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Original Research Article

Method development and validation of RP-HPLC for simultaneous estimation of cilnidipine and valsartan in synthetic mixture

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ABSTRACT

Reverse phase-high performance liquid chromatography (RP-HPLC) method have been developed and validated for the estimation of Cilnidipine and Valsartan in bulk drug and synthetic mixture. The developed method is rapid, accurate, precise, simple and economical. The separation was carried out using Luna C_{18} 100A° (250 mm×4.6 mm i.d.) 5 μm reverse phase column (phenomenex, luna®) in gradient mode, with mobile phase containing Acetonitrile: Water (85:15, v/v). The flow rate is 1.0 ml/min and effluents are monitored at 240 nm. Chromatogram showed peak at a retention time of 2.083 min for Cilnidipine and 5.458 min for Valsartan. The method is validated for system suitability, linearity, precision, accuracy specificity, ruggedness, robustness, LOD and LOQ. Recovery of Cilnidipine and Valsartan is found to be 100.36% and 100.14% respectively. The LOD and LOQ for estimation of Cilnidipine and Valsartan are found to be 0.037μg/ml, 0.31μg/ml and 0.206μg/ml, 0.62μg/ml respectively. Proposed method can be successfully applied for the quantitative determination of Cilnidipine and Valsartan in bulk drug and in synthetic mixture.

1. Introduction

Cilnidipine (CIL) is a dual blocker of L-type voltage-gated Ca^{2+} channels in vascular smooth muscle and N-type Ca2+ channels in sympathetic nerve terminals that supply blood vessels. It inhibits the Ca^{2+}influx in both in vessel & in the nerve. So causes the vasodilation & inhibits the release of nor epinephrine, which causes the vasodilation and decreases the heart rate & also decreases cardiac contraction in heart. So, used in treatment of hypertension. It is chemically 3-(2-methoxyethyl) 5-[3-phenylprop-2-enyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4 dihydropyridine - 3,5- dicarboxylate. Cilnidipine is Yellow Crystalline Solid having molecular weight 492.52g/ mol. [1–3]

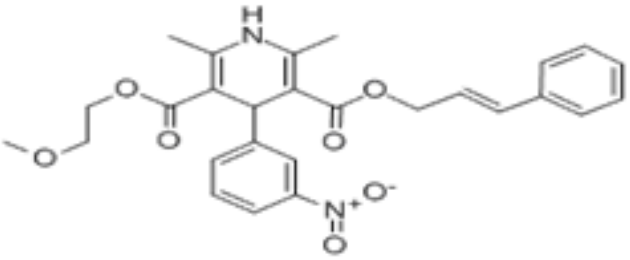

Fig. 1: Chemical structure of cilnidipine

Valsartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Inhibition of aldosterone secretion may inhibit sodium and water reabsorption in the kidneys while decreasing potassium excretion. It is chemically 3-methyl-2- [pentanoyl- [[4-[2-(2h-tetrazol-5-yl) phenyl] phenyl] methyl] amino]-butanoic acid. Valsartan is a White Crystalline Powder having molecular

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weight435.52g/ mol.[3,4]

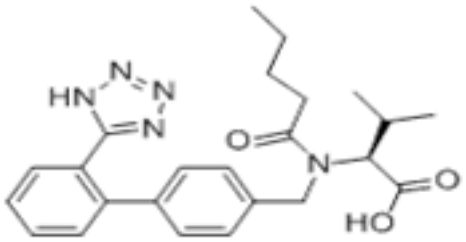

Fig. 2: Chemical structure of valsartan

Both drugs are anti-hypertensive agent. Soluble in methanol. Both drug ultimately inhibit calcium influx which causes vasodilation. So used in treatment of Hypertension.

The review of literature regarding quantitative analysis of Cilnidipine and Valsartan revealed that no Simultaneous Equation method attempt was made to develop analytical methods for Cilnidipine and Valsartan. Some spectrometric methods and chromatographic methods have been reported for the estimation of the individual and combination of drugs.[5–10] The focus of the present study was to develop and validate a rapid, stable, specific, and economic Spectroscopic method for the estimation of Cilnidipine and Valsartan in Synthetic Mixture.

2. Materials and Methods

CIL and VAL reference standard are kindly supply by Nikshan Pharmaceuticals, Ankleshwar and Cipla Pharmaceuticals, Ankleshwar as a gift sample respectively. Synthetic mixture contains 10mg Cilnidipine and 80 mg of Valsartan.

Chromatographic analysis was carried out on a prominence liquid chromatograph (UFLC Shimadzu Corporation, Tokyo, Japan) with LC-2010AHT series binary pump systems, Auto sampler injection, temperature controller (column oven, HCO-O2, PCI Analytics) system controller and a UV detector (LC-2010). CLASS-VP (version 2.31) software was used to acquire and process the data. A Semi micro analytical balance (Sartorius CD2250, Germany) was used for weighing purpose. HPLC water was obtained using arium®611VF (Sartorius). Magnetic stirrer (1 MLH, Remi) was used for mixing purpose. pH tutor (313927, Eutech Instruments) was used for pH measurement. Sonication of solutions was done using Ultrasonic cleaner (D 120/1H, Trans-O-Sonic). Column: Luna C_{18} 100A° (250mm×4.6mm i.d.) 5 μm (Phenomenex, Luna®). Pipettes of 1, 2, 5 and 10 ml capacity. Measuring cylinders of 10, 100 ml and 500 ml capacity. Class 'B' volumetric glassware. All apparatus and instrument were calibrated before use. Acetonitrile HPLC Grade, Merck Ltd. Membrane filter: 0.22 μm nylon membrane filter (RANKEM).

2.1. Preparation of mobile phase

The mobile phase consisted of mixture of acetonitrile and water in ratio of (85:15, v/v). The mode for was gradient. The mobile phase was filtered through a 0.22μm nylon membrane filter and degassed prior to use. This mobile phase was further used for preparation of stock solution.

2.2. Preparation of standard solution

Standard stock solution was prepared by dissolving 10mg of Cilnidipine and 10 mg of Valsartan drug in sufficient amount of mobile phase in a 10 ml volumetric flask and diluted up to the mark. From that 1 ml of standard solutions from Cilnidipine and 5 ml standard solution from Valsartan stock solution were pipette out in to a clean and dry volumetric flask and it was made up to 10 ml using mobile phase. The solution containing 100μg/ml of Cilnidipine and 500μg/ml of Valsartan. From Cilnidipine stock (100μg/ml) take 1 ml and from Valsartan stock (500μg/ml) take 1.6 ml in to a clean and dry volumetric flask and it was made up to 10 ml using mobile phase. Now, finally the solution has concentration 10μg/ml for Cilnidipine and 80μg/ml for Valsartan.

2.3. Sample preparation

It was prepared as per the patent.[11]

1. Cilnidipine:10 mg
2. Valsartan: 80 mg
3. Crosscamellose Sodium: 10 mg
4. HydroxyPropyl Cellulose: 10 mg
5. Hydrated Silicone Dioxide: 10 mg
6. Macrogol (PEG) 6000: 30 mg

All the excipients were mixed in 100ml volumetric flask and Sonicate for 15min. make up the volume with Methanol. The solution was filtered through Whatman filter paper No. 42.

Finally, the solution had concentration 100μg/ml for CIL and 800μg/ml for VAL from that pipette out 1ml in 10 ml volumetric flask and volume was made up to mark with mobile phase - ACN: Water (85:15 v/v) to make final concentration CIL (10μg/ml) and VAL (80μg/ml). Chromatogram of the Test solution containing 10μg/ml of CIL and 80μg/ml of CIL was recorded and peak areas were noted for estimation of CIL and VAL.

3. Results and Discussion

3.1. Validation parameters

3.1.1. Linearity

Five points calibration curve was obtained in the concentration range of 5-25μg/ml for Cilnidipine and 40-200μg/ml for Valsartan. The response of drug was found to

be linear in investigation range and the regression equations was found to be y = 16847x +105742 (n = 6) for CIL and y = 6688x + 359883 (n = 6) for VAL, with the correlation coefficient 0.9993 and 0.9996 (n = 6) respectively, is listed in Table 3. Chromatogram for linearity was as per Figure 3.

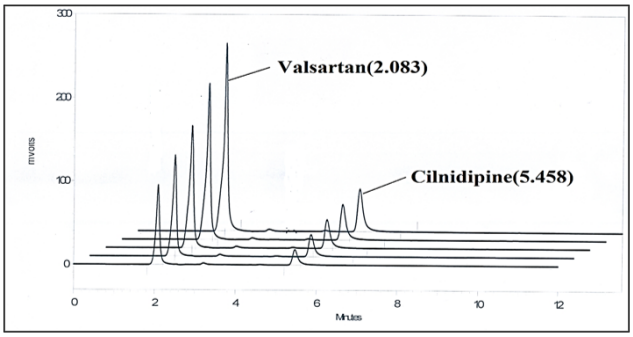

Fig. 3: Overlain chromatogram for five concentrations of CIL (5-25μg/ml) and VAL (40-200μg/ml)

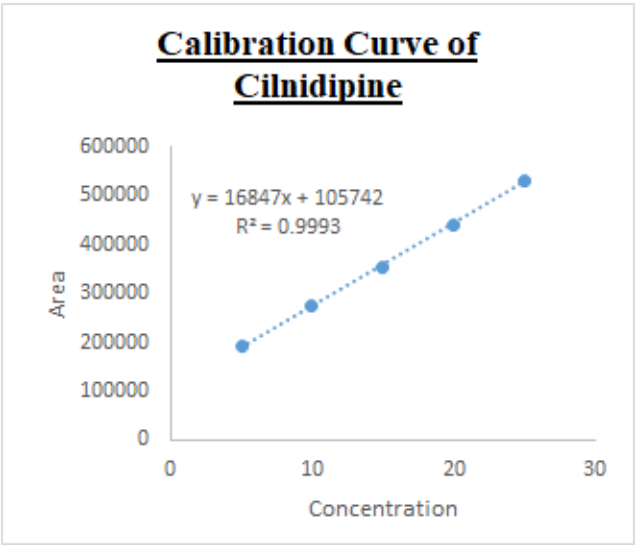

Fig. 4: Calibration curve of cilnidipine

Table 1: Chromatographic condition

Parameter	Condition
Method	Gradient reverse phase technique
Stationary Phase	Luna C_{18} 100A° (250mm×4.6mm i.d.) 5μm reverse phase column (Phenomenex, Luna®)
Mobile Phase	Acetonitrile : Water (85:15 v/v)
Flow Rate	1.0 min/ml
Wavelength	240nm
Total Run Time	12min

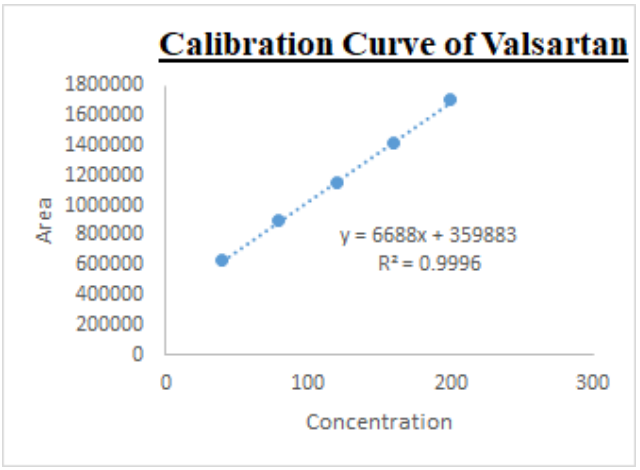

Fig. 5: Calibration curve of valsartan

3.1.2. Precision

The precision of the method was evaluated in terms of inter-day and intra-day by carrying out independent assays of three concentrations chosen from range of the standard curves (15, 20 and 25μg/ml of CIL and 120, 160, 200μg/ml of CIT) and the %RSD of assay (inter-day and intra-day) was calculated. The results of study are shown in Table 4 and Table 5.

3.1.3. Accuracy

The accuracy of the method was determined by spiking of CIL and VAL to pre quantified sample solutions of CIL (10μg/ml) and VAL (80μg/ml) in triplicate at three concentration level of 80, 100, and 120% of the specified limit. The percentage recoveries of CIL and VAL were calculated and the result is nearer to 100% shown in Table 6 and Table 7.

3.1.4. Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were evaluated by standard deviation of response and slope method. LOQ and LOD were calculated by the equation LOD = 3.3 × N/B and LOQ = 10 × N/B, where "N" is standard deviation of the absorbance and "B" is the slope of the corresponding calibration curve. The limit of detection (LOD) were found to be 0.103μg/ml for CIL and 0.206μg/ml for VAL and respectively and limit of quantitation (LOQ) were found to be 0.314μg/ml for CIL and 0.626μg/ml for VAL presented in Table 8.

3.1.5. Robustness and Ruggedness

Robustness was done by change in flow rate and mobile phase composition. The result was decided by % RSD which is in the limit which is mentioned in Table 9.

Table 2: System suitability parameters

Parameters	Observed Values		IP'2010 Specification
	CIL*	VAL*	
Theoretical plates	7605.74	2191.34	Not less than 2000
Asymmetry (10%)	1.57	0.89	Not greater than 2
Resolution	15.76	-	> 2

Table 3: Calibration data for CIL and VAL (n=6)

S. No	Concentration (μg/ml)		Peak Area* ± SD CIL	Peak Area* ± SD VAL
	CIL	VAL		
1	5	40	192773	630748
2	10	80	273960	898418
3	15	120	354792	1156403
4	20	160	439573	1418166
5	25	200	531144	1708471

Table 4: Intraday precision data for estimation of CIL and VAL *(n=3)

Conc. (μg/ml)		Peak area* ± SD CIL	% RSD	Peak area* ± SD VAL	% RSD
CIL	VAL				
5	125	192677.7±105	0.054	630868±162	0.025
10	250	354753.3±129	0.036	1156667±256	0.022
15	375	531227.7±136	0.025	1707480±477	0.027

Table 5: Inter day precision data for estimation of CIL and VAL *(n=3)

Conc. (μg/ml)		Peak area* ± SD CIL	% RSD	Peak area* ± SD VAL	% RSD
CIL	VAL				
5	125	192902±125	0.065	631095±528	0.083
10	250	354862±144	0.040	1156859±308	0.026
15	375		0.032	1708878±535	0.031

Table 6: Recovery data of CIL *(n=3)

Conc.of CIL from formulation (μg/ml)	Amount of Std. CILadded (μg/ml)	Total amount of CIL (μg/ml)	Total amount of CIL found (μg/ml)Mean* ± SD	% Recovery (n=3)	% RSD CIL
10	8.0	18	18.02±375	100.11	0.123
10	10	20	20.15±325	100.75	0.095
10	12	22	22.05±366	100.22	0.098

Table 7: Recovery data of VAL*(n=3)

Conc.of VAL from formulation (μg/ml)	Amount of Std. VALadded (μg/ml)	Total amount of VAL (μg/ml)	Total amount of VAL found (μg/ml)Mean* ± SD	% Recovery (n=3)	%RSD VAL
80	64	144	144.08±339	100.05	0.035
80	80	160	160.14±816	100.08	0.076
80	96	176	176.53±475	100.30	0.040

Table 8: LOD and LOQ data of CIL and VAL

Parameter	CIL	VAL
LOD (μg/ml)	0.103	0.206
LOQ (μg/ml)	0.314	0.628

Table 9: obustness and Ruggedness data of CIL and VAL *(n=3)

No.	Factor	Level	Peak area* ± SD	%RSD	R_{t*} ± SD	%RSD
CIL (25μg/ml)						
1.	Change in the Flow Rate (ml/min)	0.8	531627±230	0.043	5.646±0.020	0.356
		1.2	513593±961	0.187	5.237±0.021	0.410
2.	Change in Mobile Phase Composition (v/v)	87:13	552774±236	0.042	5.523±0.017	0.309
		13:87	503253±252	0.050	5.386±0.010	0.109
VAL (200μg/ml)						
1.	Change in the Flow Rate (ml/min)	0.8	1708588±993	0.058	2.322±0.016	0.693
		1.2	1507106±971	0.064	1.983±0.010	0.517
2.	Change in Mobile Phase Composition (v/v)	87:13	2055887±955	0.046	2.453±0.015	0.647
		13:87	1405991±231	0.016	2.282±0.013	0.607

Table 10: Analysis data of commercial formulation *(n=3)

S. No.	Formulation (synthetic mixture)		Peak area CIL	% Assay* CIL±SD	%RSD	Peak area VAL	% Assay* VAL±SD	%RSD
	CIL	VAL						
1			170052			539980		
2	10	80	169599	100.80 ± 276	0.162	539759	100.90 ± 0.021	0.021
3			170099			539820		

3.1.6. Assay

The assay was done by the synthetic mixture and the result was calculated as per the Table 10.

4. Conclusion

The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, LOD and LOQ. As there was no interference due to excipients and mobile phase, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Temperature separately and analysis being performed by different analysts. Good agreement was seen in the assay results of formulation by developed method. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Cilnidipine and Valsartan in Bulk drug and in synthetic mixture. The method was validated by employment of ICH[12] guidelines.

5. Source of Funding

None.

6. Conflict of Interest

None.

7. Acknowledgement

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