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Simultaneous Determination And Validation Of Nebivolol And S-Amlodipine By Zero Order Derivative And First Order Derivative Method In Bulk Drug And Pharmaceutical Formulation

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Abstract: The proposed method involves Zero order derivative and First order derivative spectroscopy method. A novel, simple and rapid UV Spectrophotometric determination method for Simultaneous estimation of Nebivolol and S-amlodipine was successfully developed and validated in bulk and pharmaceutical formulation. First method is zero order derivative method where the solutions were scanned in the range from 400-200 nm and the peaks were observed at a λmax of 280 and 364 nmfor Nebivolol and S-amlodipine respectively. Second method is First order derivative spectroscopy method, where the measurement of absorbances of one drug at zero crossing point of other drug i.e., 294 nm and 279.7 nm were selected for the estimation of Nebivolol and S-amlodipine respectively in bulk drug and formulation. Both the methods showed linearity from 10–60 µg/ml and 5-30 µg/ml for Nebivolol and S-amlodipine respectively. Recovery studies showed that the method is accurate. Precision of the proposed methods were found to be within the acceptable limits. Thus the two proposed methods and results were validated according to ICH guidelines. So, the methods can be applied for routine analysis in bulk and pharmaceutical formulation.

Keywords: Nebivolol, S-amlodipine, First order derivative method, Zero order derivative method.

INTRODUCTION:

Nebivolol hydrochloride is a long acting cardio selective β_1-receptor antagonist without partial agonist activity[1]. It may prevent arterial dilation and inhibit renin secretion although precise mechanism of action is not known. Negative chronotropic effects may slow resting heart rate and negative ionotropic effects may reduce cardiac output, myocardial contractility and myocardial oxygen consumption during stress or exercise. All of these actions may work together to lower systolic and diastolic blood pressure[2].

Nebivolol chemically is (1RS,1'RS)-1,1'-[(2S, 2'RS)-bis(6-fluorochoman-2-yl)]-2,2'iminodi ethanolHCl with a molecular formula of $C_{22}H_{25}F_2NO_4$ HCl and molecular weight of 441.9gm/mol. It is an official drug in Indian Pharmacoepia[3] and British Pharmacopoeia[4] Fig 1.

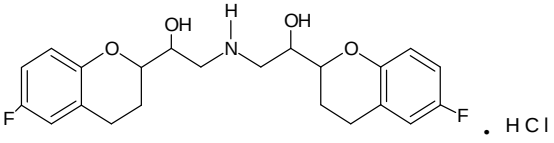

Fig.1.Chemical structure of Nebivolol Hydrochloride

S(-)Amlodipine besylate is a long-acting second generation 1,4-dihydropyridine derivative of proto typical molecule Nifedipine. It has greater selectivity for vascular smooth muscle than myocardial tissue[5]. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing total peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle[6]. It is an official drug in I.P[3] and B.P[4].

S-amlodipine chemically is (S)-2-[(2-Aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6- methyl-3,5-pyridine dicarboxylic acid 3-ethyl 5-methyl ester benzene sulphonate with a molecular formula $C_{20}H_{25}ClN_2O_5$. $C_6H_6O_3S$ and molecular weight of 567.1 gm/mol. It is an official drug in Indian Pharmacopoeia and British Pharmacopoeia. Fig.2.

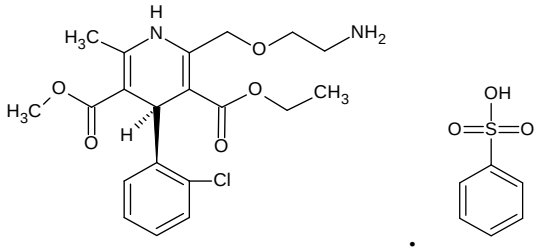

Fig.2.Chemical structure of S-amlodipine Besylate

Combination of Nebivolol and S-amlodipine are used in treatment of hypertension.

The combination of S (-)Amlodipine besylate and Nebivolol hydrochloride is very useful in the treatment of hypertension. On literature survey, it was found that only RP-HPLC, HPTLC, UV-Spectrophotometric methods have been reported for the simultaneous estimation of S(-)Amlodipine besylate and Nebivolol hydrochloride in combined dosage forms and no method is available in the pharmacopoeia. In the view of the need in the industry for routine analysis of Nebivolol and S-amlodipine in formulation, attempts are being made to develop simple and accurate analytical methods for simultaneous estimation of Nebivolol and S-amlodipine and extend it for their determination in formulation.

EXPERIMENTAL

Instrument used:

For both the methods, Shimadzu model 1700 double beam UV-VIS spectrophotometer with spectral bandwidth of 1.8nm, wavelength accuracy of 2nm and a pair of 1 cm matched quartz cells of 10 mm optical path length was used as an instrument for spectral measurements. An analytically pure sample of Nebivolol and S-amlodipine was procured as gift sample from Micro Labs Pvt. Ltd, Bangalore, India. Tablet formulation NEBICARD-SM containing Nebivolol (5 mg) and S-amlodipine (2.5 mg) was purchased from local market.

Solvent Used:

Methanol AR grade and distilled water was used as solvent.

Preparation of standard stock solution:

100 mg each of Nebivolol and S-amlodipine were weighed separately and transferred into two different 100 ml volumetric flasks. Both the drugs were dissolved in 10 ml of methanol by ultrasonication and then volume was made upto the mark with water to obtain final concentration of 1000 µg/ml of each component (stock 'A' solution). From the above stock 'A' of each solution, 5 ml of aliquot was pipetted out in a 50 ml volumetric flask and the volume was made upto the mark with water to obtain the final concentration of 100 µg/ml of each component (stock 'B' solution).

Preparation of sample stock solution using formulation:

From the powder of twenty tablets, a quantity equivalent to 100mg of Nebivolol was weighed accurately and transferred to a flask containing 10ml of methanol, ultrasonicated for 15mins, solution was filtered through membrane filter into a 100ml volumetric flask, volume was made upto mark with water to get 1000 µg/ml (stock I). Aliquots were further prepared by diluting stock II (100 µg/ml) in water to get a concentration of 10-60 µg/ml.

Methodology:
Calibration curve
Method A: Zero order derivative method[7,8]

Zero order derivative method involved the normal absorption spectrum referred to as the fundamental, zero order or D^0 spectrum. The solution was scanned in the range from 400-200 nm and the wavelength selected for the analysis were 280 nm and 364 nm for Nebivolol and S-amlodipine respectively (Fig.3 and 4). Beer's law is obeyed in the concentration range of 10-60 µg/ml and 5-30 µg/ml for Nebivolol and S-amlodipine respectively and the calibration curve

was plotted. (Fig.5 and 6). Similarly absorbances of sample solutions were measured and amount of Nebivolol and S-amlodipine was determined from standard calibration curve (Fig.7 and 8).

Method B: First order derivative method[9-13]

Using appropriate dilutions of the standard stock solution, the solution was scanned in the wavelength region of 400 – 200 nm. The absorbance spectrum, thus obtained was derivatized to remove the interference of absorbing species. From the examination of first derivative spectra zero crossing point of one drug was selected for estimation of another drug. 294 nm and 279.7 nm were selected as analytical wavelength for the estimation of Nebivolol and S-amlodipine (Fig.9 and 10). The calibration curve was plotted (Fig.11 and 12). Similarly absorbances of samples solution were measured and amount of Nebivolol and S-amlodipine was determined from standard calibration curve (Fig.13, 14 and 15).

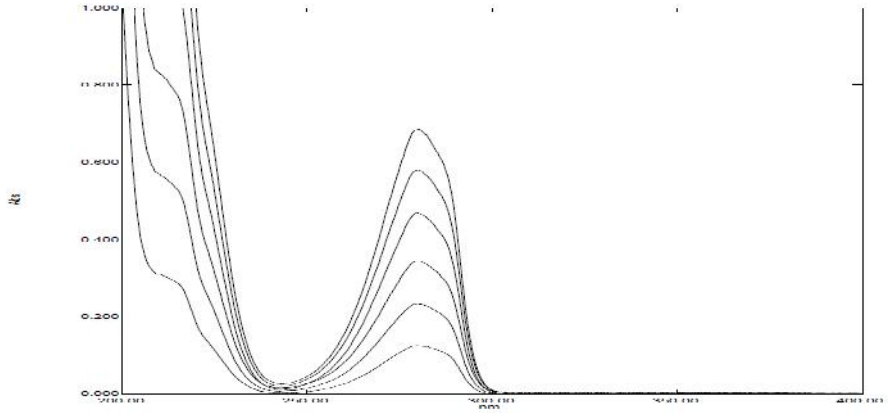

Fig.3. Linearity overlain spectra of Nebivolol from 10 to 60 µg/ml at 280 nm

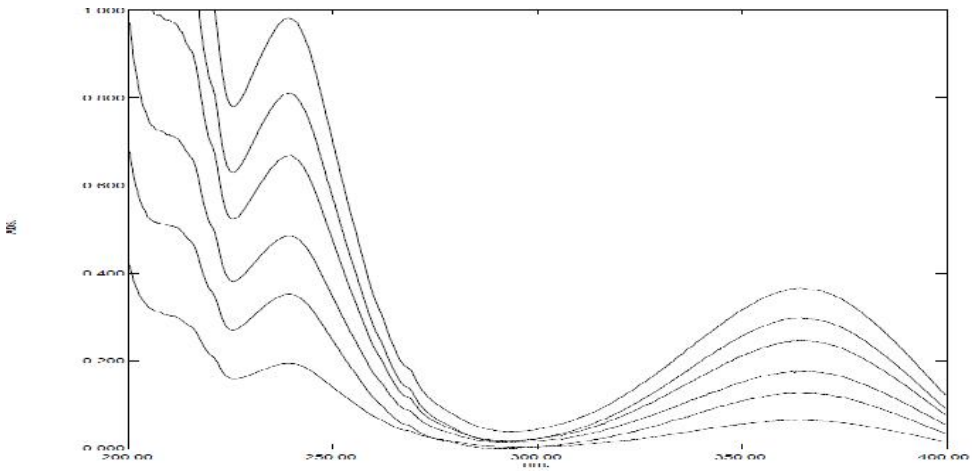

Fig.4. Linearity overlain spectra of S-amlodipine from 5 to 30 µg/ml at 364 nm

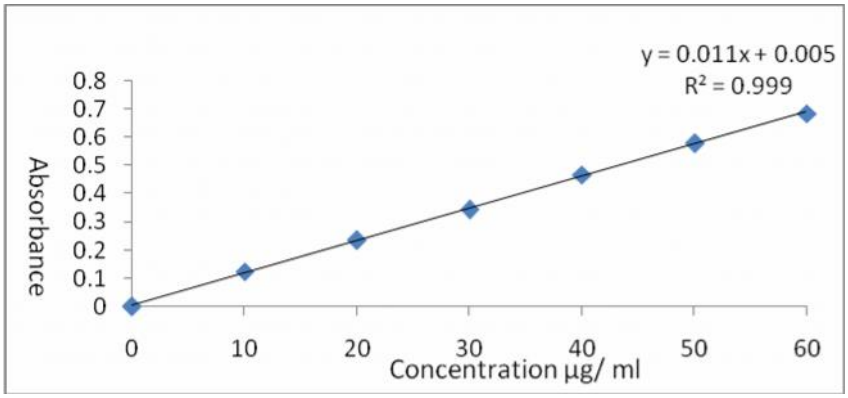

Fig.5. calibration curve of Nebivolol at 280 nm by Zero order derivative method

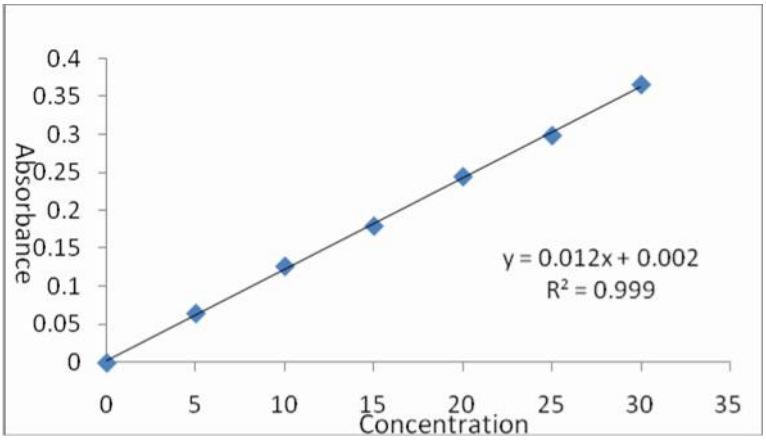

Fig.6. calibration curve of S-amlodipine at 364 nm by Zero order derivative method

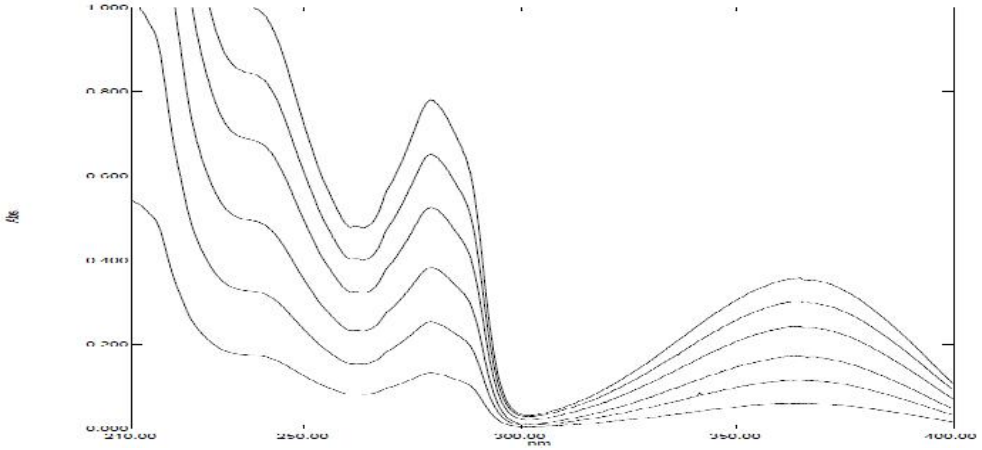

Fig.7. Linearity overlain spectra of Formulation from 10 to 60 µg/ml at 280 and 364 nm

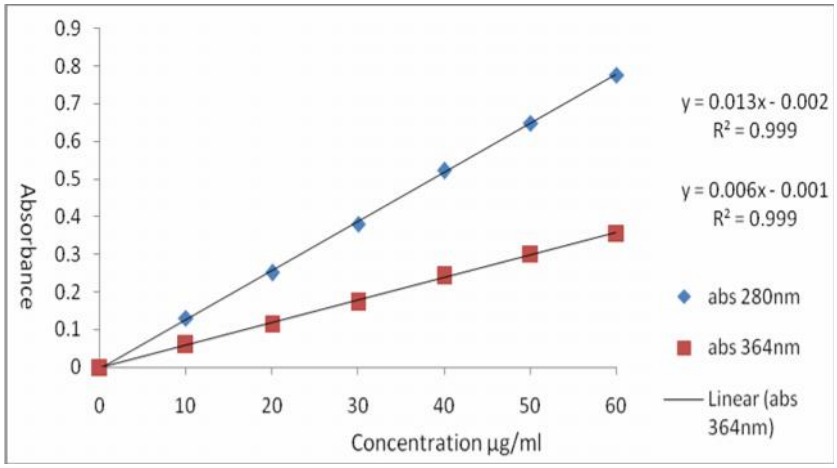

Fig.8. calibration curve of formulation at 280 and 364 nm by Zero order derivative method

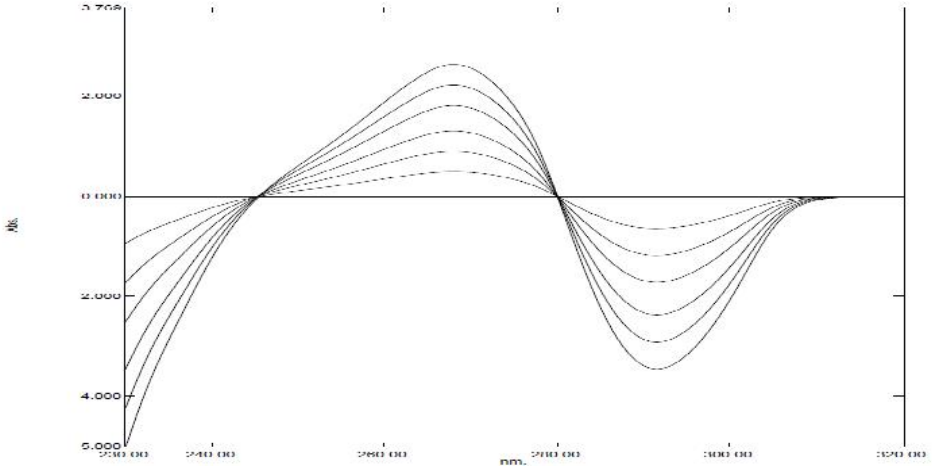

Fig.9. Derivatized overlain spectra of Nebivolol at 294 nm, which is zero crossing point of S-amlodipine in water

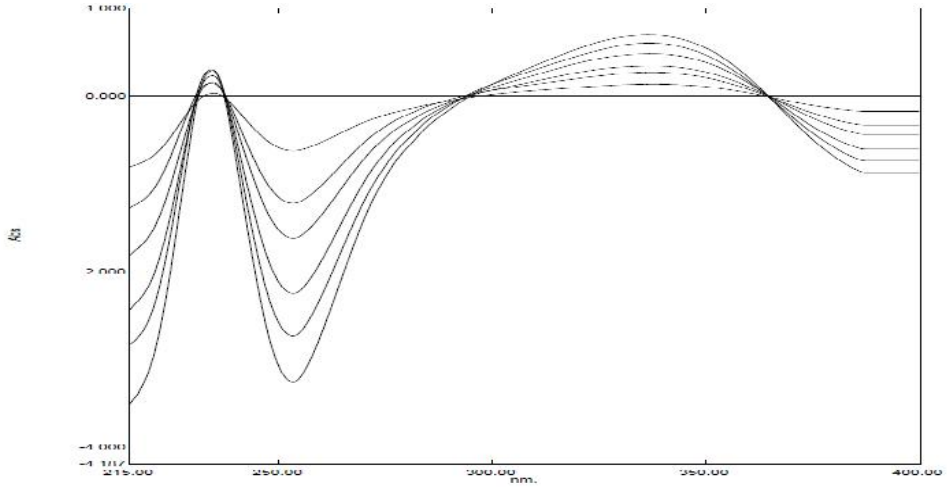

Fig.10. Derivatized overlain spectra of S-amlodipine at 279.7 nm, which is zero crossing point of Nebivolol in water

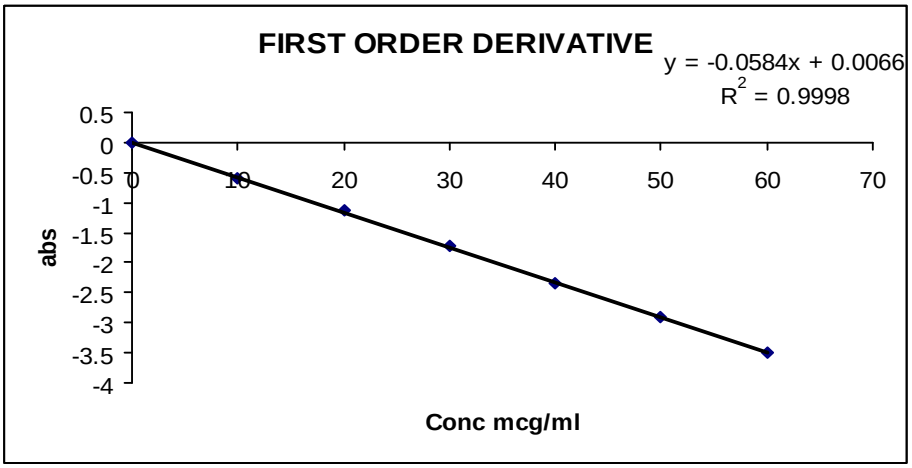

Fig.11. Calibration curve for Nebivolol at 294 nm in water by First order derivative method

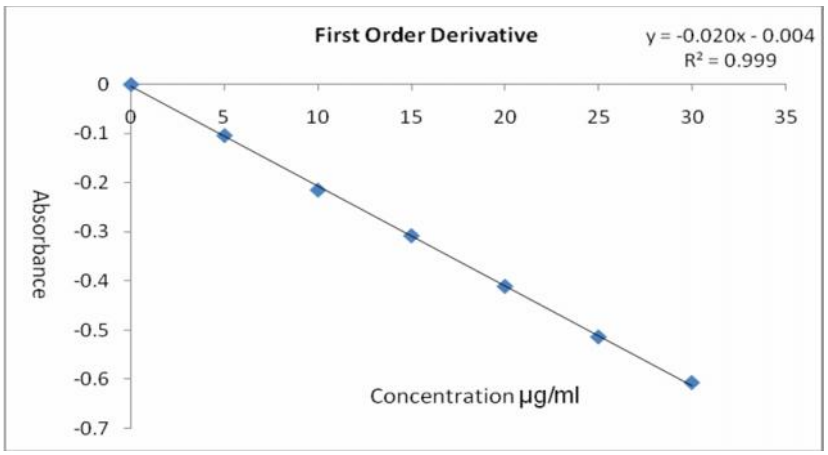

Fig.12. Calibration curve for S-amlodipine at 279.7 nm in ethanol by first order derivative method

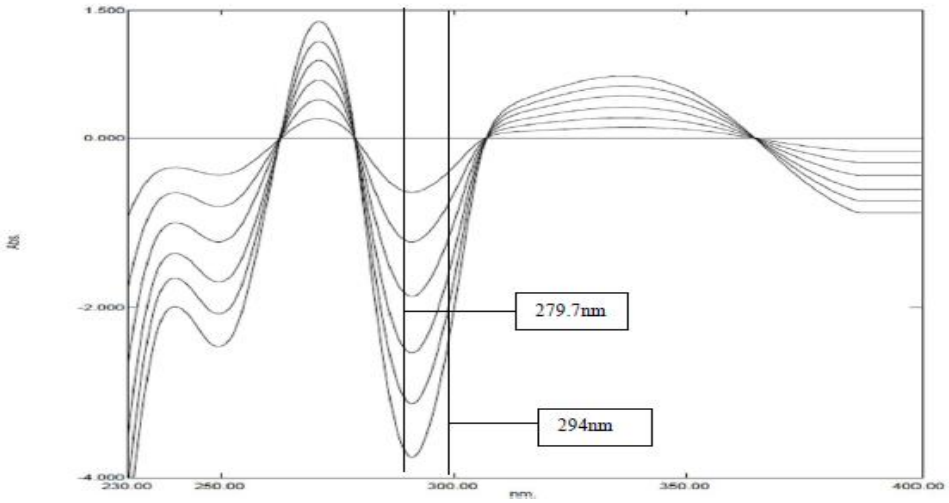

Fig.13. Derivatized overlain spectra of formulation at 294 nm and 279.7 nm

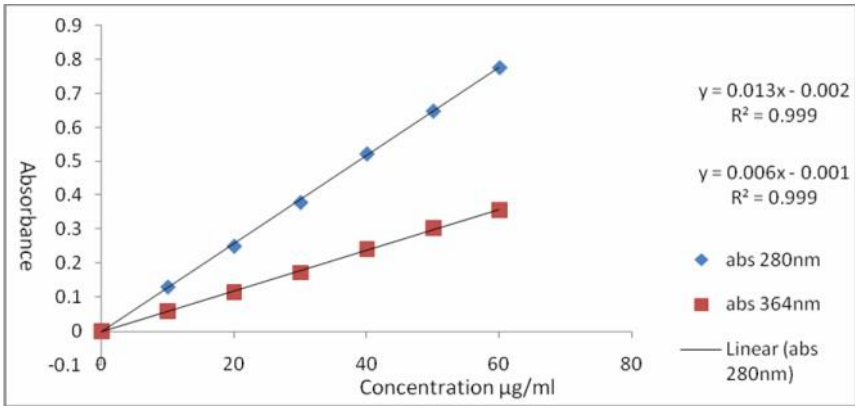

Fig.14. Calibration curve for Formulation at 294 nm and 279.7 nm in water by first order derivative method

Validation of methods[14,15]

The above methods were satisfactory in accordance to the ICH guidelines. Accuracy studies were carried out at three different levels i.e. 80%, 100% and 120% by adding the pure drug to previously analyzed tablet sample and the percentage recovery was calculated.

RESULTS AND DISCUSSION:

The absorption spectra for Nebivolol and S-amlodipine were recorded in the wavelength region of 200-400 nm for both method A and method B. The spectra were reported. These methods were found to be economic, simple, precise and accurate. Beer-Lambert's Law was obeyed in the concentration range of 10-60 µg/ml and 5-30 µg/ml for Nebivolol and S-amlodipine respectively. The accuracy was found by recovery studies at three different levels i.e.80%, 100% and 120%. The %RSD values are less than 2 for both the methods. The optical characteristics such as Beer's law limit, Sandell's sensitivity, % relative standard deviation, limit of detection, limit of quantitation and range of errors in each method were calculated and the results were reported in Table 1 and Table 4. Also the regression characteristics like slope (m), intercept (c), and correlation coefficient (r) were calculated and are presented in same tables mentioned above. The results showed that the methods have reasonable precision and the results were reported in Table 3 and Table 6. The accuracy of the methods was confirmed by the recovery studies by adding known amount of the pure drug to the pharmaceutical formulation previously analyzed by this method and the results were reported in Table 2 and Table 5.

Table.1. Spectral characteristics of Nebivolol , S-amlodipine and formulation at 280 nm and 364 nm by zero order derivative method.

Parameter	Nebivolol at 280 nm	S-amlodipine at 364 nm	Formulation At 280 nm	Formulation at 364 nm
Linear Range (µg/ml)	10-60	5-30	10-60	5-30
Slope	0.011x	0.012x	0.013x	0.006x
Intercept	-0.005	0.002	-0.002	-0.001
Regression co-efficient	0.999	0.999	0.999	0.999
Standard deviation	0.00122	0.00112	0.00107	0.00105
Sandell's sensitivity	0.0392	0.0407	-	-
Limit of Detection (µg/ml)	0.16	0.17	0.13	0.11
Limit of Quantification (µg/ml)	0.5	0.53	0.41	0.363

Table.2. Determination of Accuracy of Nebivolol and S-amlodipine.

Level of % recovery	Amount of formulation(mg/tab)		Amount of standard drug added (mg)		Total amount recovered (mg)		% Recovery	
	NEB	S-AB	NEB	S-AB	NEB	S-AB	NEB	S-AB
80%	10	5	8	4	17.89	8.90	99.38	99.44
	10	5	8	4	17.90	8.87	99.44	99.31
	10	5	8	4	17.99	8.99	99.94	99.94
100%	10	5	10	5	20.02	9.98	100.1	99.90
	10	5	10	5	19.99	9.96	99.94	99.29
	10	5	10	5	19.87	9.99	99.31	99.94
120%	10	5	12	6	21.98	10.97	99.90	99.86
	10	5	12	6	21.87	10.99	99.31	99.31
	10	5	12	6	21.97	11.02	99.86	100.1

Statistical validation Data for Recovery studies.

Components	Mean* (%)	Standard Deviation*	%Relative standard deviation*	Standard Error*
NEB	99.68666	0.350243	0.349559	0.143015
S-AB	99.67667	0.331431	0.333568	0.135333

*n = 3

Table.3. Statistical validation Data for Intra-day Precision.

Components	Mean*	Standard Deviation*	% Relative standard deviation*	Standard Error*
NEB	0.4566	0.001226	0.268511	0.00050
S-AB	0.2368	0.001122	0.517146	0.00046
FORM at 280 nm	0.4476	0.001079	0.241128	0.000442
FORM at 364 nm	0.2174	0.001054	0.196025	0.000432

*n = 6

Table.4. Spectral characteristics of Nebivolol , S-amlodipine and formulation at 294 nm and 279.7 nm by first order derivative method.

Parameter	**Nebivolol at 294 nm**	**S-amlodipine at 279.7 nm**	**Formulation At 294 nm**	***Formulation at 279.7 nm***
Linear Range (µg/ml)	10-60	5-30	10-60	*5-30*
Slope	-0.0584x	-0.020x	-0.0013x	*-0.006x*
Intercept	-0.0066	-0.004	0.002	*0.001*
Regression co-efficient	0.9998	0.999	0.999	*0.99*
Standard deviation	0.000512	0.0006837	0.0005564	*0.000606*
Sandell's sensitivity	-0.7335	-1.222	-	-
Limit of Detection (µg/ml)	0.0027	0.0046	0.0025	*0.0043*
Limit of Quantification (µg/ml)	*0.0083*	*0.0140*	*0.0080*	*0.0137*

Table: 5. Determination of Accuracy of Nebivolol and S-amlodipine.

Level of % recovery	Amount present (µg/2 tab)		Amount of standard drug added (µg)		Total amount recovered (µg)		% Recovery	
	NH	S-AB	NH	S-AB	NH	S-AB	NH	S-AB
80%	10	5	8	4	17.91	8.85	99.4	98.3
	10	5	8	4	17.79	8.89	98.6	98.7
	10	5	8	4	17.89	8.98	99.3	99.7
100%	10	5	10	5	19.89	9.95	99.3	99.5
	10	5	10	5	19.83	9.98	99.0	99.8
	10	5	10	5	19.85	9.89	99.1	98.9
120%	10	5	12	6	21.95	10.97	98.6	99.7
	10	5	12	6	21.88	10.86	99.3	98.7
	10	5	12	6	21.89	10.93	99.4	99.36

Statistical validation Data for Recovery studies.

Components	Mean* (%)	Standard Deviation*	% Relative standard deviation*	Standard Error*
NEB	99.77889	0.581032	0.582086	0.237253
S-AB	98.75469	0.915041	0.916275	0.373638

*n = 3

Table.6. Statistical validation Data for Intra-day Precision.

Components	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
NH	100.10	0.9350	0.9340	0.3818
S-AB	99.99	0.5729	0.5721	0.2339
Formu at 294 nm	99.89	0.9187	0.9154	0.3743
Formu at 279.7 nm	98.98	0.4827	0.4821	0.2271

*n = 6

CONCLUSION

For routine analytical purpose it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision. Chromatographic technique coupled with multivariate algorithms can generate large amount of quality data which serve as highly powerful and convenient analytical tool. In view of the need for a suitable method for routine analysis of Nebivolol and S-amlodipine in bulk and formulation, in the present work, an attempt was made to develop a newer, simple, accurate, precise and economic two spectrophotometric methods.

The methods was developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence, it can be concluded that the developed two spectrophotometric methods, zero order derivative and first order derivative methods are new, simple, accurate, precise and economic and can be employed successfully for the estimation of Nebivolol and S-amlodipine in bulk and formulation.

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