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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF VALSARTAN AND CILNIDIPINE IN TABLET DOSAGE FORMS BY RP-HPLC

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ABSTRACT

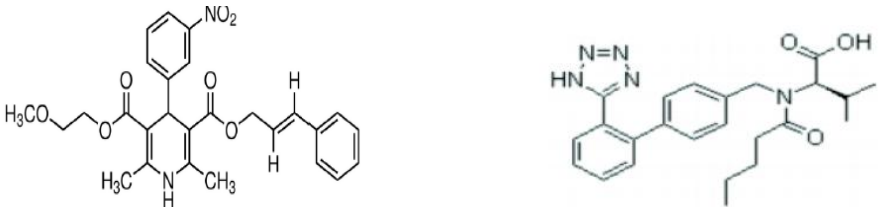
The proposed Research work is simple, precise and accurate RP-HPLC method was developed for the simultaneous estimation of Valsartan and Cilnidipine. The current RP-HPLC method utilizes stationary phase and consists of symmetry C18 (250 × 4.6 mm, 5 µm in particle size) with a mobile phase comprising of Methanol : Pottassium di hydrogen Phosphate buffer (80:20%v/v) pH 3.5 adjusted by Orthophopharic acid at a flow rate of 1.2 mL/min, column temperature of 25 °C and UV detection at 260 nm. The retention time of Valsartan and Cilnidipine were 2.05 and 5.13 min respectively. The linearity was found to be in the range of 10–60 g/mL and 5–30 µg/mL for Cilnidipine and Valsartan respectively. The % recovery was found to be 99.76 ± 0.426 for Cilnidipine and 98.93 ± 0.1020 for Valsartan. The proposed method was validated as per ICH guidelines and successfully applied for the determination of drugs in tablet.

KEYWORDS: Valsartan and Cilnidipine; UV-VIS Detector; Tablet dosage forms.

INTRODUCTION

Cilnidipine (CIL), chemically, 1,4-Dihydro- 2, 6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridinecarboxylic acid 2-methoxyethyl (2E)-3-phenyl-propenyl ester is a dual blocker of L-type voltage-gated calcium channels in vascular smooth muscle and N-type calcium channels in sympathetic nerve terminals.[1]

Valsartan (VAL) is 3-methyl-2-[N-({4-[2-(2H-1, 2, 3, 4-tetrazol-5-yl phenyl] phenyl} methyl) pentanamido] butanoic acid are shown in Figure 1. Valsartan lowers blood pressure by antagonizing the renin–angiotensin–aldosterone system, selectively competing with the angiotensin II receptor subtype.



(A) **(B)**

Fig. 1: Chemical Strcures of Cilnidipine (A) and Valsartan (B).

LITERATURE REVIEW

Literature review reveals that spectrophotometric,[1] reverse phase high-performance liquid chromatography (RP-HPLC),[2-5] and high performance thin layer chromatography *(HPTLC),*[6] methods for the determination of CIL either as a single or in combination with other drugs in pharmaceutical preparations. Analytical methods reported for VAL includes spectrophotometric,[7,8] HPLC,[9-11] and *HPTLC.*[11-13] either as a single drug or in combination with other drugs. No *HPTLC* method of analysis has yet been reported for simultaneous analysis of CIL and VAL. This paper describes a rapid, accurate, economical and validated high performance thin layer chromatographic *(HPTLC)* method for the simultaneous quantification of these compounds in bulk and tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.[14- 21]

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents used were of analytical grade. Cilnidipine and Valsartan were obtained as gift sample from the industry. As the commercial formulation for combined dosage form is not available in India it was formulated in in-house laboratory.

Instrumentation

A Waters HPLC system equipped with a 515 binary pump, an auto sampler and a 2487 photo UV-VIS detector was employed for the study. The output signal was monitored and processed with Empower-2 software. Ultrasonic bath (Power Sonic 405, Hwashin technology, Korea) and Electronic balance Shimadzu AX200, (Shimadzu Corporation, Japan) were used in the study.

Preparation of standard solutions

Stock solutions for measurements were prepared by dissolving Cilnidipine and Valsartan separately in methanol to obtain concentration of 1000 µg/ml and 8000 µg/ml respectively for each compound. For calibration, by diluting the stock standard solution with methanol in 10 ml standard volumetric flasks series of solutions were prepared containing 10, 20, 30, 40, 50, 60 µg/mL for Cilnidipine and 5, 10, 15, 20, 25, 30 µg/mL for Valsartan.

Preparation of Sample solution

Accurately weighed quantity 80.0 mg of VALSA and 10.0 mg of CILNI, respectively, was transferred to 10.0 ml volumetric flask, added 5.0 ml of methanol and ultra sonicated for 10 minutes, volume was then made up to the mark with methanol. (conc. 800 µg/ml and 100 µg/ml of VALSA and CILNI respectively). From this solution, 1.0 ml was diluted to 10.0 ml with methanol.

Selection of mobile phase

A trial and error method was used to select the optimised mobile phase. The solvent system of Buffer: Methanol in the ratio 80:20 was the most appropriate mobile phase for the *HPLC* analysis of Cilnidipine and Valsartan in methanol as solvent.

Method Validation

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The chromatograms are shown in Fig.No.11-13.

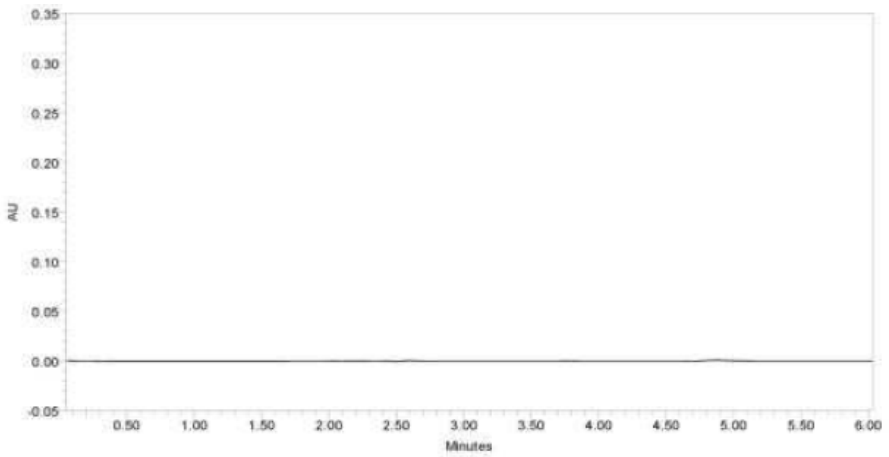


Fig. 2: Chromatogram showing blank (Solvent system).

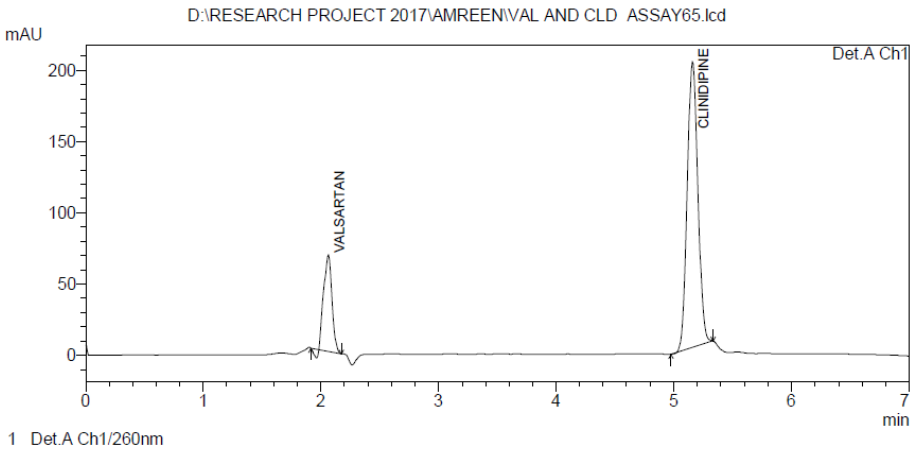


Fig. 3: Chromatogram showing standard Cilnidipine and Valsartan.

Calibration curve

Response to Cilnidipine and Valsartan was linear in the concentration ranges 5-25 µg/mL for Cilnidipine and 2.5-12.5 µg/mL for Valsartan respectively. The regression equations for Cilnidipine and Valsartan were y = 1.404x and y = 511.8x respectively, where y is response and x the concentration of drug (Figure no. 3,4). The correlation coefficients were 0.9994 and 0.9997 respectively. [Table1].

Table 1: Linearity of Valsartan and Clinidipine.

S. No.	Valsartan		Cilnidipine	
	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak Area
1	2.5	298031	5	740046
2	5	704607	10	990204
3	7.5	1026419	15	1183023
4	10	1359837	20	1439886
5	12.5	1701139	25	1682302
Correlation Coefficient		0.9994	0.9997	

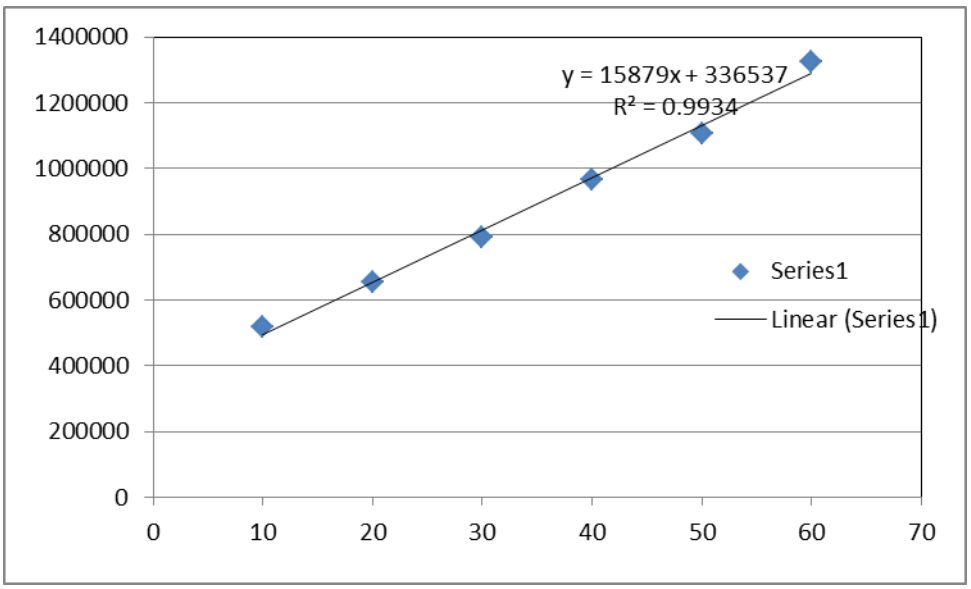


Fig. 4: Showing calibration graph for Valsartan.

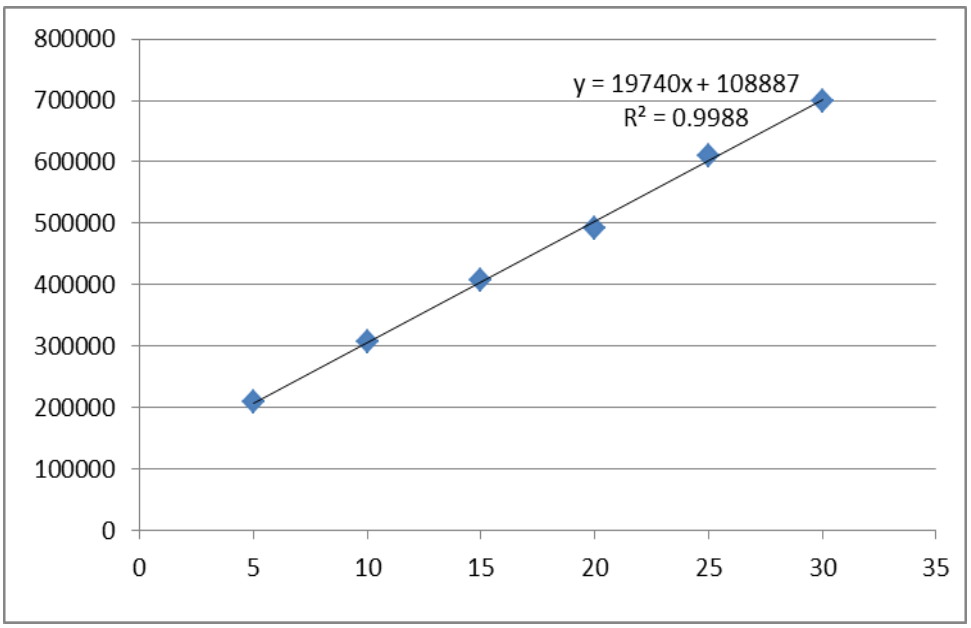


Fig. 5: Showingcalibration graph for Clinidipine.

The relationship between the concentration of Valsartan and clinidipine was linear in the specific range and the correlation coefficient was found to be within limit only. The correlation coefficient of Valsartan and clinidipine was found to be 0.9999 & 0.9999.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of Cilnidipine and Valsartan by the standard addition method. Known amount of standard of Cilnidipine and Valsartan (50%, 100%, and 150%) were added to sample solutions of tablet dosage forms. The % recovery found to be between 98.00-99.70% indicates that the method is accurate.

Precision studies

The precision of the method was checked by repeatedly scanning (n= 6) standard solutions of Cilnidipine and Valsartan 1000 µg/mL and 8 µg/mL respectively on the same day and on different days. The RSD values were found to be below 2% which indicate that the proposed methods are precise.

Table 2: Accuracy for Valsartan.

Recovery level	Accuracy of Valsartan					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	Percentage Recovery	
50%	5.05	1011326	1017498.5	101.3927	101.3927	100.599%
	5.05	1015029				
	5.05	1026141				
100%	10	1986534	1987384.8	100.0106	100.0106	
	10	1987425				
	10	1988195				
150%	15	2989367	2992493.4	100.3936	100.3936	
	15	2991556				
	15	2996557				

Table 3: Accuracy for Clinidipine.

Recovery level	Accuracy of Clinidipine					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	
50%	8.1	646754	648293.3	101.91	101.91	101.22%
	8.1	648998				
	8.1	649128				
100%	15	1172743	1174011.1	99.66	99.66	
	15	1174031				
	15	1175259				
150%	23.3	1866742	1868236.3	102.09	102.09	
	23.3	1867956				
	23.3	1870011				

Assay for marketed preparation

Weighed accurately twenty tablets of 10.0 mg of Cilnidipine and 80.0 mg of Valsartan were prepared in-house, weighed and average weight was determined; tablets were triturated to fine powder. Tablet powder equivalent to 10.0 mg of Cilnidipine and 80.0 mg of Valsartan was transferred in 25.0 ml volumetric flask and were dissolved in methanol then the solution was ultrasonicated for 20 min. and filtered through Whatman filter paper No. 41. The plate was developed under previously described chromatographic conditions.

Table 4: Summary of results of analysis of marketed formulation.

S. NO.	Drug	Amount of drug estimated (mg/tablet)*	Label Claim	S.D	%RSD
1	VAL	78.89	80	0.413	0.456
2	CIL	9.83	10	0.423	0.432

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and the LOQ of the drug were calculated using the equations 3.3 σ /S and 10 σ /S respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Table 5: LOD and LOQ.

S. No.	Drugs	LOD	LOQ
1	VAL	3.05	9.92
2	CIL	2.92	10.07

Robustness study

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters such as composition of the mobile phase, chamber saturation time, time from, time from spotting to development, time from development to scanning, and volume of mobile phase were done. The composition of the mobile phase and chamber saturation time were varied in the range of ±0.1 ml and ±2 min, respectively, of the used optimized conditions. The volume of mobile phase was varied by ±1 ml, the time from spotting to development and time from development to scanning was also varied. The effect of these changes on the retention time values and peak area were studied. The results of robustness studies are summarized in Table 6 & 7.

Table 6: System suitability (Different Flow rate).

Drugs	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
CIL	0.8	7187	1.2
	1.0	6381.5	1.2
	1.2	6471	5.0
VAL	0.8	5752	1.4
	1.0	5026.5	1.3
	1.2	4476	1.2

Table 7: Table System suitability (Different solvent ratio).

S. No	Change in organic composition in the mobile phase	System suitability results			
		VAL		CIL	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	5 % less	6498	1.2	4577	1.3
2	***Actual**	5026.5	1.3	6381.5	1.2
3	5 % more	6471	1.2	4476	1.3

RESULTS AND DISCUSSION

Results were found to be linear in the concentration range of 10-60 µg/mL for CILNI and 2.5-12.54 µg/mL for VAL with r^2 = 0.9993 and 0.9097 respectively in mobile phase buffer: Methanol 80:20. The detection was done at 260.0 nm. Retention times were found to be 2.59 and 5.06 for Valsartan and Cilnidipine respectively. The proposed method was also evaluated by the assay of commercially available tablet and % assay was found to be 98.65% for VAL and 99.45% For CIL. The accuracy of the proposed method was studied by recovery studies at three levels (50%, 100% and 150%). The % recovery was found to be in the range of 98.00-100.05 for CIL and 98.90-99.14 for VAL. The precision of the proposed method was studied by interday and intraday precision.

CONCLUSION

The developed and validated RP-HPLC method is found to be rapid, accurate, precise and economical, thus can be used for routine analysis of Valsartan and Cilnidipine in combined tablet dosage form.

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