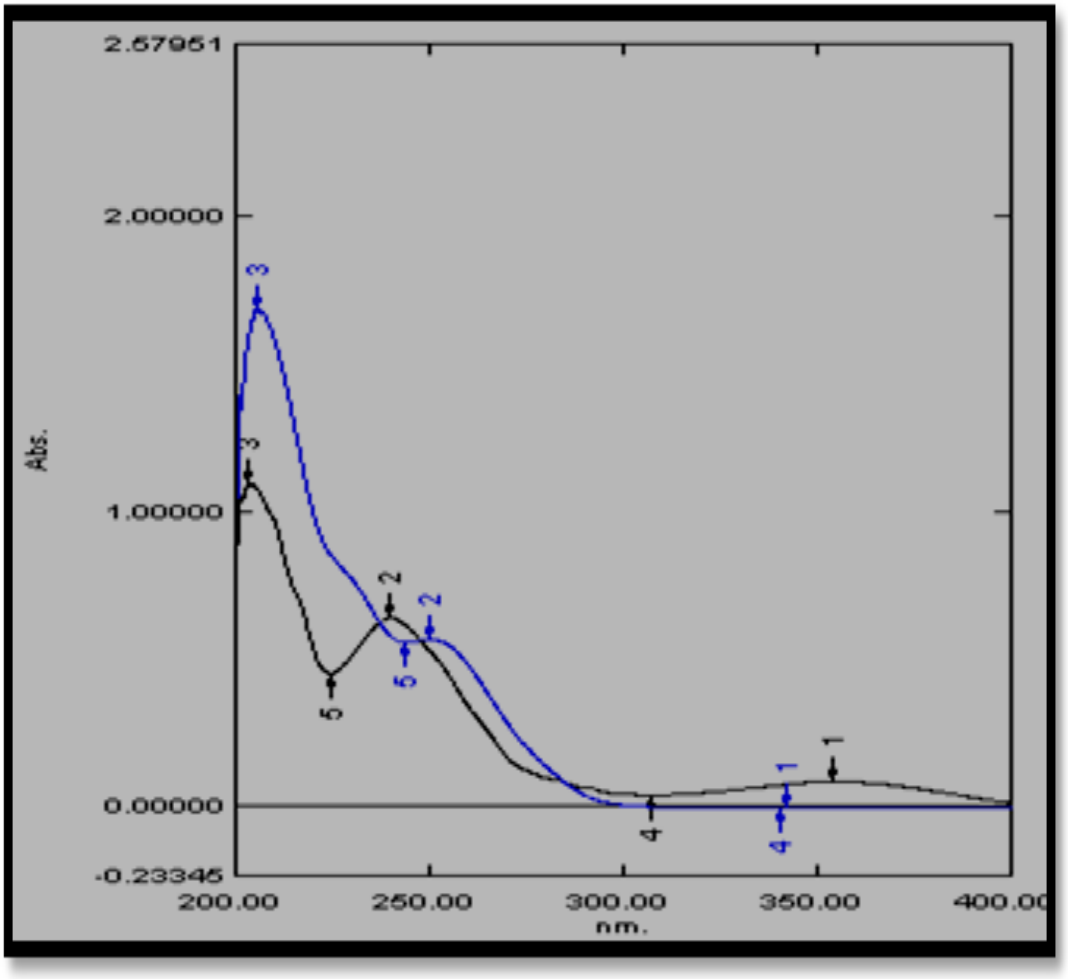


Figure 2. Overlay Spectra of CLD and VAL

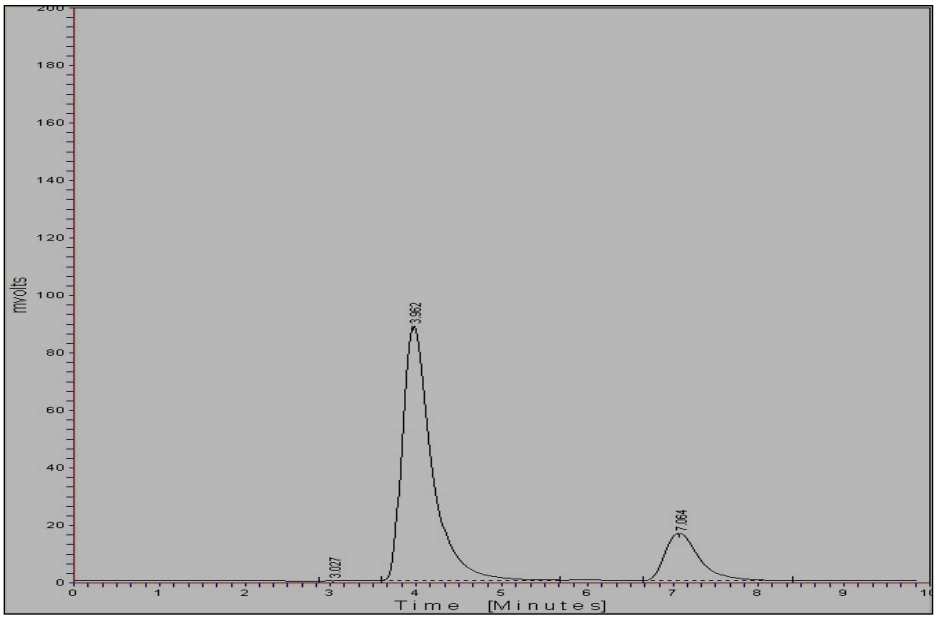


Figure 3. Representative Chromatogram of standered CLD ans VAL

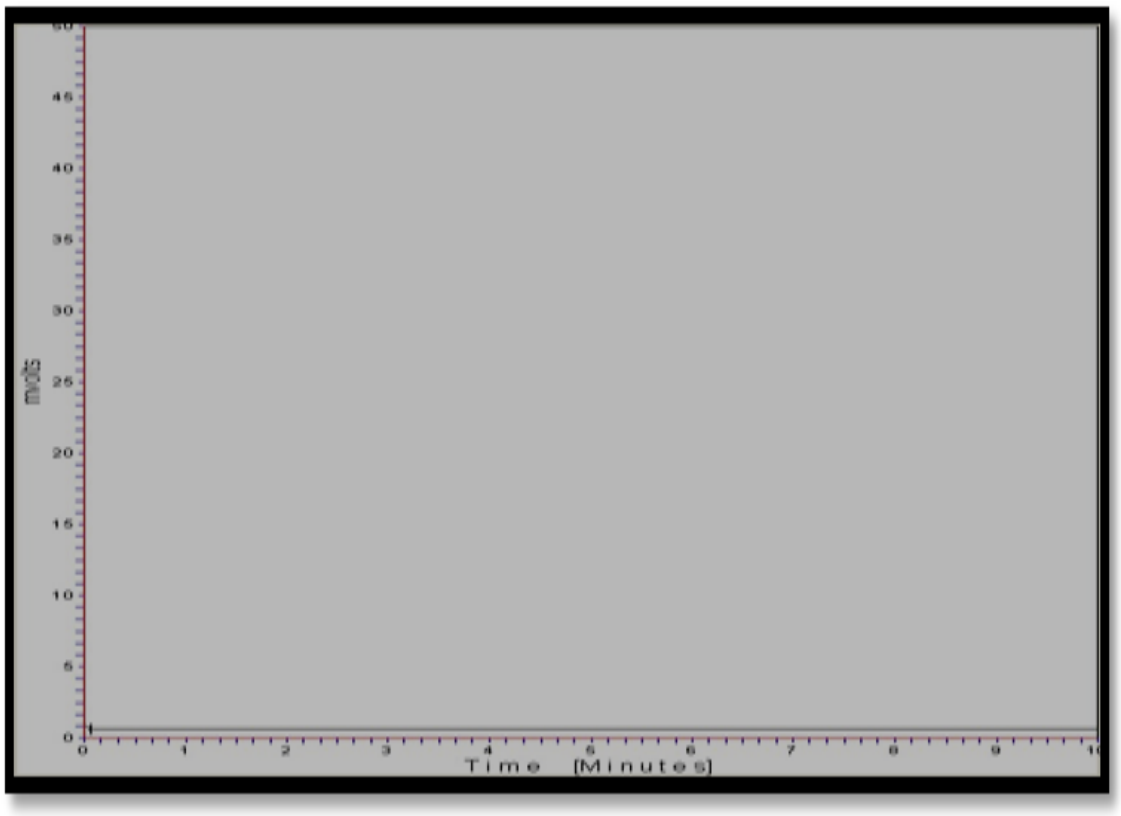


Figure 4. Repesentative Chromatogram of Blank

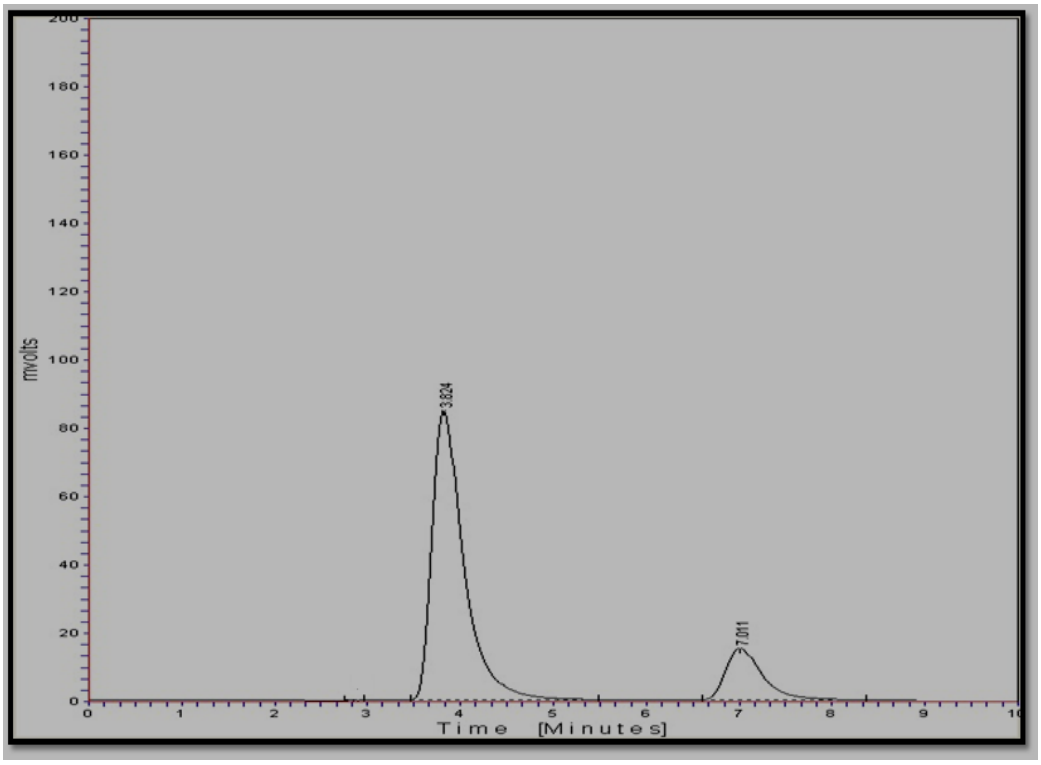


Figure 5. Repesentative chromatogram of tablet formulation

RESULTS AND DISCUSSION

System Suitability

System suitability parameters such as the number of theoretical plates, HETP and peak tailing are determined. Separation Variable was set and mobile phase was allowed to saturate the column at flow rate 1.0mL/min. After complete saturation of column, six replicates of working standard of CLD (2µg/mL) and VAL (16 µg/mL) were injected. The results obtained are shown in Table 1.

Table 1. Results of system suitability

Parameter	VAL	CLD
Retention time (min)	3.962	7.064
Tailing factor	1.7	1.5
HETP	0.0943	0.00137
Rs	-	36.60
N	877.9792	782.6912
SD	7146.396	2928.608
R.S.D. %	1.39	1.76

Linearity

To establish the linearity of analysis method, a series of dilution ranging from 1-5µg /mL for CLD and 8-40µg/ mL for VAL were prepared. All the solution was filtered through 0.2µm membrane filter and injected, chromatograms were recorded and it was repeated six times. The calibration curve of peak area vs. respective concentration was plotted. A Calibration and regression equation was derived. The results obtained are shown in Table 2.

Y (Cilnidipine) = 85739 X+1566 (r^2 =0.999)

Y (Valsartan) = 45684 X +1652 (r^2 =0.999)

Table 2. Results of linearity

Parameter	VAL	CLD
SD of Slope	1137.184	892.6661
SD of Intercept	8678.57	1403.964
Regression (r^2)	0.999	0.999

Accuracy

Recovery studies were performed to validate the accuracy of developed method to the preanalyzed sample solution, a definite concentration of standard drug was added to and recovery was studied. Different amount of pure drug solutions was added preanalyzed samples, then solution were analysed. The result of recovery studies and statistical data of CLD and VAL are reported in Table 3.

Table 3. Results of recovery study

Sr.No.	Conc. of drug in Preanalyzed samples (µg/mL)		Std. drug sol. added (µg/mL)		Recovered Amount* (µg/mL)		Recovered (%)	
	CLD	VAL	CLD	VAL	CLD	VAL	CLD	VAL
01	2	16	1.6	12.8	1.59	12.68	99.37	99.0
02	2	16	2	16	1.97	15.9	98.5	99.39
03	2	16	2.4	24	2.37	23.70	98.96	98.75
			Mean				98.94	99.04
			S.D.				0.35	0.26
			R.S.D. %				0.353	0.262

Mean of Three Readings*

Precision

Five standard dilution was prepared for the repeatability and three replicates where analysed in same day for repeatability, results were found within acceptable limits (R.S.D. %< 2), as shown in Table 3.

Day to day precision five standard dilution was prepared and three replicates where analysed in different day for intermediate precision and statistically validated. Analyst to Analyst Variation for five standard dilutions was prepared and three replicates where analysed in same day for different analyst for intermediate precision and statistically validated. Although the R.S.D. % value for as shown in Table 4.

Table 4. Results of precision

Sr. No.	Validation Parameter	Mean* %		S.D		R.S.D. (%)	
		CLD	VAL	CLD	VAL	CLD	VAL
1	Repeatability	99.98	100.01	0.41	0.37	0.41	0.36
2	Intermediate precision day to day	100	97.17	0.44	0.579	1.64	0.51
3	Intermediate precision analyst to analyst	99.54	99.18	0.42	0.26	1.62	0.22

Average of six determinations*

Robustness

Standard dilution was prepared and three replicates where analysed by Change pH at two levels (± 1). The pH of mobile phase was change 2.9 and 3.1. also change in flow rate (± 1) . The flow rate was change 0.9 min/mL and 1.1 min/mL. Results of the analysis are summarized in Table 5.

Table 5. Results of robustness

Sr. No.	Validation Parameter	Mean* %		S.D		R.S.D. (%)	
		CLD	VAL	CLD	VAL	CLD	VAL
1	Robustness (pH-2.9)	97.98	99.70	0.43	0.11	0.438	0.1196
2	Robustness (pH-3.1)	97.23	99.80	0.33	0.1010	0.344	0.1012
3	Robustness(Flow Rate0.9 mLmin-1)	98.56	98.68	0.4895	0.408	0.496	0.408
4	Robustness (Flow Rate1.1 mLmin-1)	98.60	98.60	0.128	0.135	0.130	0.137

Tablet Analysis

Contents of CLD and VAL found in the tablets by the proposed method are shown in Table 6. Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 10mg CLD and 80mg VAL was accurately weighted and transferred into a10ml of volumetric flask,7ml of diluent was added. The content was ultrasonicated for 15min. The volume was then diluted to the mark and mixed well. A small portion was with-drawn and filtered through a 0.25µm filter to ensure the absence of particulate matter. The low values of R.S.D. % indicate that the method is precise and accurate.

Table 6. Results of tablets

Sr.No.	Parameter	Cilnidipine(CLD)	Valsartan (VAL)
1	Mean* (%)	97.33	98.33
2	S.D.	0.395	0.219
3	R.S.D. (%)	0.4058	0.22

Average of Six Determinations*

CONCLUSIONS

An RP-HPLC method was developed and validated for simultaneous estimation of Cilnidipine and Valsartan in tablet dosage form. The proposed method is fast, accurate, and precise; hence, it can be employed for routine quality control of tablets containing these two drugs in industry.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

The authors wish to thank Standard Cilnidipine provided by J.B.Chemicals & Pharmaceutical limited and Lupin Laboratories Ltd Providing Valsartan as gift sample. The authors extend thanks to the School of Pharmaceutical Sciences, JNTU-K, Kakinada and Management and Principal of Amrutvahini College of Pharmacy, Sangamner for their cooperation in the present research work.

REFERENCES

1. The Merck Index an Encyclopaedia of chemicals, (2001). *Drugs, and biological*, Merck Research Laboratories, Whitehouse station. 13th Ed, New Jersey: 148,379.
2. Sarat, K., Ramesh, G., (2013). The fourth generation Calcium channel blocker Cilnidipine. *Indian Heart Journal 65*, 691-695.
3. Indian Pharmacopoeia published by the Indian pharmacopeia commission Ghaziabad 6th Ed. 2010, 2286.
4. Sethi, P. D., (2010). *High Performance Liquid Chromatography, Quantitative Analysis of Pharmaceutical Formulations*, CBS Publishers and Distributors Pvt. Ltd., 1st Ed., 1, 59-63.
5. Joel, G. H., Lee, E. L., Goodman's & Gilman's, (1996). *The Pharmacological Basis of therapeutics,* By Mc Graw Hill Publication 9th Ed..
6. Willard, M.H. (1982). *Instrumental methods of analysis*, CBS publishers, New Delhi, 6th Ed., 467-476.

7. Sethi, P. D., (2010). *High Performance Liquid Chromatography, Quantitative Analysis of Pharmaceutical Formulations*, CBS Publishers and Distributors Pvt. Ltd. 1st Ed., 11-14, 101-103.
8. Kyeong, R., Lee, Y.J., Chae, J., Hwa, L. (2012). Quantification of Cilnidipine in Human Plasma by Liquid Chromatography-Mass Spectrometry *J. of Liq. Chrome. & Related Techno.,* 308–320.
9. Haripriya, M., Antony, N., Jayasekhar, P. (2013). Development and Validation of UV Spectrophotometric Method For The Simultaneous Estimation Of Cilnidipine And Telmisartan In Tablet Dosage Form Utilising Simultaneous Equation And Absorbance Ratio Method. *Inter. J. of Pharm. and Biol. Scie., 3,* 343-348.
10. Pawar, P., Gandhi, S. V., Deshpande, P. B., Vanjari, S., Shelar, S. U. (2013). Simultaneous RP-HPLC estimation of Cilnidipine and Telmisartan in combined tablet dosage form. *Pel. Rese. Lib., 4*(2), 6-10
11. Reema, H., Joshi, H.S. (2013). *Stability Indicating Simultaneous Validation of Telmisartan and Cilnidipine with Forced Degradation Behavior Study by RP-HPLC in Tablet Dosage Form.* Hindawi Publishing Corporation ISRNC Chromatography, 1-6.
12. Safhi, M. M., (2013). Spectrophotometric Method for the Estimation of Cilnidipine in Bulk and Pharmaceutical Dosage forms Orig. *J. of Chem., 29,* 131-134.
13. Tatar, S., Saglik, S. (2002). Comparison of UV- and Second Derivative-Spectrophotometric and LC methods for the determination of Valsartan in Pharmaceutical formulation, *J. of Pharm. and Biomed. Anal., 30,* 371–375.
14. Gupta, K.R., Wadodkar, A.R., Wadodkar, S.G. (2010). UV-Spectrophotometric methods for estimation of Valsartan in bulk and tablet dosage form Inter. *J. of Chem. Tech. Research, 2,* 985-989.
15. Vinzuda, D.V., Sailor, G.U., Sheth, N. (2010). RP-HPLC Method for Determination of Valsartan in Tablet Dosage Form. *International J. of Chem. Tech. Research, 2,* 1461-1467.
16. Krishanaiah, Ch., Reddy, A.R., Kumar, A., Mukkanti, K. (2010). Stability Indicating Assay Method for Determination of Valsartan and Their degradation product in Active Pharmaceutical Ingredient and Pharmaceutical Dosage Form. *J. of Pharm. and Biomed. Anal, 53,* 483-489.
17. Santosh, P.V., Sahu, M., Prasad, K.: Chandra, M. (2011). Development and validation of Analytical Method for Estimation of Valsartan in Pure and Tablet Dosage form by RP-HPLC Method, *Int. J. of Pharm., 1*(4), 945-949.
18. Kalaimagal, A., Jerad S.A., Niraimathi, V. (2012). Spectrophotometric Methods for the Estimation of Valsartan in Bulk and Oral Dosage Form, *Int. J. of Pharm. and Pharm. Sci., 4,* 481-483.
19. Chokshi, P. V., Trivedi, K. J., Patel, N. S. (2012). Development and Validation of RP-HPLC Method for Analysis of Aliskiren Hemifumarate and Valsartan in Their Combination Tablet Dosage Form, *Int. J. of Chem. Tech. Research, 4,* 1623-1627.
20. Kharoa, M., Malkieh, N., Abualhasan, M. (2012). Tablet Formulation and Development of Validated Stability Indicating HPLC Method For Quantification of Valsartan and Hydrochlorthiazide, *Int. J. of Pharm. and Pharm. Sci., 4*(3) ,284-293.
21. Haque, M.A., Amrohi, S.H., Kumar, P., Diwan, P.V. (2012). Stability indicating RP-HPLC method for the estimation of Valsartan in pharmaceutical dosage form. *IOSR J of Pharm., 2*(4), 12 -18.
22. Nissankararao, S., Kumar, A., Sravanthi, S. (2013). Method development and validation for the estimation of Valsartan in bulk and tablet dosage forms by RP-HPLC, *Scholars Research Library, 5*(2), 206-211.
23. Sivasankara Raoa, G., Venkat Raoa, S., Ramachandrana, D. (2013). Development and validation of new UV-Spectrophotometric assay method for Valsartan in pure and in formulations, *J. of Che. and Pharma. Research, 5*(7), 229-232.
24. Rasha, A., Tarek, S., Belal, A., Fawzya, A. (2013). Validated stability-indicating HPLC-DAD method of analysis for the antihypertensive triple mixture of Amlodipine Besylate, Valsartan and Hydrochlorothiazide in their tablets. *Arabian J. of Chem.,* 1-13.
25. Jadhav, M.L., Girase, M.V., Tidme, S. K. (2014). Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Valsartan and Hydrochlorothiazide in Tablet Dosage Form, *Inte. J. of Spect.,* 1-6.

26. Gadepallia, S.G., Kunchaa, M., Sistlaa R. (2014). Simultaneous determination of Amlodipine, Valsartan and Hydrochlorthiazide by LC-ESIMS/MS and its application to Pharmacokinetics in rate. *J. of Pharm. Anal., 4*(6) 399-406.
27. Code Q2A (1994). *Text on Validation of Analytical Procedure* Step-3 Consensus Guideline, ICH Harmonised Tripartite Guideline.
28. Code Q2B (1994). *Validation of Analytical Procedure Methodology* Step-4 Consensus Guideline, ICH Harmonised Tripartite Guideline.
29. ICH (2005). *Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology* Q2 (R1), November 2005.