



RESEARCH ARTICLE

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Spectrophotometric Method Development and Validation for Simultaneous Estimation of Nebivolol hydrochloride and Valsartan in Bulk and Combined Pharmaceutical Dosage Form in Release Media

Chandani Makvana*, Satyajit Sahoo

Faculty of Pharmacy, C. U. Shah University, Surendranagar-Ahmedabad highway, Near Kothariya village, Dist. Surendranagar, Wadhwan-363030, Gujarat, India

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ABSTRACT

A simple, rapid, precise, accurate and sensitive spectrophotometric method has been developed for the simultaneous estimation and validation of Nebivolol Hydrochloride (NEB) and Valsartan (VAL) in pure and combined tablet dosage forms. Pure drug samples of NEB and VAL were dissolved in 67 mM Phosphate buffer pH 6.8 with 0.5% sodium dodecyl sulphate (SDS) and found to have absorbance maxima at 280 nm for NEB and 250 nm for VAL, respectively. The linearity lies between 10-70 µg/ml for NEB and 10-60 µg/ml for VAL in this method. The correlation coefficient (r^2) was found to be 0.9965 for NEB and 0.9960 for VAL. The % recoveries obtained were 95.65%-109.85% for NEB and 97.42%-101.43% for VAL. The % RSD found 0.271%-1.490% for intraday and 0.334%-1.917% for interday for NEB and 0.188%-0.944% for intraday and 0.392%-1.197% for interday for VAL. The limit of detection and limit of quantitation for NEB were found to be 4.608 µg/ml and 13.965 µg/ml respectively and the limit of detection and limit of quantitation for VAL were found to be 4.348 µg/ml and 13.178 µg/ml respectively. Simultaneous calibration of both drugs in 67 mM Phosphate buffer pH 6.8 with 0.5% SDS shows that λ_{max} of one drug does not interfere on the λ_{max} of other drug. Recovery study was performed to confirm the accuracy of the method. The results of analysis have been validated statistically by recovery studies as per International Conference on Harmonization guidelines. The method showed good reproducibility and recovery with % RSD <2. Hence, this proposed method was found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of NEB and VAL in pure and combined tablet dosage form.

Keywords: Nebivolol hydrochloride, Valsartan, UV spectroscopy, Simultaneous equation method, Method development and validation.

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*Corresponding author: Ms. Chandani Makvana

Address: Faculty of Pharmacy, C. U. Shah University, Surendranagar-Ahmedabad highway, Near Kothariya village, Dist. Surendranagar, Wadhwan-363030, Gujarat, India

E-mail ✉: chandani.makvana1991@gmail.com

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INTRODUCTION

NEB is a highly cardioselective vasodilatory β_1 receptor blocker used in treatment of hypertension.

NEB is a selective β_1 -receptor antagonist. Activation of α_1 -receptors by epinephrine increases the heart rate, blood pressure and the heart consumes more oxygen.

NEB blocks these receptors which reverses the effects of epinephrine, lowering the heart rate and blood pressure. In addition, beta blockers prevent the release of renin, which is a hormone produced by the kidneys which leads to constriction of blood vessels. At high enough concentrations, this drug may also bind β_2 receptors. [1-3]

VAL is an Angiotensin receptor blocker that selectively inhibits the binding of angiotensin II to angiotensin I, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the angiotensin I-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. [4]

The majority of patients with hypertension require two or more medications to achieve their blood pressure goals. So, the fixed dose combination of NEB (5 mg) and VAL (80 mg) which was approved in June 06, 2016 by US FDA was selected that reduce blood pressure through multiple mechanism of action. It is first and only fixed dose combination of a beta blocker and angiotensin II receptor blocker available in US. [5] The addition of NEB counteracts the effect of increased angiotensin II concentrations resulting from potent AT1 blockage and give better anti-hypertensive effect than monotherapy effect. [6]

Different analytical methods have been reported in the literature for the assay of NEB and VAL in bulk and pharmaceuticals which include spectrophotometry, TLC, HPLC, HPTLC and LC-MS using different solvents. [7-28] The present investigation reports a simple UV Spectrophotometric method for the analysis of NEB and VAL in bulk as well as in tablet dosage form using 67 mM phosphate buffer pH 6.8 with 0.5% SDS (finished dosage form release media) as a solvent for developing and proving the method. [29] The developed method was validated as per ICH guidelines Q2(R1) Validation of Analytical procedures: Text and methodology. [30-31]

MATERIALS AND METHODS

Instrument

A Shimadzu UV-visible spectrophotometer (Lab India, UV3092, 20-1950-21-0004) was employed with a spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm with automatic wavelength correction with a pair of 10 mm quartz cells.

Chemicals and reagents

An analytically pure sample of NEB and VAL were received as gift sample from Torrent research centre, Ahmedabad, India. All the chemicals used were of analytical grade.

Preparation of 67 mM Phosphate buffer pH 6.8 with 0.5 % SDS: Accurately weighted 10.45 g Sodium dihydrogen orthophosphate (NaH_2PO_4), 0.9 g NaOH and 5 g SDS and dissolved in 1000 ml Purified water. This solution was then used for further method development and validation study.

The commercial sample of Nebicard-V containing NEB 5 mg and VAL 80 mg was purchased from local market.

Preparation of standard curve of NEB and VAL

Preparation of standard stock solution (1000 $\mu\text{g/ml}$)

The standard stock solutions of 1000 $\mu\text{g/ml}$ of NEB and VAL were prepared separately. 100 mg of both the drugs was separately taken in 100 ml volumetric flask and dissolved in 67 mM Phosphate buffer pH 6.8 with 0.5% SDS and then volume made up to the mark with 67 mM Phosphate buffer pH 6.8 with 0.5% SDS to get a concentration of 1000 $\mu\text{g/ml}$.

Preparation of working standard solution (100 $\mu\text{g/ml}$)

The working standard solution of 100 $\mu\text{g/ml}$ of NEB and VAL were prepared separately. 10 ml standard stock solution of both drugs was separately transferred to a 100 ml volumetric flask and volume was adjusted to 100 ml with 67 mM Phosphate buffer pH 6.8 with 0.5 % SDS to get a concentration of 100 $\mu\text{g/ml}$.

Preparation of dilutions for calibration curve

By appropriate dilution of working standard solution of NEB and VAL with 67 mM Phosphate buffer pH 6.8 with 0.5% SDS, solutions containing 10 $\mu\text{g/ml}$ of NEB and 10 $\mu\text{g/ml}$ of VAL were scanned separately in the range of 200 nm-400 nm. Wavelength of maximum absorption was determined for both the drugs. NEB showed maximum absorbance at 280 nm and VAL at 250 nm. These dilutions gave 10, 20, 30, 40, 50, 60 and 70 $\mu\text{g/ml}$ concentration of NEB and 10, 20, 30, 40, 50 and 60 $\mu\text{g/ml}$ concentration of VAL. The absorbance of prepared solutions of NEB and VAL in 67 mM Phosphate buffer pH 6.8 with 0.5% SDS was measured at wavelength maximum 280 nm and 250 nm using Shimadzu UV-visible spectrophotometer against 67 mM Phosphate buffer pH 6.8 with 0.5% SDS as blank. The experiment was performed in triplicate and based on average absorbance; the equation for the best line was generated. The results of standard curve preparation are shown in Table 1 and Fig. 1 for NEB and Table 2 and Fig. 2 for VAL.

Validation of Analytical method of NEB and VAL

Analytical measurement of NEB and VAL in 67 mM Phosphate buffer pH 6.8 with 0.5% SDS by UV spectrophotometry was validated separately as per ICH guideline, Q2(R1). The UV spectrophotometric method was validated for the quantification of NEB and VAL in samples. Intraday and Interday precision and accuracy were determined by analysis of five concentrations. The overall precision of the method was expressed as relative standard deviation (RSD) and the accuracy of the method was expressed in terms of relative error.

Linearity and range: Linearity is expressed in terms of correlation coefficient of linear regression analysis. The linearity response was determined by analyzing independent levels of calibration curve in the range of 10-70 $\mu\text{g/ml}$ for NEB and 10-60 $\mu\text{g/ml}$ for VAL. Plot the calibration curve of absorbance vs concentration and determines correlation coefficient and regression line equations for NEB and VAL separately.

Accuracy preparation of sample solution: The accuracy study was determined by standard addition method. 100 mg of NEB and VAL was weighed and transferred into a 100 ml of volumetric flask, dissolved and diluted up to mark with 67 mM Phosphate buffer pH 6.8 with 0.5% SDS separately. Pipette out 10 ml of the above solution in 100 ml volumetric flask and diluted to mark with 67 mM Phosphate buffer pH 6.8 with 0.5% SDS to get 100µg/ml solution of NEB and VAL, from that 20µg/ml of solution for NEB and 10µg/ml of solution for VAL were prepared. To one ml of the above solution, increasing aliquots of standard solution (10, 20 and 30µg/ml of NEB, 5, 10 and 15µg/ml of VAL) were added and diluted to 10 ml with 67 mM Phosphate buffer pH 6.8 with 0.5% SDS. Absorbance of solution was measured at selected wavelength. The amount of NEB and VAL was calculated at each level and % recoveries were computed.

Precision

Repeatability: The absorbance of same concentration was measured three times and RSD was calculated.

Intraday Precision: Solutions containing 10-70µg/ml of NEB and 10-60µg/ml of VAL were analyzed three times on the same day and % RSD was calculated.

Interday Precision: Solutions containing 10-70µg/ml of NEB and 10-60µg/ml of VAL were analyzed three times on the different 3 days and % RSD was calculated. It is a measure of either the degree of reproducibility or repeatability of the analytical method.

Limit of detection (LOD) and Limit of quantitation (LOQ): The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under standard experimental condition. The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under standard experimental condition. LOD and LOQ were calculated using the following formula:

$$\text{LOD} = 3.3 \sigma / S$$

$$\text{LOQ} = 10 \sigma / S$$

Where σ is Standard deviation of the response and S is slope of the calibration curve.

Simultaneous estimation of NEB and VAL

Preparation of standard stock solution

Stock solutions (1000µg/ml) of NEB and VAL were prepared by dissolving separately 100 mg of drug in 67 mM Phosphate buffer pH 6.8 with 0.5% SDS. Then make up the volume with 67 mM Phosphate buffer pH 6.8 with 0.5% SDS. The stock solution were suitably diluted to produce solution of concentration 100µg/ml, these working standard solutions were scanned in the entire UV range (200 nm-400 nm) to determine the λ max. Absorption maxima of NEB and VAL were detected at 280 nm (λ_2) and 250 nm (λ_1), respectively and overlain spectra was recorded. A series of standard dilutions of each drug were prepared having concentration range of 10- 70µg/ml. NEB and VAL

showed linearity with absorbance in the range 10-70µg/ml and 10-60µg/ml respectively. The absorbances were measured at 250 nm and 280 nm and calibration curves were plotted at these wavelengths.

Simultaneous equations method

Method is based on simultaneous equations method of Vierordt. Absorption maxima of NEB and VAL were 280 nm (λ_2) and 250 nm (λ_1), respectively. Calibration curve for NEB and VAL was prepared in the concentration range 10-70µg/ml and 10-60µg /ml respectively. The absorptivity coefficients of the two drugs were determined by using Beer's law: $A = E (1\%, 1 \text{ cm}) CL$ and their average value taken. The overlain spectra of NEB and VAL are represented in [Fig. 3]. A set of two simultaneous equations was developed using these absorptivity coefficients. These are: $A_1 = 0.03372 C_x + 0.0011 C_y \dots (1)$; and $A_2 = 0.0074 C_x + 0.0156 C_y \dots (2)$, where A_1 and A_2 are absorbances at 250 nm and 280 nm respectively, and C_x and C_y are concentrations of VAL and NEB respectively.

Analysis of tablet formulation

For the estimation of drugs in the commercial formulations, 20 tablets containing 5 mg of NEB and 80 mg of VAL were weighed and average weight was calculated. The tablets were crushed and powdered in a glass mortar. For the analysis of drugs, quantity of powder equivalent to 5 mg of NEB and 80 mg of VAL was transferred to 100 ml volumetric flasks and dissolved in sufficient quantity of 67 mM Phosphate buffer pH 6.8 with 0.5 % SDS. It was sonicated for 30 minutes and volume was made up to the mark to obtain a stock solution of 1000µg/ml of NEB and 1000µg/ml of VAL. This solution was then filtered through whatman filter paper (grade one). Further dilutions were made from this stock solution to get required concentration.

RESULTS AND DISCUSSION

The optimized UV method for Simultaneous estimation was validated according to the procedures described in ICH guidelines Q2 (R1) for the validation of analytical method.

Preparation of standard curve of NEB and VAL

The drug was scanned in wavelength range of 200 nm-800 nm by UV double beam spectrophotometer to determine the absorption maxima. The absorption maxima found to be at wavelength of 280 nm and 250 nm for NEB and VAL in 67 mM Phosphate buffer pH 6.8 with 0.5% SDS respectively.

NEB and VAL in the concentration of 10µg/ml in 67 mM Phosphate buffer pH 6.8 with 0.5% SDS scanned in the UV region of 200 nm-800 nm showed absorption maxima of 280 nm for NEB and 250 nm for VAL using 67 mM Phosphate buffer pH 6.8 with 0.5% SDS as blank. This confirmed the authenticity of the sample. Therefore, the wavelength of 280 nm and 250 nm were used for the quantitative estimation of NEB and VAL respectively.

NEB and VAL exhibits maximum absorbance at 280 nm and 250 nm respectively and obeyed Beer's law in the range of 10-70µg/ml and 10-60µg/ml respectively. The results of calibration curve preparation were showed in Table 1 and Fig. 1 for NEB and Table 2 and Fig. 2 for VAL.

Table 1: Standard calibration curve data of NEB

S. No.	Concentration (µg/ml)	Absorbance			Absorbance (Mean $\pm$ SD)
1	10	0.139	0.141	0.14	0.140 $\pm$ 0.001
2	20	0.281	0.283	0.284	0.283 $\pm$ 0.0015
3	30	0.396	0.392	0.395	0.394 $\pm$ 0.0021
4	40	0.592	0.593	0.59	0.592 $\pm$ 0.0015
5	50	0.756	0.755	0.756	0.756 $\pm$ 0.0005
6	60	0.923	0.924	0.921	0.923 $\pm$ 0.0015
7	70	1.059	1.062	1.06	1.060 $\pm$ 0.0015

SD: Standard deviation, *: Mean of each 3 reading

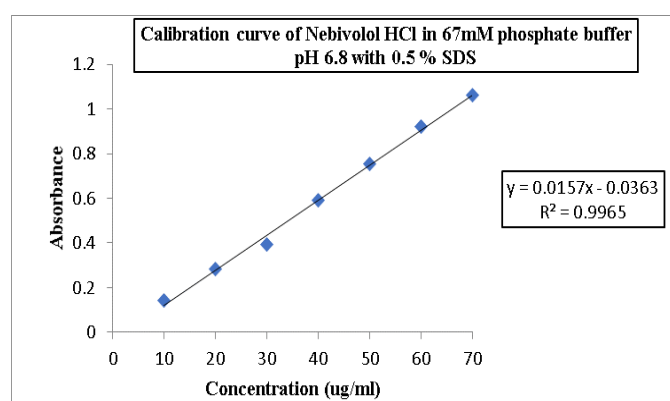


Fig. 1: Standard calibration curve of NEB

Table 2: Standard calibration curve data of VAL

S. No.	Concentration (µg/ml)	Absorbance			Absorbance (Mean $\pm$ SD)
1	10	0.220	0.221	0.219	0.220 $\pm$ 0.001
2	20	0.352	0.35	0.351	0.351 $\pm$ 0.001
3	30	0.509	0.511	0.509	0.509 $\pm$ 0.0011
4	40	0.662	0.660	0.663	0.662 $\pm$ 0.0015
5	50	0.817	0.818	0.815	0.817 $\pm$ 0.0015
6	60	1.023	1.019	1.020	1.020 $\pm$ 0.0021

SD: Standard deviation, *: Mean of each 3 reading

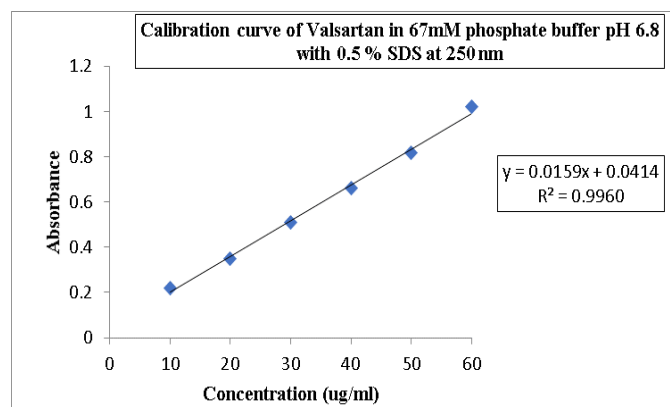


Fig. 2: Standard calibration curve of VAL

Validation of Analytical method of NEB and VAL

Linearity and range

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of NEB and VAL. The data obtained in the

calibration experiments when subjected to linear regression analysis showed a linear relationship between absorbance and concentrations in the range 10-70µg/ml for NEB and 10-60µg/ml for VAL. The results of calibration curve preparation were showed in Table 1 and Table 2 for NEB and VAL respectively. The respective linear equation for NEB was $y = 0.0157x - 0.0363$ and VAL equation $y = 0.0159x + 0.0414$, where x is the concentration and y is the absorbance. The correlation coefficient was found to be 0.9965 at 280 nm and 0.9960 at 250 nm for NEB and VAL respectively. The calibration curve of NEB and VAL is depicted in Fig. 1 and Fig. 2.

Accuracy (% recovery)

To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method. To pre analyzed tablet solution, a definite concentration of standard drug (50%, 100% and 150%) was added and then its recovery was analyzed. The % recoveries were found to be within 95.65%-109.85% and 97.42%-101.43% for NEB and VAL respectively. The results of recovery study were showed in Table 3 and Table 4 for NEB and VAL respectively.

Table 3: Data of recovery study for NEB

S. No	Amount of drug taken(µg/ml)	Amount of drug added (µg/ml)	Total amount of drug (µg/ml)	Amount of drug found (µg/ml)	% Recovery
1	20	-	20	-	-
2	20	10	30	28.69	95.65
3	20	20	40	42.39	105.97
4	20	30	50	54.92	109.85

Table 4: Data of recovery study for VAL

S. No	Amount of drug taken(µg/ml)	Amount of drug added (µg/ml)	Total amount of drug (µg/ml)	Amount of drug found (µg/ml)	% Recovery
1	20	-	20	-	-
2	20	10	30	29.22	97.42
3	20	20	40	39.14	97.85
4	20	30	50	50.71	101.43

Precision

The precision UV methods was obtained by analyze on the same day (intra-day) and analyze on the different days by triplicate analysis (inter-day) and expressed as relative standard deviation percentage. For NEB the % RSD found 0.271%-1.490% for intraday and 0.334%-1.917% for interday. For VAL the % RSD found 0.188 %-0.944% for intraday and 0.392%-1.197% for interday. Precision revealed that the proposed method is precise. Results were showed in Table 5 and Table 6 for NEB and Table 7 and Table 8 for VAL.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were performed on samples containing concentration of analytes, based on the standard deviation of the response and the slope of calibration curve. The slope and standard deviation (SD) values were calculated using the linearity graph. The LOD was

found 4.608µg/ml and 4.348µg/ml for NEB and VAL respectively. The LOQ was found 4.608µg/ml and 4.348µg/ml for NEB and VAL respectively.

Table 5: Intraday precision data for NEB

S. No	Concentration (µg/ml)	Absorbance			Absorbance (Mean* ± SD)	% RSD
		1	2	3		
1	10	0.142	0.138	0.139	0.140 ± 0.0020	1.490
2	20	0.278	0.279	0.281	0.279 ± 0.0015	0.546
3	30	0.391	0.395	0.393	0.393 ± 0.002	0.508
4	40	0.591	0.589	0.587	0.589 ± 0.002	0.339
5	50	0.752	0.75	0.755	0.752 ± 0.0025	0.334
6	60	0.926	0.928	0.931	0.928 ± 0.0025	0.271
7	70	1.061	1.054	1.055	1.056 ± 0.0037	0.358

SD: Standard deviation, RSD: Relative standard deviation, * : Mean of each 3 reading

Table 6: Interday precision data for NEB

S. No	Concentration (µg/ml)	Absorbance			Absorbance (Mean* ± SD)	% RSD
		1	2	3		
1	10	0.137	0.141	0.136	0.138 ± 0.0026	1.917
2	20	0.281	0.28	0.28	0.280 ± 0.0005	0.205
3	30	0.389	0.391	0.394	0.391 ± 0.0025	0.643
4	40	0.585	0.589	0.593	0.5894 ± 0.004	0.679
5	50	0.751	0.749	0.754	0.751 ± 0.0025	0.334
6	60	0.92	0.926	0.921	0.922 ± 0.0035	0.348
7	70	1.058	1.067	1.065	1.063 ± 0.0047	0.444

SD: Standard deviation, RSD: Relative standard deviation, * : Mean of each 3 reading

Table 7: Intraday precision data for VAL

S. No	Concentration (µg/ml)	Absorbance			Absorbance (Mean* ± SD)	% RSD
		1	2	3		
1	10	0.222	0.221	0.218	0.220 ± 0.0021	0.944
2	20	0.348	0.349	0.351	0.349 ± 0.0015	0.437
3	30	0.512	0.513	0.509	0.511 ± 0.0021	0.407
4	40	0.665	0.660	0.661	0.662 ± 0.0026	0.399
5	50	0.810	0.809	0.812	0.810 ± 0.0015	0.188
6	60	1.031	1.029	1.034	1.031 ± 0.0025	0.244

SD: Standard deviation, RSD: Relative standard deviation, * : Mean of each 3 reading

Table 8: Interday precision data for VAL

S. No	Concentration (µg/ml)	Absorbance			Absorbance (Mean* ± SD)	% RSD
		1	2	3		
1	10	0.219	0.224	0.22	0.221 ± 0.0026	1.197
2	20	0.345	0.348	0.347	0.347 ± 0.0015	0.440
3	30	0.51	0.508	0.512	0.510 ± 0.002	0.392
4	40	0.66	0.664	0.669	0.664 ± 0.0045	0.678
5	50	0.815	0.821	0.811	0.816 ± 0.0050	0.617
6	60	1.022	1.029	1.032	1.027 ± 0.0051	0.499

SD: Standard deviation, RSD: Relative standard deviation, * : Mean of each 3 reading

Summary of Validation Parameters

The proposed method was found to be linear over concentration range of 10-70µg/ml and 10-60µg/ml for NEB and VAL respectively. The method was found to be accurate and precise as indicated by the results of recovery studies and precision studies whose % RSD is not more than 2%. The results of the analysis of NEB and VAL by the proposed method were highly reproducible and reliable which conclude that the proposed method is highly simple, sensitive, reproducible, economic, less time consuming and easy to apply for routine analysis of NEB and VAL in 67 mM Phosphate buffer pH 6.8 with 0.5% SDS.

Simultaneous estimation of NEB and VAL

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures.

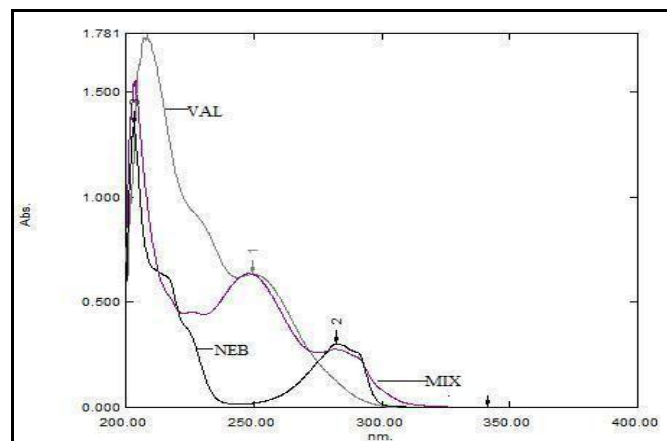


Fig. 3: The overlain spectra of NEB and VAL

Table 9: Summary of validation parameters of NEB and VAL

S. No	Validation parameters	Results	
		NEB	VAL
1	Absorption maxima (nm)	280	250
2	Linearity range (µg/ml)	10-70	10-60
3	Linearity equation	y=0.0157x+0.0363	y=0.0159x+0.0414
4	Linearity (R ² , Correlation coefficient)	0.9965	0.9960
5	Precision Intraday (% RSD)	0.271%-1.490%	0.188%-0.944%
	Interday	0.334%-1.917%	0.392%-1.197%
6	Accuracy (% Recovery)	95.65%-109.85%	97.42%-101.43%
7	LOD(µg/ml)	4.608	4.348
8	LOQ(µg/ml)	13.965	13.178

RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

Table 10: Accuracy and Precision data for determination of NEB and VAL

Accuracy and Precision data for determination of VAL in the presence of NEB		
Added amount of VAL (ug/ml)	Within day * (Amount found ± SD)	Between day * (Amount found ± SD)
10	10.05 ± 0.04	10.08 ± 0.05
20	20.02 ± 0.02	20.13 ± 0.11
30	30.16 ± 0.21	30.22 ± 0.18
Accuracy and Precision data for determination of NEB in the presence of VAL		
Added amount of NEB (ug/ml)	Within day * (Amount found ± SD)	Between day * (Amount found ± SD)
2	2.05 ± 0.06	2.03 ± 0.18
4	4.06 ± 0.15	4.10 ± 0.03
6	6.07 ± 0.12	6.15 ± 0.04

SD: Standard deviation, * : Mean of each 6 reading

Table 11: Assay results of NEB and VAL in Nebicard-V tablet

Drugs	Amount (mg/tablet)		% Label claim (% Found ± SD)*
	Labelled (mg)	Found (Mean ± SD)	
NEB	5 mg	4.98 ± 0.06	99.6 ± 0.92
VAL	80 mg	79.96 ± 0.24	99.7 ± 1.16

SD: Standard deviation, * : Mean of each 6 reading

The results of the analysis of both drugs by the proposed method are highly reproducible and reliable.

This UV spectrophotometric method was adopted for determination of the NEB and VAL release in the dissolution medium for *in vitro* dissolution testing of the combined dosage form. Simultaneous calibration of both drugs in 67 mM Phosphate buffer pH 6.8 with 0.5 % SDS shows that λ_{max} of one drug does not interfere on the λ_{max} of other drug. The developed method was found to be simple, sensitive, accurate and reproducible and can be used for routine analysis of NEB and VAL.

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