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Development and Validation of Dual Wavelength Spectrophotometric Method for Simultaneous Estimation of Valsartan and Nifedipine in Synthetic Mixture

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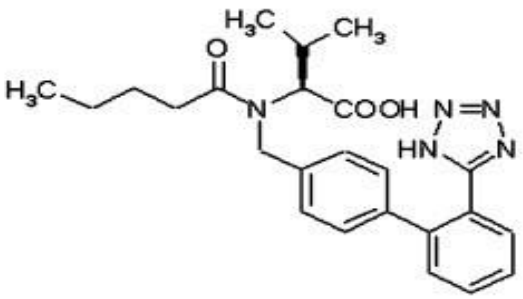
ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economic dual wavelength method for simultaneous estimation of Valsartan and Nifedipine in synthetic mixture. The principle of dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest".Nifedipine was determined directly at 327.5nm in methanol. The wavelengths selected for determination of Valsartan were 327.5 nm and 262.6 nm in methanol. The two drugs follow Beer-Lambert's law over the concentration range of 2-20 µg/ml. The method was successfully applied to synthetic mixture. No interference from the synthetic mixture excipients was found. The suitability of this method for the quantitative determination of Valsartan and Nifedipine was proved by validation. The results of analysis have been validated statistically and by recovery studies according to ICH guidelines.

INTRODUCTION

Valsartan (VAL) is chemically N-Pentanoyl-N-{[2'-(1H-tetrazol-5-yl) biphenyl-4-yl] methyl}-L-valine(Figure 1) is an angiotensin II receptor antagonist. It is mainly used for treatment of high blood pressure, congestive heart failure[1]. It is official in Indian Pharmacopeia[2] and United State Pharmacopeia[3]. Literature survey reveals UV Spectrophotometry[4], HPLC[5] and HPTLC[6] methods for determination of VAL in pharmaceutical dosage forms. Literature survey also reveals Spectrophotometric[7], HPLC[8] and HPTLC[9] methods for determination of VAL with other drugs in combination. Nifedipine (NIF) is chemically3, 5-dimethyl 2, 6-dimethyl-4-(2-nitrophenyl) - 1, 4dihydropyridine-3, 5-dicarboxylate (Figure 2), is a dihydropyridine calcium channel blocker that primarily blocks L-type calcium channels. Its main uses are as an antianginal and antihypertensive[10]. It is official in Indian Pharmacopeia[11], United State Pharmacopeia[12], British Pharmacopeia[13], European Pharmacopeia[14] and Japanese Pharmacopeia[15]. Literature survey reveals UV Spectrophotometry[16], HPLC[17] and HPTLC[18] methods for determination of NIF in pharmaceutical dosage forms. Literature survey also reveals Spectrophotometric[19], HPLC[20] and HPTLC[21] methods for determination of NIF with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation of VAL and NIF in their combined mixture. The combination of VAL and NIF are studied under clinical trial phase identifier no: NCT00993109 by Bayer Pharmaceuticals[22] (Chine & Korea FDA). Combination of VAL and NIF are useful in reducing the blood pressure. Literature survey reveals only one reported spectrophotometric method for simultaneous estimation of VAL and NIF in synthetic mixture[23]. The present communication describes simple, sensitive, rapid, accurate and economical spectrophotometric method for simultaneous estimation of VAL and NIF in synthetic mixture.



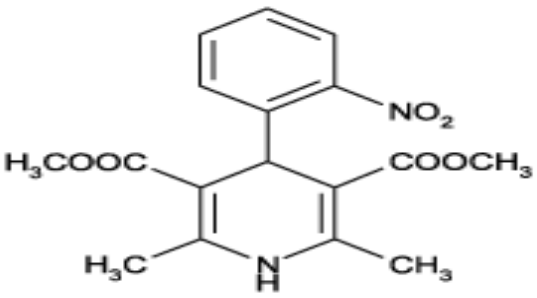


Figure 1: Chemical structure of Valsartan **Figure 2: Chemical structure of Nifedipine**

MATERIALS AND METHODS

Reagents and Materials

VAL and NIF bulk powder were kindly gifted by Torrent Pharmaceuticals Ltd. Ahmedabad, Gujarat, India. Methanol AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India. Whatman filter paper no 41 (Millipore, USA) was also used in the study.

Apparatus

A Shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2nm, wavelength accuracy of 0.5nm and a pair of 10mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe 2.0 system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Preparation of standard stock solutions

Standard stock solution of VAL and NIF (100 µg/ml) was prepared separately by dissolving an accurately weighed quantity of VAL (10 mg) and NIF (10 mg) to a separate 100 ml volumetric flask and diluted up to mark with methanol to obtained standard solution having concentration of 100µg/ml for both drugs. For NIF preparation amber colored volumetric flask were used.

Preparation of synthetic mixture

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

Development of method

The utility of dual wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest, independent of the interfering components. From the overlain spectra of

two drugs (Figure 3), it is evident that direct determination of NIF at 327.5nm (no absorbance of VAL at 327.5nm). For estimation of VAL, two wavelengths selected (327.5nm and 262.6nm) where NIF shows same absorbance. The determination of VAL is carried out by subtracting absorbance due to NIF at 262.6nm and the difference between 262.6nm and 327.5nm is directly proportional to concentration of VAL in the mixture.

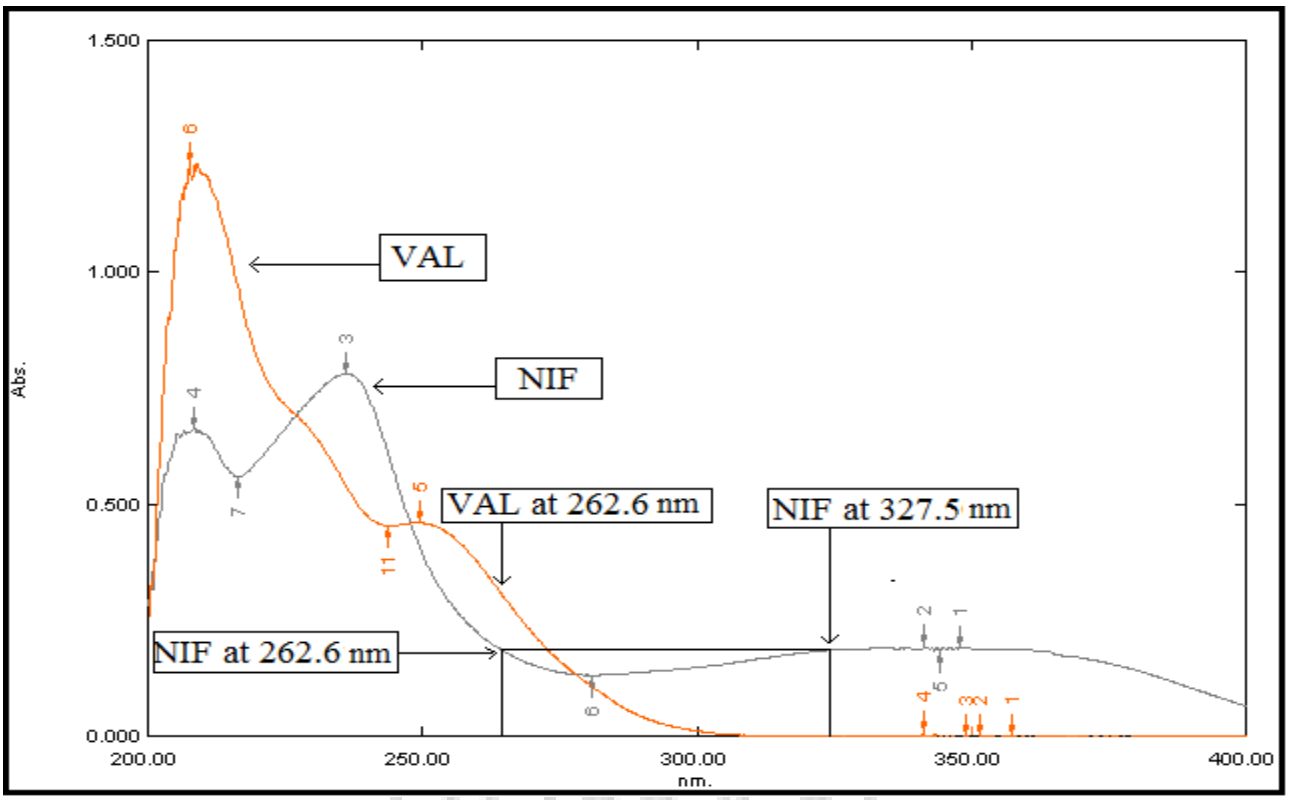


Figure 3: Overlain zero order absorption spectra of VAL and NIF in methanol

METHOD VALIDATION

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.[24]

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-20 µg/ml for each VAL and NIF. Accurately measured standard stock solutions of each VAL and NIF (0.2, 0.4, 0.8, 1.2, 1.6, and 2.0 ml) were transferred to a series of 10 ml volumetric flask separately and diluted up to the

mark with methanol. The absorbances of solution were then measured at 262.6nm and327.5nm. The calibration curves were constructed by plotting absorbances versus concentration and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbances of solutions (n = 6) of VAL and NIF (12 µg/ml for both drugs) without changing the parameters of the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of VAL and NIF (4, 8 and 12 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Accuracy (% recovery study)

The accuracy of the method was determined by calculating recoveries of NIF and VAL by the standard addition method. Known amounts of standard solutions of NIF and VAL were added at 80%, 100% and 120% level to prequantified sample solutions of VAL (8 µg/ml) and NIF (3 µg/ml). The amounts of VAL and NIF were estimated by putting obtained values in the respective regression line equation. The experiment was repeated three times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug was derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of VAL and NIF in synthetic mixture

VAL (80 mg) and NIF (30 mg) standard drug powder were weighed and then mixed with commonly used formulation additives like starch, lactose, magnesium stearate and talc. The synthetic mixture was then transferred to 100 ml volumetric flask containing 50 ml methanol and sonicated for 15 minutes. The solution was filtered through Whatman filter paper No. 41 and the volume were adjusted up to mark with methanol. The solution was suitably diluted with methanol to get a final concentration of 8 µg/ml of VAL and 3 µg/ml of NIF. The absorbances of the sample solution were measured against methanol as blank at 262.6nm and 327.5nm for quantification of VAL and NIF. The amount of VAL and NIF present in the sample solutions were determined by putting obtained values in respective regression equation for VAL and NIF.

RESULTS AND DISCUSSION

In this method, two specific wavelengths are selected, first wavelength λ1 at which minimum absorbance of NIF was observed and there was no interference of VAL at this wavelength (327.5nm). Second wavelength λ2 was the wavelength at which the absorbance of NIF was same as at λ1, and VAL was also having some absorbance at this wavelength (262.6nm). The absorbance at these two wavelengths was found to be equal for NIF. These two selected wavelengths were employed to determine the concentration of VAL from the mixture of NIF and VAL. The difference in absorbance at these two wavelengths ($A_{262.6}$– $A_{327.5}$) cancels out the contribution of absorbance of NIF in mixture.

The proposed method was found to be simple, sensitive, rapid, accurate, precise and economical for the routine simultaneous estimation of two drugs. The linearity ranges for both drugs were found to be 2-20µg/ml. Characteristic parameters of regression equation and correlation are given in (Table 1). Precision was calculated as repeatability (%RSD) and intra and inter-day variation (% RSD) for both the drugs. Accuracy was determined by calculating the recovery, and the mean was determined (Table 2). The LOD and LOQ were found to be 0.19 and 0.58µg/ml, respectively for NIF and 0.24 and 0.74µg/ml, respectively for VAL, indicates sensitivity of the proposed method. The method was successfully used to determine the amounts of VAL and NIF present in synthetic mixture. The results obtained are in good agreement with the corresponding labeled amount (Table 3). By observing the validation parameters, the method was found to be

sensitive, accurate and precise. Hence, the method can be employed for the routine analysis of these drugs in combinations.

Table 1: Regression analysis data and summary of validation parameters for the proposed method

PARAMETERS	VAL	NIF
Wavelength range (nm)	262.5 and 327.5	327.5
Beer's law limit (µg/ml)	2 - 20	2 -20
Regression equation (y = a+bc) Slope (b) Intercept (a)	0.0254x + 0.0267 0.0254 0.0267	0.0146x + 0.0122 0.0146 0.0122
Correlation Coefficient (r^2)	0.9994	0.9976
Method precision (Repeatability) (% RSD), (n = 6)	0.55	0.84
Intraday (n = 3) (%RSD)	0.59–0.80	0.46 – 0.86
Interday (n = 3) (%RSD)	0.74 – 1.58	0.51–1.47
LOD (µg/ml)	0.24	0.19
LOQ (µg/ml)	0.74	0.58
Accuracy (Mean % Recovery± S.D.) (n =3)	99.91 ± 0.49	99.99 ± 1.17
% Assay ± S.D. (n = 5)	100.2 ± 0.55	100.4 ± 1.68

RSD = Relative standard deviation. LOD = Limit of detection. LOQ = Limit of quantification. SD = Standard deviation.

Table 2: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
VAL	I	8	80	99.03 ± 0.61
	II	8	100	100.3 ± 0.48
	III	8	120	100.4 ± 0.40
NIF	I	3	80	99.85 ± 0.77
	II	3	100	99.84 ± 1.47
	III	3	120	100.3 ± 1.29

S.D. is Standard deviation and n is number of replicates.

Table 3: Analysis of VAL and NIF by proposed method

Synthetic mixture	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 5)	
	VAL	NIF	VAL	NIF	VAL	NIF
I	80	30	80.20	30.12	100.2± 0.55	100.4± 1.68

S.D. is Standard deviation and n is number of replicates.

CONCLUSION

The proposed dual wavelength spectrophotometric method was found to be simple, sensitive, accurate, precise and economical and can be employed for the routine analysis of these two drugs in combined synthetic mixture.

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