DEVELOPMENT AND VALIDATION OF *HPTLC* METHOD FOR SIMULTANEOUS ESTIMATION OF CILNIDIPINE AND VALSARTAN IN BULK AND TABLET DOSAGE FORM

Ritesh P. Bhole*.Vinayak C. Pawara, Sohan S. Chitlange, Sager B. Wankhede

Department of Pharmaceutical Chemistry, Pad. Dr. D.Y.Patil,
Institute of Pharmaceutical Sciences and Research, Pimpri, Pune - 411018, Maharashtra,

***Corresponding author:**
E-mail: ritesh.edu@gmail.com

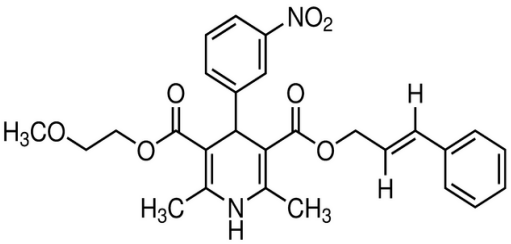
ABSTRACT

Cilnidipine and Valsartan in combined dosage form has been developed and validated. Sample and standard solutions of Cilnidipine and Valsartan were applied to precoated silica gel G 60 F254 HPTLC plates and the plates were developed with Toluene: Methanol: Ethyl acetate: Glacial Acetic acid in the ratio 8:1:1:0.1 (v/v/v/v) as mobile phase. The Rf value for cilnidipine and Valsartan was found to be 0.29 min and 0.56 min respectively. The detection was performed at 240 nm. The calibration curve was found to be linear between 1000 to 6000 ng/band for cilnidipine and 8-48 µg/band for Valsartan with correlation coefficients 0.993 and 0.993 for Cilnidipine and Valsartan respectively. Accuracy and precision of the proposed method was evaluated by recovery studies (% recovery for Valsartan and Cilnidipine was 99.03 % and 99.86 %).The results have been validated statistically as per ICH guidelines.

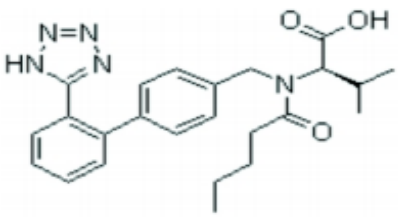
Keywords: *Validation, Valsartan, Cilnidipine, HPTLC*

INTRODUCTION

Cilnidipine (CILNI), 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 terminals1. **Valsartan** (VALSA) 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.



Structure of CILNIDIPINE



Structure of VALSARTAN

Fig. 1: Typical structures of Valsartan and Cilnidipine

Literature survey reveals 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 CILNI either as a single or in combination with other drugs in pharmaceutical preparations. Analytical methods reported for VALSA 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 CILNI and VALSA. 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

Pre-coated silica gel 60F254 aluminium plates (10 x 10 cm, 250 µm thickness; Merck, Germany), Automatic TLC sampler 4 (Camag, Switzerland), twin trough chamber (10 x 10 cm; Camag, Switzerland), UV chamber (Camag, Switzerland), TLC scanner 4 (Camag, Switzerland),

winCATS version 1.4.6 software (Camag, Switzerland) were used in the study. Ultrasonic bath (PowerSonic405, 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 1000, 2000, 3000, 4000, 5000, 6000 ng/band for Cilnidipine and 8, 16, 24, 32, 40, 48 µg/band 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 ultrasonicated 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 Toluene: Methanol: Ethyl acetate: Glacial Acetic acid in the ratio 8:1:1:0.1 (v/v/v/v) was the most appropriate mobile phase for the *HPTLC* analysis of Cilnidipine and Valsartan in methanol as solvent.

Application of standard solutions

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 ultrasonicated for 10 minutes, volume was then made up to the mark with methanol. (conc. 8000 µg/ml and 1000 µg/ml of VALSA and CILNI, respectively).

Application of sample solution

10µl of the mixed standard solution of 10 µg/ml for cilnidipine and 80 µg/ml for Valsartan was applied. The same procedure was repeated with the sample solution prepared from tablet dosage form. After application the position of spots were visualized and confirmed under UV cabinet at 240 nm.

Development of spot

Twin Trough chamber containing 10 ml of mobile phase system was used for developing the spotted plates and saturated for 15 minutes. The plates were dried after development and viewed under UV lamp to evaluate the spot obtained. The spots were uniform and there was no tailing.

Scanning by *HPTLC* scanner

After setting up the instrument parameters, the spot was scanned from 200-400 nm and the spots showed maximum absorption at 240 nm using the Camag TLC scanner 4. (Figure 1) The Rf values were found to be 0.56 for cilnidipine and 0.29 for Valsartan. Typical chromatograms obtained for Cilnidipine and Valsartan of sample (Figure 2).

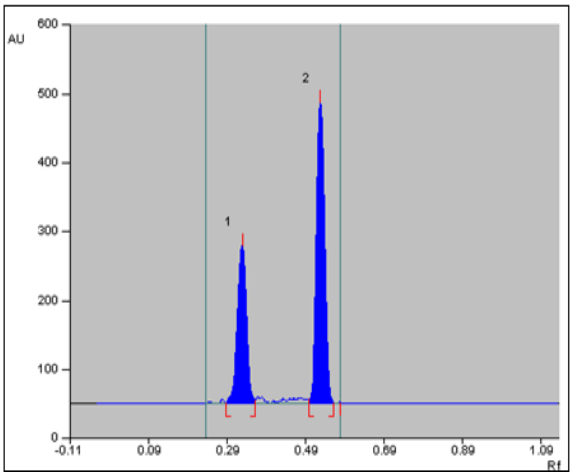


Fig. 2: Typical densitogram of Valsartan and Cilnidipine

The method was validated for linearity, accuracy and intra-day and inter-day precision and specificity, in accordance with ICH guidelines [16].

Calibration curve:

Response to Cilnidipine and Valsartan was linear in the concentration ranges 1000-6000 ng/band for Cilnidipine and 8-48 µg/band for Valsartan respectively. The regression equations for Cilnidipine and Valsartan were y = 1.694x and y = 211.8x respectively, where y is response and x the concentration of drug (Figure no. 3,4). The correlation coefficients were 0.993 and 0.993 respectively. [Table1]

Table 1: Standard calibration data for Valsartan and Cilnidipine

Sr. No.	VALSA		CILNI	
	Concen-tration µg/band	Mean peak area 240.0 nm	Concen-tration ng/band	Mean peak area 240.0 nm
1.	8	2568.13	1000	1081.93
2.	16	4345.70	2000	1808.39
3.	24	5357.74	3000	2436.11
4.	32	6644.86	4000	3341.28
5.	40	7940.55	5000	3994.06
6.	48	9120.23	6000	5181.85

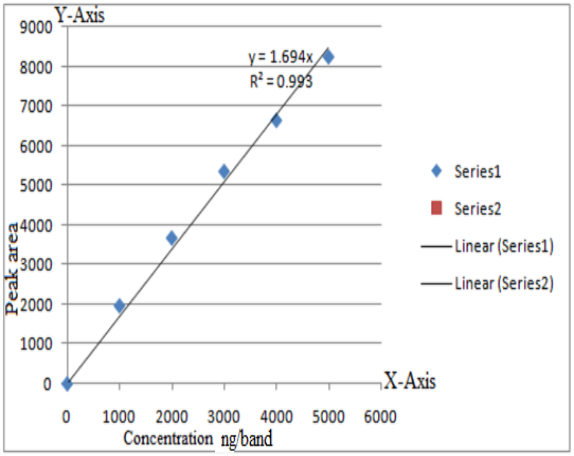


Fig. 3: Calibration curve of CILNIDIPINE at 240.0 nm

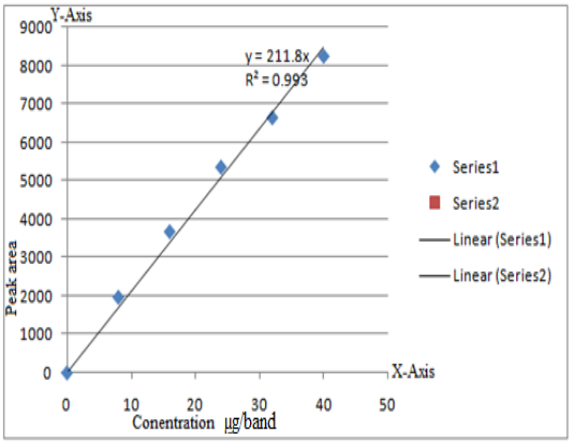


Fig. 4: Calibration curve of VALSARTAN at 240.0 nm

Assay for marketed preparation

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 2: Summary of results of analysis of marketed formulation

Sr. no	Drug	Amount of drug estimated (mg/tablet)*	% Label Claim*	S.D. (±)	R.S.D.
1.	VALSA	78.92	98.65	0.440	0.454
2.	CILNI	9.92	99.45	0.447	0.472

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 (80%, 100%, and 120%) were added to sample solutions of tablet dosage forms. The % recovery found to be between 96.00-100.10 % 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 ng/band and 8 µg/band 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.

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.

Specificity

Specificity study was performed by analyzing standard of drugs and samples. The spot for VALSA and CILNI in sample was confirmed by comparing the Rf value and spectra of the spot with that of standards.

Table 3: Summary of results of method validation

Content		*HPTLC* Method	
		VALSA	CILNI
Linearity Range		8-48 µg/band	1000-6000 ng/band
Accuracy	Percent Recovery* S.D. (±)	96.61 ± 0.4975	96.94 ± 0.479
Precision	Intra-day Precision		
	% Label Claim** (±) S.D.	99.86 ± 0.5432	99.45 ± 0.2821
	Inter-day Precision		
	% Label Claim** S.D. (±)	99.67 ± 0.5121	99.10 ± 0.2789
LOD (µg/band)		1.324	0.00083
LOQ (µg/band)		3.678	0.00757

*Mean of nine determinations

** Mean of three determinations

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 Rf values and peak area were studied.
The results of robustness studies are summarized in Table 4

Table 4: Summary of result of robustness study

Sr. No.	Factor	R.S.D. for peak area	
		VALSA	CILNI
1.	Mobile phase composition (± 0.1 ml)	0.584	0.4088
2.	Duration for chamber saturation (± 2 min)	0.470	0.3149
3.	Spotting to development	1.234	1.205
4.	Development to scanning	0.2743	0.694
5.	Volume of mobile phase (± 1 ml)	0.329	3.284
6.	Stability of solution	3.135	3.769

There was no significant change in the Rf value of both the drugs as the Rf were within ± 5.0 Rf units of standard values. As there was no significant change in Rf value and peak area for both the drugs, it indicates the robustness of the method.

Degradation study:

In forced degradation studies, intentional degradation was tried by exposing tablet powder sample to following stress condition, acidic (0.1M HCl), alkaline (0.1M NaOH), oxidation (3 % H_2O_2), and UV exposure (for 24 hrs). For acidic, alkaline and oxidation conditions the content in the flasks were kept in water bath at 80^0C for 3 hr. After the respective time intervals all the flasks were removed and allowed to cool. The samples were then analyzed in similar manner as described under analysis of tablet formulation.

The Results of forced (stress) degradation studies are summarized in Table 5.

Table 5: Summary of results of forced degradation studies

Sr. No.	**Stress Condi-tion**	**Temperature and time**	**% assay of active substance**		**Rf values of degraded peaks**	
			VALSA	**CILNI**	**VALSA**	**CILNI**
1.	Acid (0.1 M HCl)	80^0C for 3 hr	90.75	92.50	0.54,0.47,0.14	0.58,0.74,0.86
2.	Alkali (0.1m NaOH)	80^0C for 3 hr	96.33	84.53	0.40	0.59,0.99
3.	Oxide (3 % H_2O_2)	80^0C for 3 hr	97.85	94.17	0.39,0.58	0.78, 0.89, 1.03
4.	Photo degradation	24 hr	99.09	98.72	-	-

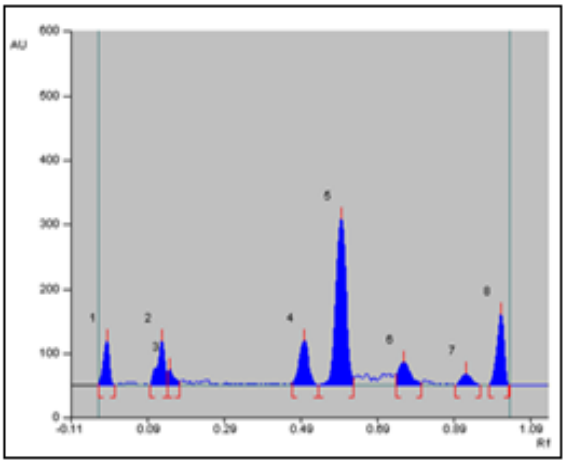


Fig. 5: Densitogram of acid (0.1 M HCl) treated sample

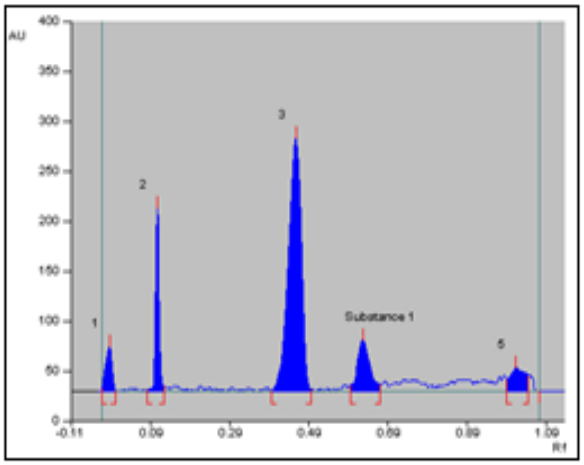


Fig. 6: Densitogram of alkali (0.1 M NaOH) treated sample

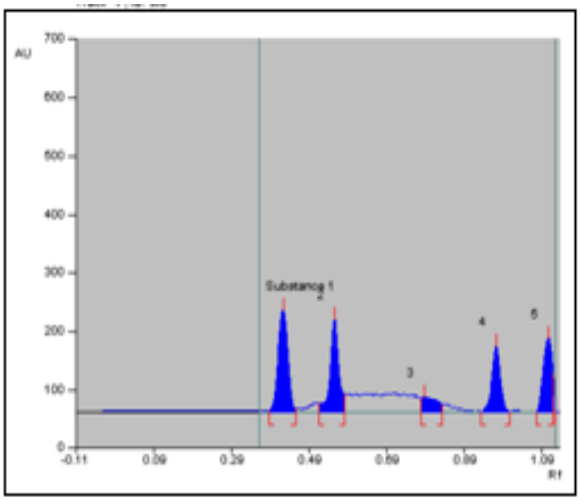


Fig. 7: Densitogram of oxide (3% H_2O_2) treated sample

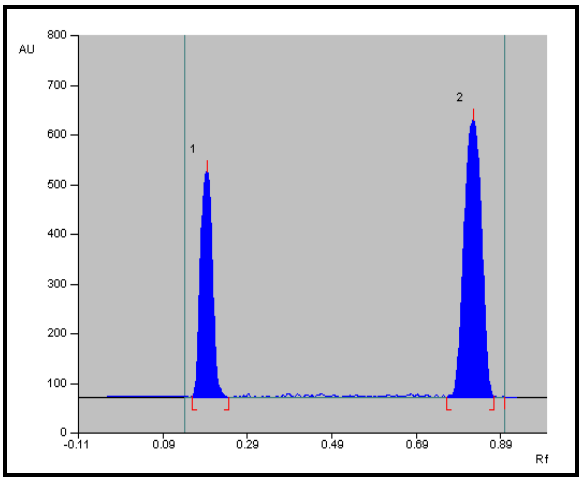


Fig. 8: Densitogram of sample exposed to UV

Cilnidipine and Valsartan was found to degrade in acidic, alkaline and oxide conditions. Degradation in alkaline condition was more as compared to acidic stress condition. However, Valsartan and Cilnidipine was found stable under photodegradation conditions. The method was able to resolve the peak of degradation products from the peak of VALSA and CILNI.

RESULTS AND DISCUSSION

Results were found to be linear in the concentration range of 1000-6000 ng/band for CILNI and 8-48 µg/band for VALSA with r2 = 0.993 and 0.993 respectively in mobile phase Toluene: Methanol: Ethyl acetate: Glacial Acetic acid in the ratio 8:1:1:0.1 (v/v/v/v). The detection was done at 240.0 nm. Rf value was found to be 0.29 and 0.56 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 VALSA and 99.45% For CILNI. The accuracy of the proposed method was studied by recovery studies at three levels (80%, 100% and 120%). The % recovery was found to be in the range of 96.00-101.01 for CILNI and 96.00-100.14 for VALSA. The precision of the proposed method was studied by interday and intraday precision.

The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. The summary of validation parameters of proposed *HPTLC* method is given in Table 6.

Table 6: Summary of validation parameters CILNI and VALSA

Parameter	CILNI	VALSA
Linearity range (ng/spot)	1000-6000	8-48
Correlation co-efficient	0.993	0.993
Precision (intraday) %RSD	0.2519	0.4438
Precision (interday) %RSD	0.7556	0.4289
Accuracy (S.D)	96.00-101.0	96.00-100.0
LOD (µg/band)	0.00083	1.324
LOQ (µg/band)	0.00757	3.678

CONCLUSION

The developed and validated *HPTLC* 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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