

Research Article



Zero-Crossing Point Derivative Simultaneous Spectrofluorimetric Method for Quantification of Nebivolol Hydrochloride and Valsartan Combination in Tablets

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ABSTRACT

A first derivative spectrofluorimetric method has been developed and validated for simultaneous quantification of Nebivolol hydrochloride (NEB) and Valsartan (VAL) in combined tablet dosage form without any prior separation of components from the sample. NEB was determined at emission wavelength of 294 nm (zero-crossing wavelength point of VAL). Similarly, VAL was measured at 404 nm (zero-crossing wavelength point of NEB). The first derivative amplitude- concentration plots were rectilinear over the range of 0.1-2.1 µg/ml; for both drugs. Analytical performance of the proposed spectrofluorimetric procedure was statistically validated with respect to linearity, range, precision, accuracy, selectivity, detection and quantification limits as per ICH guidelines. No interference was observed from common pharmaceutical additives. The % assay in commercial formulation was found to be 101.6 and 98.5 for NEB and VAL, respectively by the proposed method and % RSD values for precision and accuracy studies were found to be less than 2. The proposed method can be successfully applied for routine analysis of NEB and VAL in their pharmaceutical tablet dosage form.

Keywords: First-derivative, Nebivolol hydrochloride, Spectrofluorimetry, Valsartan.

INTRODUCTION

Nebivolol hydrochloride (NEB) (Figure 1) chemically known as, α , α' -[1minobis (methylene)] bis[6-fluoro-3, 4-dihydro-2H-1-benzopyran-2-methanol] is a selective β_1 receptor antagonist that is used in the treatment of hypertension and myocardial infarction. Valsartan (VAL) (Figure 2) chemically known as N-(1-Oxopenty)-N-[[2'-(1H-tetrazol-5-yl) [1, 1' biphenyl]-4-yl] methyl] - L-valine, is an angiotensin II receptor antagonists used in the treatment of hypertension. NEB and VAL have been formulated in a fixed-dose combination and used in the treatment of hypertension.¹ Few analytical methods include spectrophotometry and high performance liquid chromatography's (HPLC) were developed for simultaneous quantification of NEB and VAL in fixed- dose combinations.²⁻⁴

To the best of our knowledge, no spectrofluorimetric method has been reported yet for quantification of NEB and VAL in combined tablet form.

Spectrofluorimetric methods have found more selective than normal UV-spectroscopy due to quantification of substance at specific excitation and emission wavelengths.⁵⁻⁷ Derivative Spectrofluorimetry/spectrophotometry provides a greater selectivity and spectral discrimination than normal techniques. It is the powerful approach for resolution of one analyte whose peak is hidden by a large overlapping peak of another analyte in multi-component analysis.⁷⁻¹¹ The fusion of scanning Spectrofluorimetry with derivative techniques is advantageous in terms of sensitivity, spectral discrimination and more reliable identification of chemical species in multi component analysis.¹²⁻¹⁵ The aim of the present work was to develop a simple, economic,

sensitive and rapid method for the simultaneous determination of NEB and VAL in tablet dosage form by first derivative fluorimetry (FDF) based on their native fluorescence. The emission spectra of NEB and VAL were overlapped. It was difficult to analyze and determine their contents by conventional fluorimetry. These problems were minimized by using FDF.

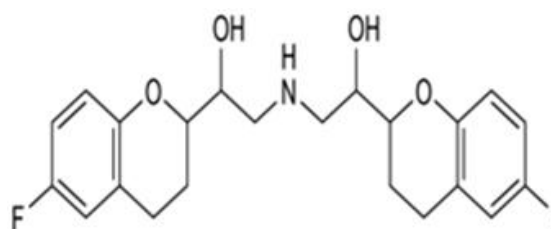


Figure 1: Chemical structure of NEB

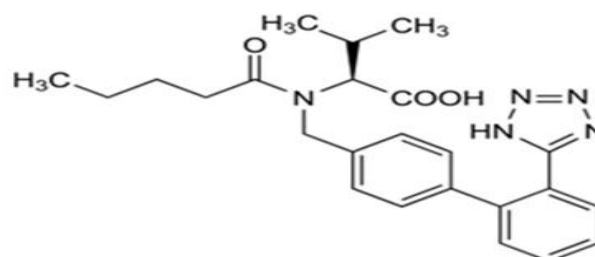


Figure 2: Chemical structure of VAL

MATERIALS AND METHODS

Chemicals

All chemicals and reagents were of analytical grade. NEB and VAL were gift samples from Dr Reddy's Laboratories Limited (Hyderabad, India). Nebicard-V formulation (NEB- 5 mg and VAL- 80 mg) were purchased from the local



pharmacies. Glacial acetic acid (GAA) was purchased from SD Fine Chemicals Ltd, Mumbai, India. Triple distilled water was used wherever required.

Instrumentation

The fluorescence spectra and measurements were recorded using a Shimadzu (Japan) RF-5301 PC spectrofluorimeter connected to RFPC software, equipped with 150 watt Xenon arc lamp, 1cm non-fluorescent quartz cell and analytical balance (Shimadzu AUX 220, Japan) were used for the study. The instrument was operated both at low and high sensitivity with excitation and emission slit width set at 5 nm.

Preparation of standard solutions

Each of standard NEB (10 mg) and VAL (10 mg) were weighed and transferred into two separate 10 ml volumetric flasks and dissolved in 5 ml glacial acetic acid. The flasks were shaken and volume was made up to the mark with acetic acid. These stock solutions of NEB and VAL were further diluted with water to get the final concentration having 10 µg/ml of each drug and fluorescence intensity quantified by spectrofluorimeter.

Procedure for commercial tablets

Twenty tablets of marketed formulation (Nebicard-V), containing 5 mg of NEB and 80 mg of VAL were taken and accurately weighed. Average weight was determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 2.5 mg NEB and 40 mg VAL was transferred to volumetric flask of 10 ml capacity. Glacial acetic acid was added to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with acetic acid. The above solution was filtered through whatman filter paper (No.41) filtrate contain 250 µg/ml and 4000 µg/ml of NEB and VAL respectively. This solution was further diluted with water to get the final concentration with in linearity range to quantify.

The amount of NEB and VAL present in the sample solution were determined by substituting derivative fluorescence responses into the equation of the straight line representing the calibration curves for NEB and VAL.

Method Validation

The method was validated for linearity, sensitivity, selectivity, precision and accuracy as per International Conference on Harmonization (ICH) guidelines.¹⁶

Linearity

The prepared standard dilutions of NEB (0.1-2.1 µg/ml) and VAL (0.1-2.1 µg/ml) were quantified by first derivative spectrofluorimetric technique and fluorescence intensities were recorded. The calibration curve was constructed by plotting the analyte intensity against the concentration.

Sensitivity

The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on standard calibration curve.

Specificity

The method specificity was assessed by comparing the spectra obtained from the commercial formulation and the synthetic mixture from standard solution in order to check if any component of the formulation could generate a response.

Precision

Precision of the method was determined by intra-day precision and inter-day precision variations as per ICH guidelines. For both intra-day and inter-day precision, the samples containing NEB (0.1, 1.3, 2.1 µg/ml) and VAL (0.1, 1.3, 2.1 µg/ml) were analyzed six times on the same day (intra-day precision) and for three consecutive days (inter day precision). The % RSD was calculated.

Accuracy

The accuracy was carried out by recovery studies using standard addition method; known amount of standard drug was added to pre analyzed sample of NEB and VAL in according to 80%, 100% and 120% levels of labeled claim and then subjected to the proposed method. The experiment was conducted in triplicate. The percentage recovery and percentage relative standard deviation (% RSD) were calculated for each concentration.

RESULTS AND DISCUSSION

Method optimization

NEB drug molecule contains polycyclic aromatic systems like two aromatic rings in which more number of π electrons are available and electron donating group $-NH_2$, to exhibit fluorescence. Similarly, VAL show fluorescence due to presence of imidazoline ring and conjugated aromatic ring. Different solvent systems were tested in order to find out the best conditions like solubility, fluorescence activity, stability and spectral discrimination (clear separation) of both the drugs. NEB exhibit native fluorescence at emission wave length 294 nm after excitation at 256 nm and similarly VAL exhibit fluorescence at emission wavelength 404 nm after excitation at 256 nm in water as solvent (Figure 3), revealed that, the fluorescence spectra of these drugs overlap considerably and as a result, the conventional spectrofluorimetric method does not permit the simultaneous determination of both the drugs. This overlap was highly discriminated by using first-order derivative spectrofluorimetric method, this method used to choose the suitable wavelengths that make the estimations proportional to NEB and VAL concentrations was the "zero crossing". The first-order derivative spectrum of NEB has zero intensity at 404 nm, where VAL gives the significant derivative response, while the



derivative spectrum of VAL has zero intensity at 294 nm, where NEB gives significant derivative response (Figure 4). Therefore, 294.0 nm selected for estimation of NEB and 404 nm selected for the estimation of VAL in synthetic mixture and tablet dosage form.

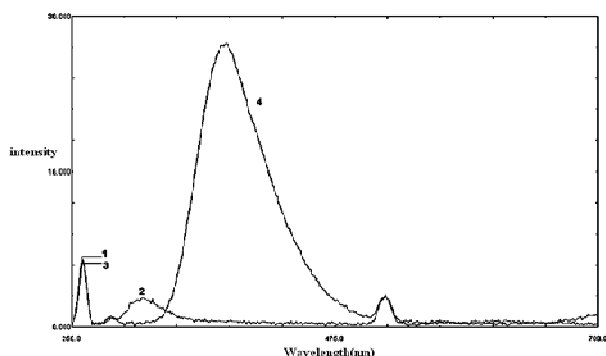


Figure 3: Zero-order fluorescence overlaid excitation (1, 3) and emission (2, 4) spectra's of NEB (0.9 µg/ml) and VAL (0.9 µg/ml) in water.

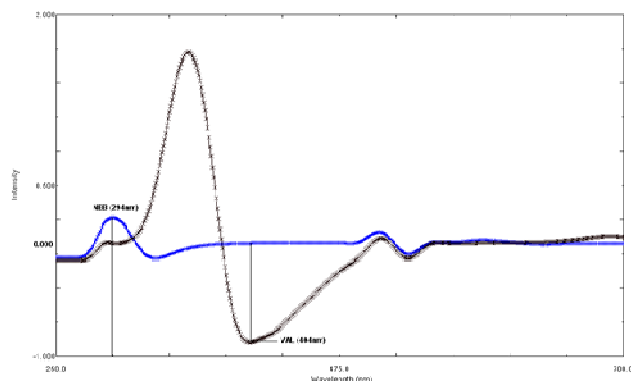


Figure 4: First order fluorescence overlaid spectrum of NEB (2.1 µg/ml) and VAL (2.1 µg/ml) in water

Method validation

The linearity was evaluated by the least square regression method. The responses for NEB at 294 nm were found to be linear in the concentration range of 0.1-2.1 µg/ml with a correlation co-efficient (r^2) value of 0.999. Similarly the responses for VAL at 404 nm were linear in the concentration range of 0.1-2.1 µg/ml with a correlation

coefficient (r^2) value of 0.999. The range of linearity spectra and calibration plots are shown in Figure.5. Optimum conditions of proposed method mentioned in Table 1. The sensitivity of the method indicated by LOD and LOQ values of the proposed method, reported in Table 1, which indicates the high sensitivity of the method. The % recoveries of NEB and VAL were found to be in the range 98 – 108.7 and 98.43 – 99.58, respectively which are satisfactory, and the % RSD at each concentration level was less than 2, thus indicates the accuracy of the method (Table 2). There was no significant difference between the % RSD values of intra-day and inter-day precision, revealed that the method is reproducible (Table 3).

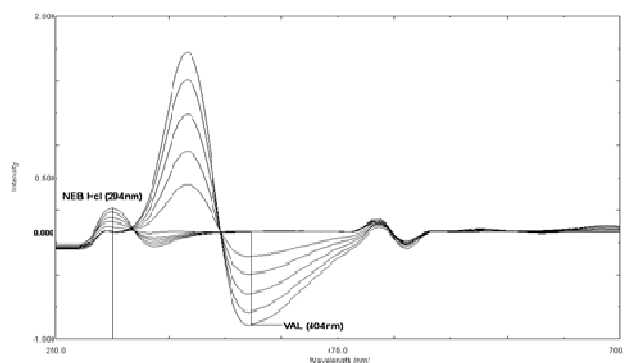


Figure 5: Fluorescence first-derivative linearity range of NEB (0.1-2.1 µg/ml) and VAL (0.1-2.1 µg/ml)

Table 1: Optimum conditions of proposed method

Parameter	NEB	VAL
Emission maxima (nm)	294	404
Range (µg/ml)	0.1 – 2.1	0.1 – 2.1
Slope	0.102	-0.402
Intercept	-0.003	-0.008
Correlation coefficient	0.999	0.999
LOD (µg/ml)	0.016	0.015
LOQ (µg/ml)	0.049	0.047
Regression equation	$y = 0.102x - 0.003$	$y = -0.402x - 0.008$

* $Y = a + bX$. Y is the absorbance, X is the concentration in µg/ml, a is the intercept and b is the slope.

Table 2: Accuracy studies of proposed method

Formulation	Recovery level (%)	Recovery of analyte	Theoretical content (mg)	Amount found (mg) A.M* ± SD	Recovery (%)	% RSD
NEBICARD-v	80	NEB	4	4.35 ± 0.0006	108.7	0.013
		VAL	72	71.70 ± 0.013	99.58	0.018
	100	NEB	5	4.92 ± 0.0007	98.40	0.014
		VAL	80	78.75 ± 0.016	98.43	0.020
	120	NEB	6	5.95 ± 0.0007	99.16	0.012
		VAL	88	87.16 ± 0.015	99.04	0.017

* Mean of three determinations

Table 3: Precision studies of proposed method

NEB				
Concentration (µg/ml)	Intra-day precision		Inter-day precision	
	Concentration estimated(µg/ml) A.M* ± SD	% RSD	Concentration estimated(µg/ml) A.M* ± SD	% RSD
0.1	0.0882± 0.0008	0.907	0.0889±0.0009	1.012
1.3	1.333± 0.0015	0.112	1.320±0.0014	0.106
2.1	2.107± 0.0058	0.275	2.109± 0.0060	0.284
VAL				
0.1	0.087±0.001	1.149	0.085± 0.0012	1.411
1.3	1.338±0.0254	1.898	1.341± 0.0252	1.879
2.1	2.067±0.0369	1.785	2.081± 0.0366	1.758

*Mean of three determinations

Table 4: Results of analysis of marketed tablets by proposed method

Formulation	NEB				VAL			
	Label claim (mg)	Amount found(mg) A.M* ± SD	% Assay	% RSD	Label claim (mg)	Amount found (mg) A.M* ± SD	% Assay	% RSD
NEBICARD-V	5	5.08 ± 0.0011	101.6	0.021	80	78.8 ± 0.0351	98.5	0.048

* Mean of three determinations

Assay of marketed tablet formulations

The application of proposed method was applied to the assay of commercially available tablets (Nebicard-V) containing NEB (5 mg) and VAL (80 mg). The results obtained for NEB and VAL were compared with the corresponding labeled amounts and reported in Table 4. The % assay in commercial formulations was found to be 101.6% for NEB and 98.5% for VAL by the proposed method. The % RSD for the formulations (Nebicard-V) was less than 2.

CONCLUSION

In the present study, a new simple, sensitive and time saving first derivative spectrofluorimetric method was explored for the simultaneous quantification of NEB and VAL in binary mixture and pharmaceutical dosage forms. This spectrofluorimetric method has been found to be superior, because of its high specific, spectral discrimination, readily available solvent, economical, lack of extraction procedure, reliable, precise and accurate. The assay values were in good concurrence with their respective labeled claim, which suggested no interference of formulation excipients in the estimation and obtained results from validation proved the proposed method was scientifically sound. These advantages promote that; the developed method can be routinely employed in quality control for analysis of NEB and VAL combined dosage forms.

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