

**DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE
THIN LAYER CHROMATOGRAPHIC METHOD FOR
SIMULTANEOUS ESTIMATION OF VALSARTAN AND NIFEDIPINE
IN SYNTHETIC MIXTURE**

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ABSTRACT

A simple, precise, sensitive, rapid high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of the Valsartan and Nifedipine in synthetic mixture. The stationary phase used was precoated silica gel 60F₂₅₄ plate. The mobile phase used was a mixture of toluene: acetonitrile: formic acid (8:2:0.2, v/v/v). The detection of spots was carried out densitometrically using a UV detector at 227 nm in absorbance mode. This system was found to give compact spots for Valsartan with R_f value of 0.25. and Nifedipine and with R_f value of 0.49. The method was validated according to ICH guidelines in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 300 to 2100 ng/spot for both the drugs with significantly high value of correlation coefficient ($r^2 > 0.99$). The limits of detection and limits of quantitation were found to be 16.86 and 51.17 ng/spot respectively for Valsartan and 18.18 and 55.10 ng/spot respectively for Nifedipine. All the validation parameters were found

to be within acceptable limits. The method was found to be rapid, cost effective, accurate and precise therefore be utilized for the routine analysis of both the drugs in synthetic mixture. The proposed method can be used in the quality control of both the drugs in synthetic mixture.

KEYWORDS: Valsartan, Nifedipine, HPTLC, Synthetic mixture, Validation.

INTRODUCTION

Valsartan (VAL) is chemically N-[p-(o-1H-Tetrazol-5-ylphenyl) benzyl]-N-valeryl-L-valine (Figure 1) is an angiotensin II receptor antagonist. It is mainly used for controlling high blood pressure and for treatment heart disease, stroke and heart attack.^[1] VAL is officially mentioned in Indian Pharmacopeia^[2] and United State Pharmacopeia.^[3] Literature review reveals UV Spectrophotometry^[4], HPLC^[5] and HPTLC^[6] methods for analysis of VAL in tablet dosage forms. Literature review also shows UV Spectrophotometry^[7], HPLC^[8] and HPTLC^[9] methods for determination of VAL in combination with other drugs. Nifedipine (NIF) is chemically 3, 5-Pyridinedicarboxylic acid, 1, 4-dihydro-2, 6-dimethyl-4-(2-nitophenyl)-, dimethyl ester (Figure 2), is a calcium channel blocker. NIF blocks L-type calcium channels. It is mainly used as an antianginal and antihypertensive^[10]. NIF is officially mention in Indian Pharmacopeia^[11], United State Pharmacopeia^[12], British Pharmacopeia^[13], European Pharmacopeia^[14] and Japanese Pharmacopeia.^[15] Literature review reveals UV Spectrophotometry^[16], HPLC^[17] and HPTLC^[18] methods for determination of NIF in tablet and capsule dosage forms. Literature review also shows Spectrophotometric^[19], HPLC^[20] and HPTLC^[21] methods for determination of NIF in combination with other drugs. The combination of VAL and NIF has no official status in any pharmacopoeia. The combination of VAL and NIF is under clinical trial phase study (identifier no: NCT00993109) conducted by Bayer Pharmaceuticals²² (Chine & Korea FDA). Combination of VAL and NIF is found to be useful in reduction of morning blood pressure and also shows greater reduction in diastolic blood pressure. Literature survey uncover only few reported spectrophotometric method for simultaneous estimation of VAL and NIF in synthetic mixture.^[23-24] The present work describes simple, rapid, accurate, precise and cost effective HPTLC method for simultaneous estimation of VAL and NIF in synthetic mixture.

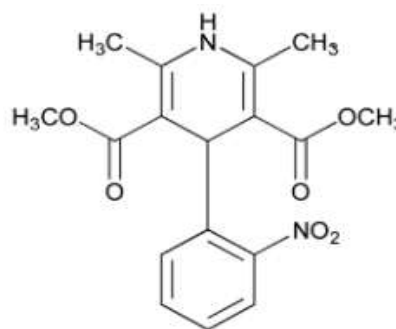
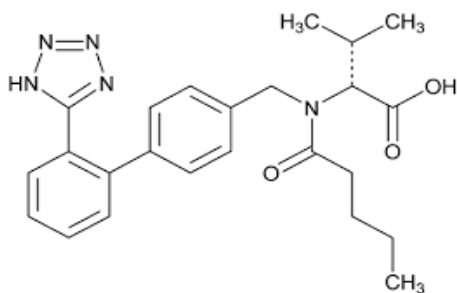


Figure 1: Chemical structure of Valsartan **Figure 2: Chemical structure of Nifedipine**

MATERIALS AND METHODS

Apparatus

Pre-coated silica gel aluminum plate 60F₂₅₄, (10 x 20 cm, layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) was used as stationary phase, Camag (Muttens, Switzerland) flat bottom and twin-trough flat-bottom TLC developing chamber (10 x 20 cm), Volumetric flasks and pipettes.

Materials and Reagents

VAL and NIF standard drug powder kindly procure from Torrent Pharmaceutical Pvt. Ltd., Ahmedabad. Chemical used during experimental works were Methanol, Toluene, Acetonitrile and Formic acid (85%). All chemicals and reagents used were of analytical grade.

Instruments

A Camag HPTLC system (Switzerland) with Linomat V automatic sample applicator and Camag TLC Scanner IV, Camag TLC Visualizer, Ultrasonic bath (Frontline ultrasonic bath, Mumbai, India), Analytical balance (CP224S, Sartorius, and Gottingen, Germany).

Software

Win-CATS 1.4.6 planar chromatography manager software.

Preparation of standard stock solution

VAL (3 mg) and NIF (3 mg) were individually weighed accurately, dissolved and diluted with methanol to obtain the final concentration of 300 µg/ml for both VAL and NIF in separately 10 ml volumetric flask. For NIF preparation amber colored volumetric flask was used.

Preparation of Synthetic mixture

Synthetic mixture (180 mg) was prepared by using VAL (80 mg), NIF (30 mg) and excipients (70 mg) like starch, magnesium stearate, lactose and talc.

Chromatographic conditions

The experiment was performed on stationary phase which was silica gel 60F₂₅₄ aluminum sheets (10 x 20 cm) layer thickness 0.2 mm. Mobile phase was consist of mixture of Toluene: Acetonitrile : Formic acid (85%) (8:2:0.2, v/v/v). TLC plates were prewashed with methanol and activated in an oven at 50° for 5 min prior to experimental work. The sample solutions were applied on TLC plate in the form of bands of 6 mm width under a stream of nitrogen

gas using a Camag Linomat V automatic sample applicator. A constant application rate of 0.1 $\mu\text{l/s}$ was employed and space between two bands was fixed at 16 mm. Ascending development to 70 mm was performed in 10 cm x 20 cm Camag twin trough glass chamber (Muttenez, Switzerland) saturated with the mobile phase for 20 min at room temperature. The developed TLC plate was dried by dryer and then scanned between 200 to 400 nm using Camag TLC scanner IV using WinCATS 1.4.6 planar chromatography manager software. Both the drugs show reasonably good response at 227 nm keeping the slit dimension of 4×0.30 mm and scanning speed of 20 mm/s. The monochromatic band width was set at 20 nm, each track was scanned thrice and baseline correction was used. Two and five microliters of standard and sample solutions of VAL and NIF were spotted and developed.

Calibration curves of VAL and NIF

Calibration curve were plotted over a concentration range of 300- 2100 ng/spot for both the drugs. For this aliquots of 1, 2, 3, 4, 5, 6, and 7 μl from 300 $\mu\text{g/ml}$ standard stock solution of VAL and NIF were over spotted on the TLC plate. TLC plate was dried by dryer, developed in TLC chamber and analyzed photometrically as described earlier. The calibration curves were obtained by plotting peak area Vs concentration (ng/spot) corresponding to each spot.

VALIDATION OF THE DEVELOPED METHOD^[25]

Linearity

Calibration curve were plotted over a concentration range of 300-2100 ng/spot for both the drugs. For this, 1, 2, 3, 4, 5, 6 and 7 μl of 300 $\mu\text{g/ml}$ of stock solution was spotted in band width 6 mm using Hamilton syringe (100 microliter) for both the drugs individually on precoated silica gel aluminum plate 60 F₂₅₄ using automatic application device.

Precision

The repeatability of the method was checked by repeated scanning and measuring the peak area of solutions (n = 6) of VAL (1200 ng/spot) and NIF (1200 ng/spot) without changing the parameters of the developed method It showed low % RSD of peak area of VAL and NIF. The inter-day and intra-day variation was determined at three different concentration levels on three different days over a period of one week (interday precision) and three different times on same day (intraday precision) .Concentration level taken were 900, 1200, 1500 ng/spot for both drugs.

Limit of detection and limit of quantification

LOD and LOQ of the drug were calculated by using the following equations designated by ICH guideline: $LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma/S$.

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

Specificity

The specificity of the method was ascertained by analyzing standard drugs and the sample. The spots for VAL and NIF in the samples were confirmed by comparing the R_f and spectra of the spots with that of the standards.

Recovery studies

The accuracy of the method was determined by calculating recoveries of VAL and NIF by the standard addition method. Known amounts of standard solution of VAL and NIF were added at 50 %, 100 % and 150 % levels to pre quantified sample solutions of VAL and NIF. Accuracy was determined in terms of percentage recovery. The experiment was conducted in triplicate.

Analysis of VAL and NIF in synthetic mixture

The amount equivalent to 80 mg VAL and 30 mg NIF from synthetic mixture was transferred to 100 ml volumetric flask and 50 ml methanol was added to it and sonicated for 15 minutes. The solution was filtered through Whatman filter paper No.41 and volume was diluted up to mark with methanol. The resulting sample solution had final concentration of 800 $\mu\text{g/ml}$ of VAL and 300 $\mu\text{g/ml}$ of NIF. The peak area of sample solution was measured densitometrically at 227 nm for quantitation of VAL and NIF. The amount VAL and NIF present in the sample solutions were determined by fitting the response into the respective regression line equation for VAL and NIF.

RESULTS AND DISCUSSION

Development of the optimum mobile phase: The TLC procedure was optimized with a view to develop an assay method. Both the pure drugs were spotted on the TLC plates and run in different solvent systems as mention in below Table 1. Finally, the mobile phase consisting of Toluene: Acetonitrile: Formic acid (85%) (8:2:0.2, v/v/v) gave sharp and symmetrical peak with R_f values of 0.25 for VAL and 0.49 for NIF. A 3D chromatogram showing peak of VAL and NIF in different concentration at 227 nm as shown in Figure 3 and chromatogram

of standards spot and sample spot from synthetic mixture are depicted in Figure 4 and Figure 5 respectively.

Table 1: Optimization of Mobile Phase

Trial No.	Mobile phase	Observation
1	Toluene: Ethyl acetate (8:2, v/v)	Very Poor separation.
2	Toluene: Methanol (6:4, v/v/v)	Both drugs were separated with but VAL shows tailing.
3	Toluene: ACN: Trimethylamine (7:2.5:0.5, v/v/v)	Both drugs were separated with poor Rf, VAL shows tailing.
4	Toluene: Methanol: Trimethylamine (7:2.5:0.5, v/v/v)	Both drugs well separated but noise in between two peak is high.
5	Toluene: Ethanol; Trimethylamine (7:2.5:0.5, v/v/v)	Both the drugs were separated but with higher Rf (Rf >9)
6	Toluene: Acetonitrile: Acetic acid (7:3:0.2, v/v/v)	Both the drugs were separated but VAL shows tailing.
7	Toluene: Acetonitrile: Formic acid (85%) (8:2:0.2, v/v/v)	Both drugs were separated with good resolution and good intensity.

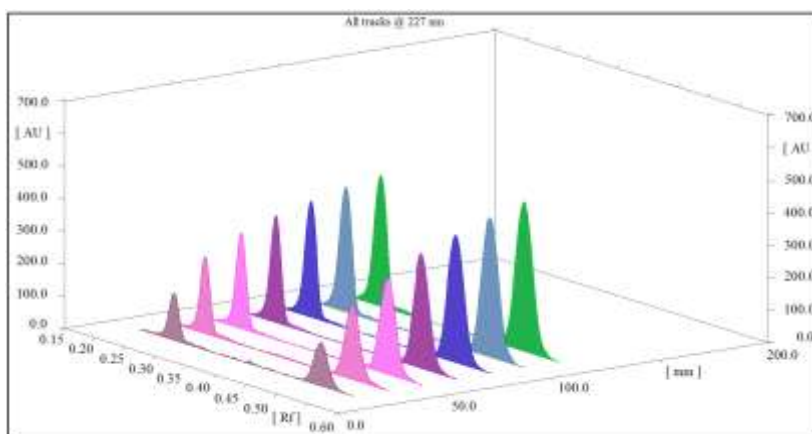


Figure 3: 3D Densitogram of VAL and NIF at 227 nm

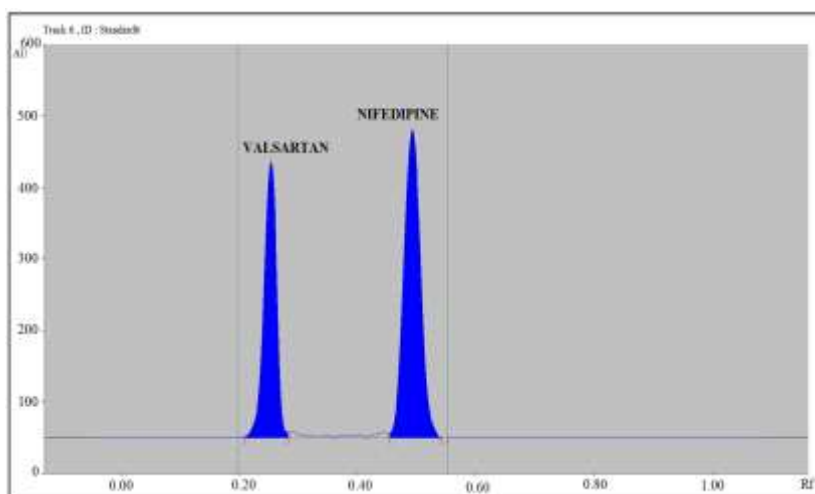


Figure 4: Chromatogram of VAL (1800 ng/spot) and NIF (1800 ng/spot)

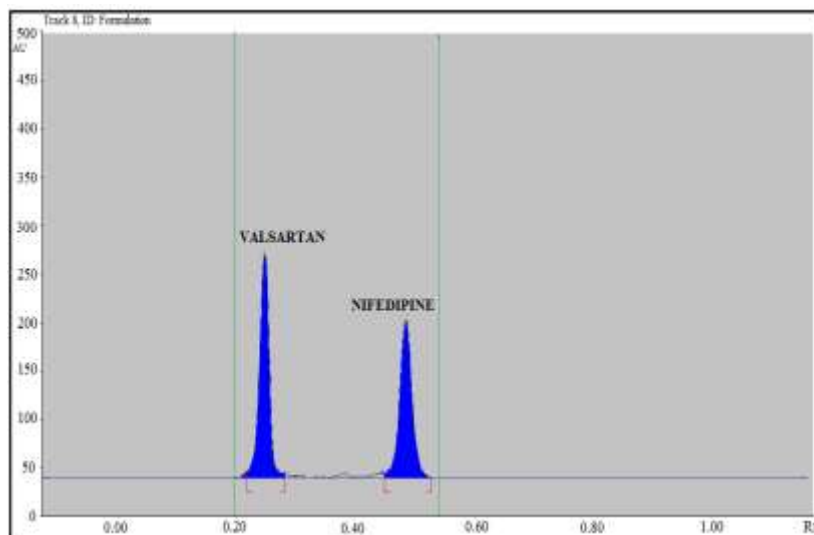


Figure 5: Chromatogram of VAL (800 ng/spot) and NIF (300 ng/spot) in synthetic mixture

Validation data of the method

Calibration graph was found to be linear over the concentration range of 300-2100 ng/spot for both the drugs as per beer lambert law²⁶. Peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equations. Equations were ($y = 2.718x + 1921$) (r^2) 0.9977 for VAL and ($y = 4.443x + 2347$) (r^2) 0.9946 for NIF. In precision the repeatability of developed were expressed in terms of % RSD. The % RSD was found to be < 2 %.The results of inter and intraday variation were found to be in acceptable limit. LOD and LOQ were found to be 16.86 and 51.17 ng/spot respectively for VAL and 18.18 and 55.10 ng/spot respectively for NIF. The summary data of validation parameter are as mentioned in Table 2. For specificity it was observed that the excipients present in the formulation did not interfere with the peaks of VAL and NIF. The peak purity of VAL and NIF were assessed by comparing the spectra of standard and sample at peak start, peak apex and peak end positions of spot. For VAL standard spot and sample spot r (S, M) = 0.99966 & 0.99950 and r (M, E) = 0.99958 & 0.99947 respectively. For NIF standard spot and sample spot r (S, M) = 0.99929 & 0.99914 and r (M, E) = 0.99949 & 0.99938. Good correlation was also found between standards and sample chromatogram of VAL and NIF as shown in figure 6 and figure 7 respectively. In recovery study recovery average was found to be 100.8 ± 1.62 for VAL and 100.4 ± 1.21 for NIF. Recovery data are listed in Table 3. Analysis of synthetic mixture was performed two spots at R_f 0.25 and R_f 0.49 were observed in chromatogram of the drugs samples extracted from synthetic mixture. There was no interference from the excipients commonly present in the synthetic mixture. The average drug content as per label

claim was found to be 100.7% with S.D of 1.42 for VAL and 100.3% with a S.D of 1.88 for NIF as mentioned in Table 4 . The low values of standard deviation indicates the suitability of this method for routine analysis of VAL and NIF in synthetic mixture.

Table 2: Regression Analysis Data And Summary of Validation Parameters For The Developed Method

PARAMETERS	VAL	NIF
Wavelength (nm)	227	227
Beer's law limit (ng/spot)	300-2100	300-2100
Regression equation (y = a+bc)	2.718x + 1921	4.443 + 2347
Slope (b)	2.718	4.443
Intercept (a)	1921	2347
Correlation Coefficient (r ²)	0.9977	0.9946
Method precision (Repeatability) (% RSD), (n = 6)	0.94	0.79
Intraday (n = 3) (%RSD)	0.82 – 1.35	0.71 – 1.23
Interday (n = 3) (%RSD)	0.86 – 1.86	0.76 – 1.74
LOD (ng/spot)	16.86	18.18
LOQ (ng/spot)	51.17	55.10
Accuracy (Mean % Recovery± S.D.) (n =3)	100.8 ± 1.62	100.4 ± 1.21
% Assay ± S.D. (n = 6)	100.7 ± 1.42	100.3 ± 1.88

Table 3: Results of Recovery Study of VAL and NIF by Developed Method.

Drug	Level	Amount taken (ng/spot)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
VAL	I	800	50	100.7 ± 1.30
	II	800	100	100.1 ± 1.52
	III	800	150	101.6 ± 2.04
NIF	I	300	50	99.44 ± 1.19
	II	300	100	100.3 ± 1.62
	III	300	150	101.5 ± 0.83

Table 4: Analysis of VAL and NIF in Synthetic Mixture by Developed Method.

Synthetic mixture	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 6)	
	VAL	NIF	VAL	NIF	VAL	NIF
I	80	30	80.60	30.09	100.7 ± 1.42	100.3 ± 1.88

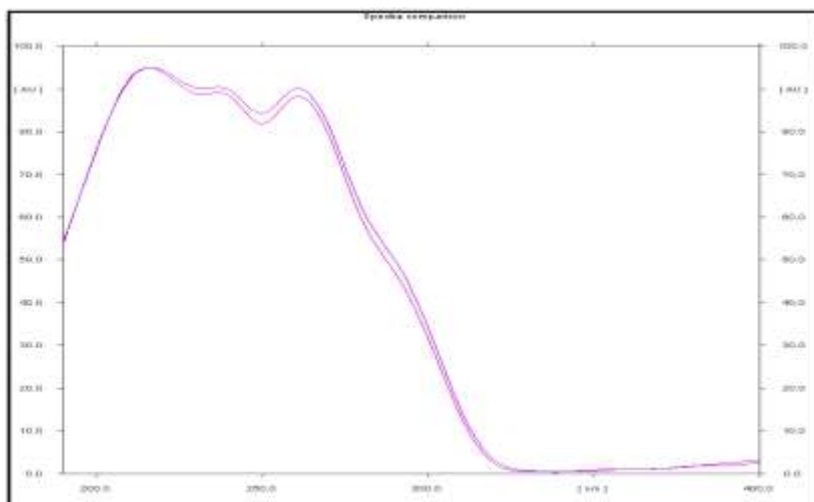


Figure 6: Spectrum comparison of standard and sample spot of VAL at 227 nm

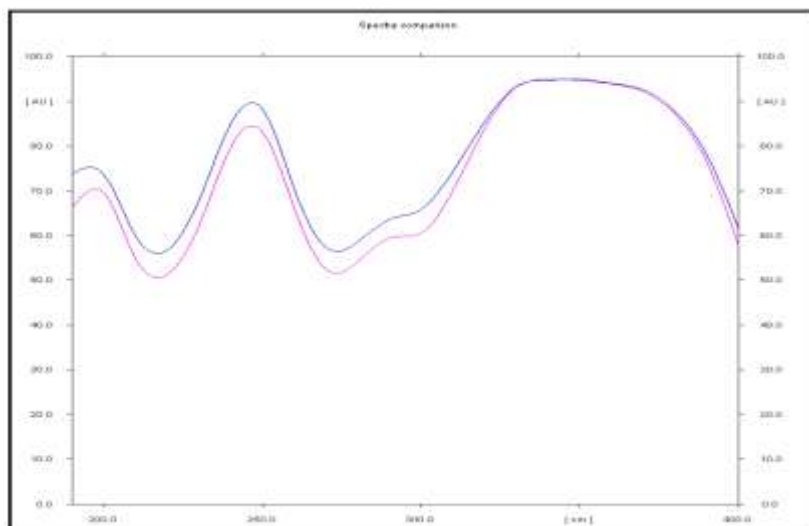


Figure 7: Spectrum comparison of standard and sample spot of NIF at 227 nm

CONCLUSION

The High performance thin layer chromatography method was developed for simultaneous determination of VAL and NIF in synthetic mixture. Method was found to be precise and accurate as can be reflected from validation parameter data. Developed method was efficiently applied for determination of VAL and NIF in synthetic mixture and there for method can be extended for the analysis of formulation.

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