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Area Under Curve Method Development And Validation For The Estimation Of Nebivolol And Valsartan In Bulk And Pharmaceutical Dosage Form.

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ABSTRACT

A simple, robust, precise, UV spectroscopic method has been developed for the simultaneous estimation of Nebivolal and Valsartan in bulk and tablet dosage forms. In this paper the estimation of those drugs was carried out by simultaneous equation method. This method is based on measurement of absorption at 281nm and 251nm i.e, λ_{max} of Nebivolal and Valsartan respectively. The linearity observed for Nebivolal is in the range of 5-25 µg/ml and for Valsartan is in the range of 5-80 µg/ml. The accuracy of methods was assessed by recovery studies and was found to be within the range of 99.28%-99.26% for both Nebivolal and Valsartan. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The method can be employed for estimation of pharmaceutical formulations with no interference from any other excipients and diluents. The results were validated as per ICH guidelines.

Keywords: Nebivolal, Valsartan, ICH, AUC, Validation etc.

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INTRODUCTION

By curing various fatal diseases, drugs play a vital role in the progress of the human civilization. Heartattack is one of the fatal and severe disease is threat for the entire world. In India, more than 62 million Indians are affected by Heart problems. Hence, it is necessary to control heart problem. Nebivolal (NEBI) and Valsartan (VAL) combination the new drug combination used to cure heart attack[1].

Chemically, NEBI is known as, 1'-Bis (6-fluoro-3,4-dihydro-2H-1-benzopyran-2-yl)-2,2'-iminodiethanolIt is an orally administered NEBI is unique as a beta-blocker. NEBI lowers blood pressure (BP) by reducing peripheral vascular resistance, and significantly increases stroke volume with preservation of cardiac output. The net hemodynamic effect of NEBI is the result of a balance between the depressant effects of beta-blockade and an action that maintains cardiac output. Antihypertensive responses were significantly higher with NEBI than with placebo in trials enrolling patient groups considered representative of the U.S. hypertensive population, in Black patients, and in those receiving concurrent treatment with other antihypertensive drugs.[2, 3]

The chemical name of VAL is (S)-N-Valeryl-N-{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]-methyl}-valine VAL is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. VAL lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II. Unlike angiotensin-converting enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough. VAL may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure, systolic dysfunction, myocardial infarction and coronary artery disease.[2, 3]

NEBI and VAL is a new drug combination. Therefore, there are very few reports of quantitative estimation methods for this new combination. Simultaneous Equation Method is typically applies for the estimation of drug combinations containing two or more than two drugs. This method is easy, simple and gives reproducible results as compared to other UV methods. Therefore, this is a attempt to develop simple, robust, reproducible method for the determination of efficacy and safety of NEBI and VAL combination. This method was fully validated according to International Conference on Harmonization (ICH) and ready for the application in routine analysis without interference of an excipients.[3,4]

Analytical methods reported for quantitative determination of NEBI individually in pharmaceutical formulations or biological fluids are high-performance liquid chromatographic HPLC,[9-11] UV[12-13]and methods reported for quantitative determination of VAL individually in pharmaceutical formulations or biological fluids are HPLC[9-11,12],UV,[12-13,14] and some of the reported for quantitative determination method in combination of NEBI and VAL but no reported AUC method was found on same combination with selected key parameters.

Figure 1: Structure of Nebivolal

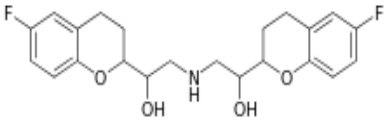
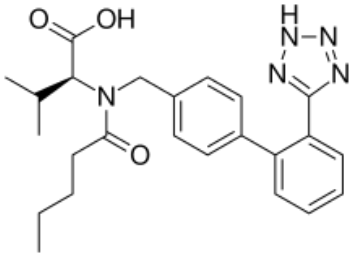


Figure 2. Structure of Valsartan



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MATERIALS AND METHODS

Instruments

Shimadzu UV-1800 double beam spectrophotometer was used to record the spectra of sample and reference solutions using pair of quartz cells of 10 mm path length. All weighing was carried out on Sansui Vibra DJ-150S-Sweighing balance. Sonicator of Fast Clean is used for the purpose of sonication, Filter papers of Sartorius Stedim Biotech of grade 292 are used for the filtration purpose.

Chemicals

NEBI(5 mg) and VAL(80 mg) pure drugs were obtained as a gift sample from Lupin Pharma and Dolphin Pharma India. The combined formulation Torrent Pharma (5 mg/80 mg) of the two drugs purchased from Local Pharmacy Medical, .Analytical grade methanol purchased from Merck Chemicals Pvt. Ltd. Mumbai.

Preparation of stock solution and selection of wavelength

NEBI stock solution

An accurately weighed quantity of NEBI (1 mg) was taken in 10 mL volumetric flask and dissolved in methanol (8 mL) with the help of ultra-sonication for about 10 min. Then the volume was made up to the mark using methanol to get NEBI standard stock solution (1 mg / mL).

NEBI working solution

NEBI standard stock solution (1 mL) was diluted to 10 mL using methanol to get working standard solution (100 µg/mL).

VAL stock solution

An accurately weighed quantity of VAL (1 mg) was taken in 10 mL volumetric flask and dissolved in methanol (8 mL) with the help of ultra-sonication for about 10 min. Then the volume was made up to the mark using methanol to get VAL standard stock solution (1 mg / mL).

VAL working solution

VAL standard stock solution (1 mL) was diluted to 10 mL using methanol to get working standard solution (100 µg / mL).

Determination of λ Max of Individual Component

An appropriate aliquot portion of NEBI (0.015 mL) and VAL (0.5 mL) were transferred to two separate 10 mL volumetric flasks, the volume was made up to the mark using methanol to obtain NEBI (0.15 µg/mL) and VAL (5 µg/mL). Drug solutions were scanned separately between 200 nm to 400 nm. NEBI shows the λ_{max} at 281nm while VAL shows λ_{max} at 251nm.

Overlay spectra of NEBI and VAL

The overlay spectra of both drugs were recorded and two wavelengths 281nm (λ_{max} of NEBI) and 251nm (λ_{max} of VAL) were selected for further study.

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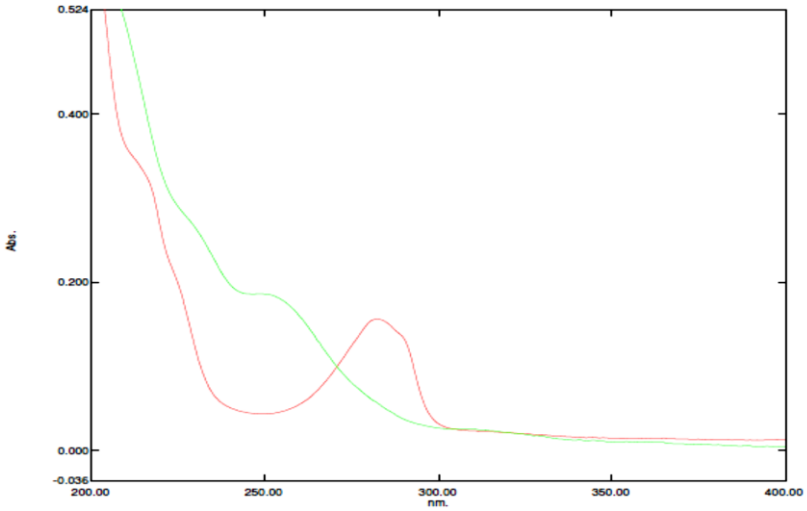

Graph 1: Overlay spectra of NEBI and VAL

Linearity study for NEBI

An accurately measured aliquot portion of working standard solution of NEBI was transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using methanol to obtain concentrations of NEBI (5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25 µg/ml). Absorbance of these solutions was measured at 281 nm, Calibration curve was plotted, absorbance Vs concentration.

Linearity study for VAL

Accurately measured aliquot portions of working standard solution of VAL were transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using methanol to obtain concentrations (5µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80 µg/ml) Absorbance of these solutions was measured at 293 nm. Calibration curve was plotted, absorbance Vs concentration. The results are shown in the Table No.1

METHODOLOGY

AREA UNDER CURVE METHOD

It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated.

This wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. For the selection of analytical wavelength suitable dilutions of NEBI (5-80µg/ml) and VAL (5-80µg/ml) of the standard stock solutions (10µg/ml) of both the drugs were prepared separately and scanned in the range of 400-200 nm. Maximum absorbance was observed at 276 nm and 293 nm for NEBI and VAL respectively. The wavelength ranges selected for the estimation of NEBI and VAL are 271-281 nm (λ1 and λ2) and 288-298 (λ3 and λ4) respectively. Aliquots were prepared for the sample solution in the concentration range of 5-80µg/ml and 5-80µg/ml for NEBI and VAL and their area under curve was measured at above selected wavelengths.

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GRAPHICAL REPORT

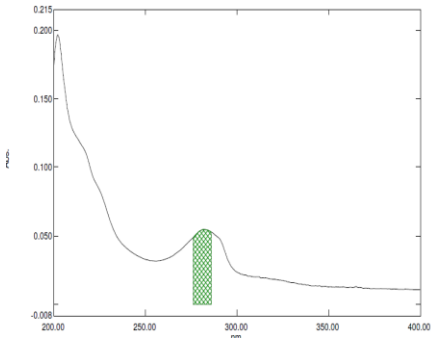

Figure 3: AUC spectrum of NEBI at 5µg/ml

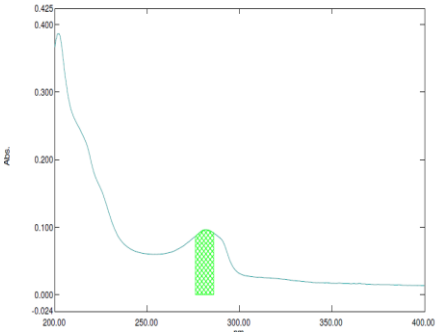

Figure 4: AUC spectrum of NEBI at 10µg/ml

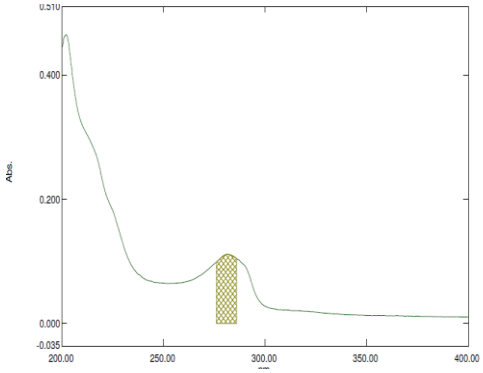

Figure 5: AUC spectrum of NEBI at 15µg/ml

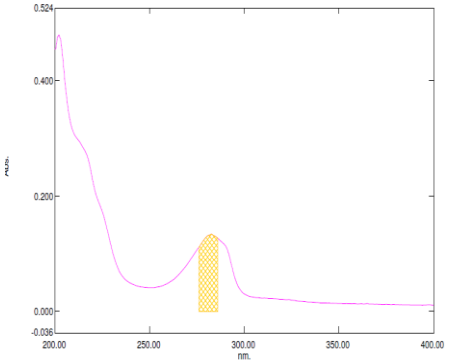

Figure 6: AUC spectrum of NEBI at 20µg/ml

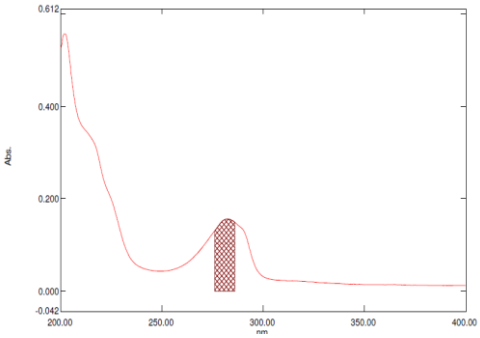

Figure 7: AUC spectrum of NEBI at 25µg/ml

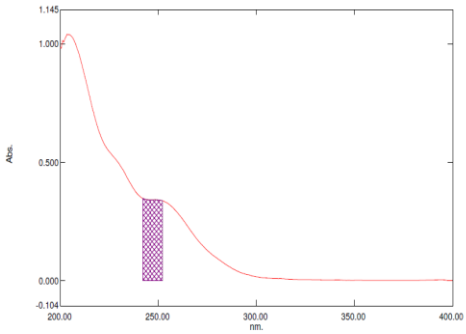

Figure 8: AUC spectrum of VAL at 5µg/ml

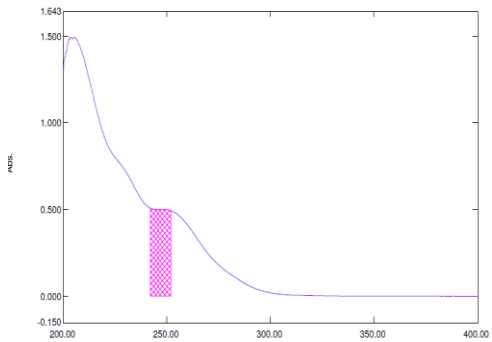

Figure 9: AUC spectrum of VAL at 20µg/ml

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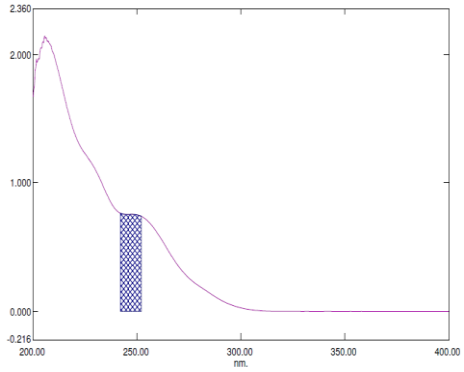

Figure 10: AUC spectrum of VAL at 40µg/ml

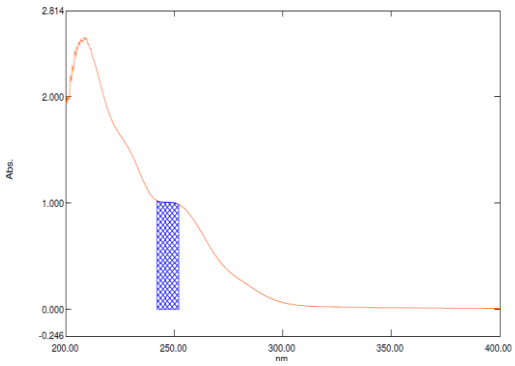

Figure 11: AUC spectrum of VAL at 60µg/ml

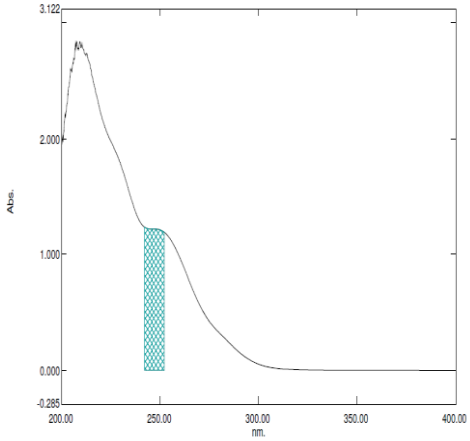

Figure 12: AUC spectrum of VAL at 80µg/ml

The Area under Curve range of NEBI and VAL was taken as 276-286 nm and 246-256 nm respectively.The absorptivity values calculated. The calibration curve was plotted with concentration v/s area under curve and regression equation was calculated.

Determination of absorptivity values:

Absorptivity = Absorbance / Concentration of the component in gm/l

Concentration of NEBI and VAL was calculated using following formula;

$$C_{NEBI} = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2 \text{ ---------- (1)}$$
$$C_{VAL} = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2 \text{ ---------- (2)}$$

Where,
C_{NEBI}= Concentrations of NEBI
C_{VAL}= Concentrations of VAL
A_1 = Area of NEBI at 276 nm to 286 nm
A_2 = Area of VAL at 246 nm to 256nm
ax_1 = Absorptivity value of NEBI at 276-286 nm,
ax_2 = Absorptivity value of NEBI at 246 - 256 nm,
ay_1 = Absorptivity value of VAL at 246-256 nm,
ay_2 = Absorptivity value of VAL at 276 -286 nm

VALIDATION OF THE PROPOSED METHOD

The Proposed method was validated as per the ICH guidelines.

ACCURACY (RECOVERY STUDY)

Accuracy of proposed method was ascertained on the basis of recovery study of NEBI and VAL performed by standard addition method. A known amount of standard drug solutions of NEBI and VAL were added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The amounts of NEBI and VAL were estimated by applying obtained values to the respective regression line equations.

LINEARITY STUDY

The calibration curves were plotted over a concentration range of 5-25µg/ml for NEBI and 5-80 µg/ml for VAL. Accurately measured standard solutions of NEBI (5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml) and VAL (5µg/ml, 10µg, 20µg/ml, 40µg/ml, and 80µg/ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbance of the solutions was measured at 281 and 253 nm against methanol as blank. The calibration curves were constructed by plotting area versus concentrations and the regression equations were calculated.

PRECISION

METHOD PRECISION (REPEATABILITY)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n =5) for NEBI and VAL (5µg/ml for NEBI and 80 µg/ml for VAL) without changing the parameter of the proposed spectrophotometry method

INTERMEDIATE PRECISION (REPRODUCIBILITY)

Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing NEBI (1,3 and 5 µg/mL) and VAL (20, 40 and 80 µg/mL) for three times on the same day. Inter-day precision was determined by analyzing the same concentration of solutions for three different days over a period of week. The results are reported in terms of relative standard deviation (RSD).

RUGGEDNESS

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two different analyst (A_1 and A_2) using same operational ((n=3)conc. at 5 µg/mL for NEBI and 80 µg/mL for VAL) and environmental conditions (Day-1 and Day-2) The results are reported in terms of relative standard deviation (RSD).

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The Limit of Detection (LOD) and the Limit of Quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) 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.

L.O.D.: Limit of detection of NEBI and VAL were found to be0.28638µg/mL and 0.13023µg/mL respectively.

L.O.Q.: Limit of Quantitation of NEBI and VAL were found to be 0.86781µg/mL and 0.39464µg/mL respectively.

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Table 1: Results Of Formulation

Tablet- NEBICART- Average Weight of Tablet = 366 mg.		
Sr. No	% of Drug Recovered (n = 3)	
	VAL	NEBI
Mean	99.28	99.26
SD	0.326	0.447
%RSD	0.328	0.451

** Average of five determinations.

Table 2. Result of Analysis of the Tablet Formulation

Drugs	Label Claim	Amount Found	%Label Claim	S.D	R.S.D
NEBI	5	4.988	99.92	0.1109	0.1111
VAL	80	79.96	99.86	0.1128	0.1129

Table 3: Result of Accuracy

Drug	Conc. [µg/mL]	Intra-day Amount Found		Inter-day Amount Found	
		Mean ±S.D [n = 3]	% R.S.D.	Mean ± S.D. [n = 3]	% R.S.D.
VAL	20	19.3 ± 0.34928	1.78025	19.26± 0.0230	0.1198
	40	39.7 ± 0.61481	0.52408	39.71± 0.0231	0.0898
	80	79.16 ± 0.78046	0.97275	79.18 ± 0.0808	0.2511
NEBI	1	0.91 ± 1.24	1.3	0.89 ± 0.02	0.3395
	3	2.69 ± 0.0251	0.920	2.88 ± 0.0264	0.3356
	5	4.97 ± 0.0707	0.975	4.97 ± 0.00826	0.1680

Table 4 Regression Analysis and Validation Parameters

Parameters	NEBI	VAL
Linearity Range	5-25 µg/ml	5-80 µg/ml
Correlation Coefficient	0.9962	0.998
Precision (% RSD)		
Intra Day	0.972– 1.780	0.63 – 0.84
Inter Day	0.110– 1.300	0.0.168 – 0.339
LOD (µg/ml)	0.28638	0.13023
LOQ (µg/ml)	0.86781	0.39464
Assay (% Accuracy)	99.28 %	99.26 %

RESULT AND DISCUSSION

The estimation of NEBI and VAL in bulk and tablet formulation was found to be accurate and reproducible with a linearity range of 5-25µg/ml and 5-80µg/ml and the correlation coefficient was found to be 0.9945 and 0.9946 for the method. The optical characteristics such as linearity study, molar absorptivity, percentage relative standard deviation of recovery studies and precision in each method were calculated and the results were reported in **Table 1** and **Table 2. Table 3.**

Also the regression characteristics like slope (m), intercept (c) and correlation coefficient (R^2) were calculated and are presented in **Table 4**. The accuracy was found by recovery studies at three different levels i.e. 80%, 100% and 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value was less than 2, an indicative of the accuracy of the methods are presented in **Table 3**. The results for formulation were reported in **Table 1**.

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The spectra of NEBI, VAL and formulation reported by (Area under Curve method) calibration curve was plotted **(Fig. 11,12)**.

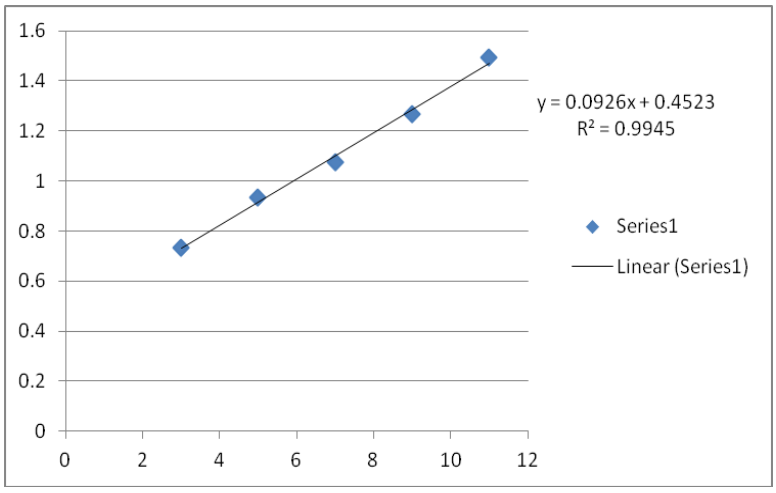

Figure 11: Calibration curve of AUC for NEBI at 271nm-281nm at different concentrations

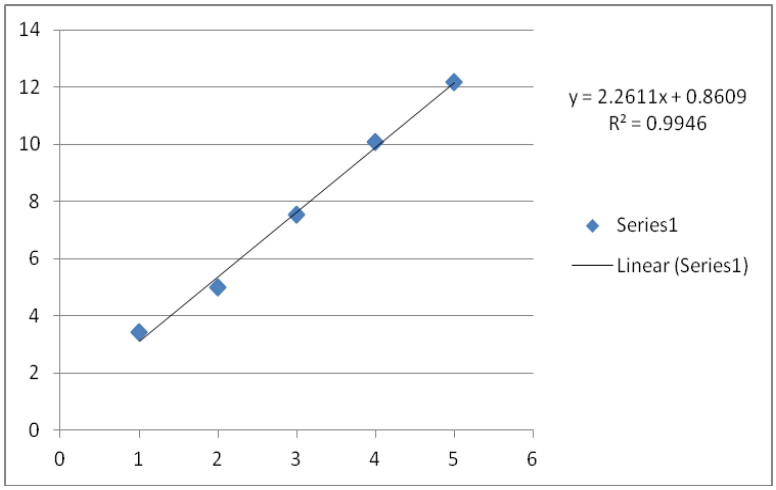

Figure 12: Calibration curve of AUC for VAL at 288nm-298nm at different concentrations

Table 5: Optical Characteristics And Parameters For Method

Parameters	NEBI	VAL
Linear range (µg/ml)	5-25	5-80
λmax / wavelength range (nm)	276-286	246-256
Coefficient of correlation(R^2)	0.9945	0.9946
Slope*(m)	0.926x	2.2611x
Intercept*(c)	0.4523	0.8609
Limit of Detection (µg/ml)	0.13023 µg	0.28638 µg
Limit of Quantification (µg/ml)	0.39464 µg	0.86781 µg

*y = mx + c; when x is the concentration in µg/ml and y is absorbance unit

CONCLUSION

The proposed spectrophotometric method was found to be simple, accurate and precise and inexpensive and can be used for routine laboratory analysis of NEBI and VAL in bulk and its formulation.

ACKNOWLEDGEMENT

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