Int J Pharm Bio Sci 2013 July; 4(3): (P) 379 - 391

Research Article **Analytical Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

DEVELOPMENT AND VALIDATION OF DERIVATIVE SPECTOSCOPIC METHOD FOR SIMULTANEOUS DETERMINATION OF NEBIVOLOL HYDROCHLORIDE AND S-AMLODIPINE BESYLATE IN COMBINED DOSAGE FORM

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ABSTRACT

A novel, simple, accurate, sensitive, reproducible, economical spectroscopic method has been developed and validated for the determination of Nebivolol hydrochloride and S-amlodipine besylate in combined dosage form. The method obeys Beer's Law in concentration range of 15-35 µg/ml for Nebivolol and 7.5-17.5 µg/ml for S-amlodipine. The method was validated for linearity, range, accuracy, precision and specificity as per ICH guidelines. Zero crossing point for Nebivolol hydrochloride and S-amlodipine besylate was 243 nm and 291 nm respectively in methanol. The developed method as successfully used for the quantitative analysis of commercially available dosage form (Nebicard SM).

KEYWORDS: Nebivolol hydrochloride, S-amlodipine besylate, Derivative Spectroscopy, Zero crossing point, combined dosage form.

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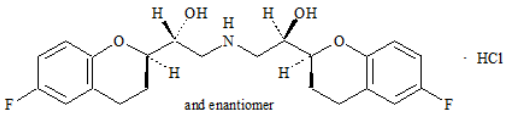
INTRODUCTION

Nebivolol hydrochloride [1-12]

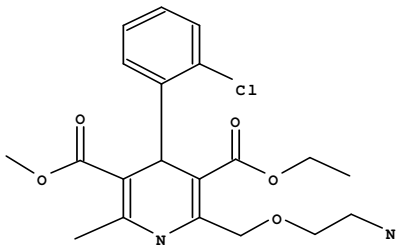
Nebivolol is α,α,[Imino bis (methylene)] bis[6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol]. Nebivolol is a β_1-adrenoceptor blocking drug that possesses certain unusual pharmacological properties by which it differs from conventional β_1-blockers in its chemical structure and in its hemodynamic profile. Nebivolol is a racemic mixture of two enantiomers in equal ratios. The D-isomer is a potent, highly selective and long acting β_1-adrenoceptor blocking agent whose hemodynamic profile resembles that of Atenolol and other usual β_1-blockers. Nebivolol is a competitive and selective β_1- adrenoceptor antagonist which attributed to the D-Nebivolol and has mild visodilating properties due to an interaction with L-arginine/nitric oxide pathway. In humans Nebivolol lowers blood pressure acutely and reduces vascular peripheral resistance, without compromising left ventricular function, but increasing stroke volume. These effects contrast those caused by traditional β-blockers. The antihypertensive activity of Nebivolol may be attributed to its β-antagonistic properties. Nebivolol improves the heart filling mechanisms and overall left ventricular performance. In patients with essential hypertension, Nebivolol 5mg daily for 1 month significantly decreases both systolic and diastolic blood pressure, lowers the mean blood pressure, significantly decreases peripheral vascular resistance, maintains or increases cardiac output, but does not modify pulmonary artery pressure.



Nebivolol hydrochloride

S-amlodipine besylate [13-19]

S-amlodipine is 3-ethyl-5-1-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylate. Optically pure S (-) isomer of amlodipine is the effective antihypertensive agent for both systolic and diastolic hypertension, particularly in mild to moderate hypertension and angina. It avoids the adverse effects such as headache, edema, dizziness, flushing, palpitation, fatigue, nausea, and somnolence associated with the administration of the racemic mixture of amlodipine. It is reported that utilization of optically pure S (-) isomer of amlodipine results in clearer dose-related definitions of efficacy, diminished adverse effects, and accordingly an improved therapeutic index. It is therefore more desirable to use the optically pure S (-) isomer of amlodipine. The optically pure S enantiomer is substantially free of R enantiomer. R enantiomer lacks or has a lower level of antihypertensive activity.



Benzene sulphonic acid salt

S-amlodipine besylate

The aim of present work is to develop analytical method for combined fixed dose formulation of Nebivolol hydrochloride with S-amlodipine besylate which is novel to the market. The formulation is novel and both drugs having immediate release fractions. The formulation of β-blockers and amlodipine is common in the Indian drug market, e.g. Atenolol with amlodipine. The formulation of Nebivolol hydrochloride and S-amlodipine besylate is novel to the drug market and no analytical method(s) are reported for the simultaneous determination of these two drugs in their combined dosage forms. Literature survey reveals that Methods were reported for Nebivolol alone or combination with other drugs [20-23]. Spectrophotometry [24-45], HPLC [46-58], HPTLC[59-63] ,Electrophoresis [64-68] methods were reported for Amlodipine alone or combination with other drugs. In this study, a simple, precise and convenient first derivative spectrophotometric method was developed and validated for its application in the simultaneous determination of Nebivolol hydrochloride and S-amlodipine besylate in their combined dosage forms. The method suggested was reported to have no interference of any common excipients. First derivative spectrophotometric method is fast and quick for the quality control release of the dosage form.

MATERIALS AND METHODS

Materials

Nebivolol hydrochloride (NEB) and S-amlodipine besylate (SAM) were supplied by Torrent Research Center (Gandhinagar, India). Tablets of Nebicard SM supplied by Torrent Research Center (Gandhi agar, India).Methanol (HPLC Grade, Ranchem) used as solvent.

Instruments

JASCO V-550 UV/VIS spectrophotometer having quartz cell with 1cm path length was used for the spectroscopic analysis. Mettler Toledo AX balance used for weighing.

Preparation of Standard Solutions

Stock solution of Nebivolol hydrochloride

27.25 mg of Nebivolol hydrochloride equivalent to about 25 mg of Nebivolol was weighed accurately and transferred to 100ml volumetric flask. 70 ml of methanol was added and sonicated to dissolve. The volume was made up to the mark with methanol (250 µg/ml of Nebivolol).

Stock solution of S-amlodipine besylate

34.67 mg of S-amlodipine besylate equivalent to about 25 mg of S-amlodipine was weighed accurately and transferred to 200ml volumetric flask. 150 ml of methanol was added and sonicated to dissolve. The volume was made up to the mark with methanol (125 µg/ml of S-amlodipine).

Determination of Absorption Maxima

Solutions of both drugs were prepared in methanol having concentration of 25 µg/ml for NEB and 12.5 µg/ml for SAM .The mixed solution of both drugs in same concentration was also prepared. First of all the three solutions were scanned between 200-400 nm by keeping methanol as a blank to determine the wavelength of maximum absorption for both drugs.

Derivative Spectroscopy

The Spectra in Fig.1 and Fig.2 revel that no method was possible in zero order. Derivative spectroscopic method was tried as an option for the simultaneous determination of both. The zero order spectra of both drugs (Fig.1 & Fig.2) were converted to first derivative spectra (Fig.3 & Fig.4) and overlapped to find out the zero crossover of both drugs. The ZCO for NEB 243 nm and 291 nm and for SAM

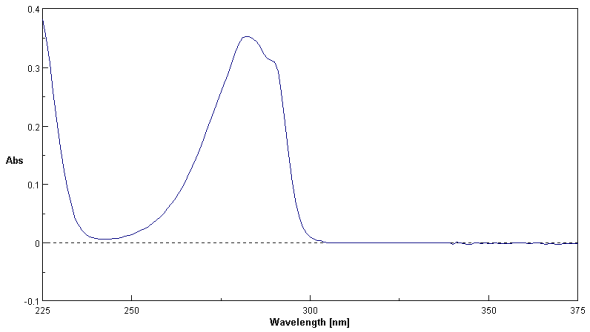


Figure 1: Zero order spectrum of NEB

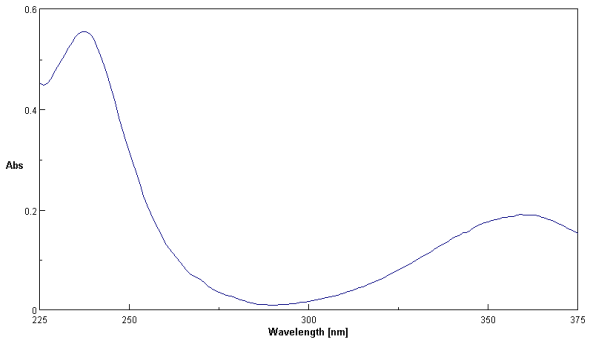


Figure 2: Zero order spectrum of SAM

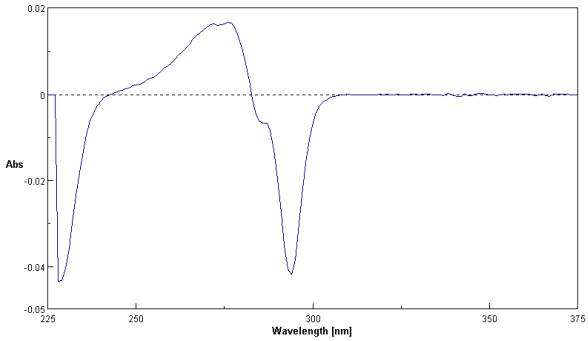


Figure 3: First order spectrum of NEB

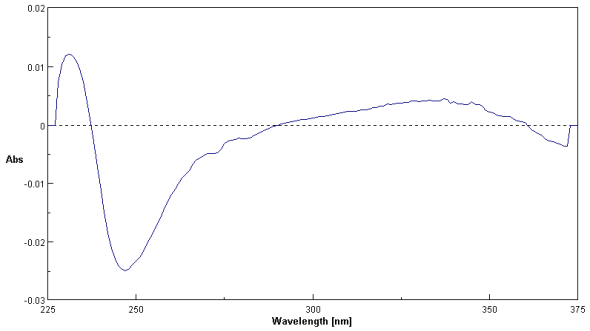


Figure 4: First order spectrum of SAM

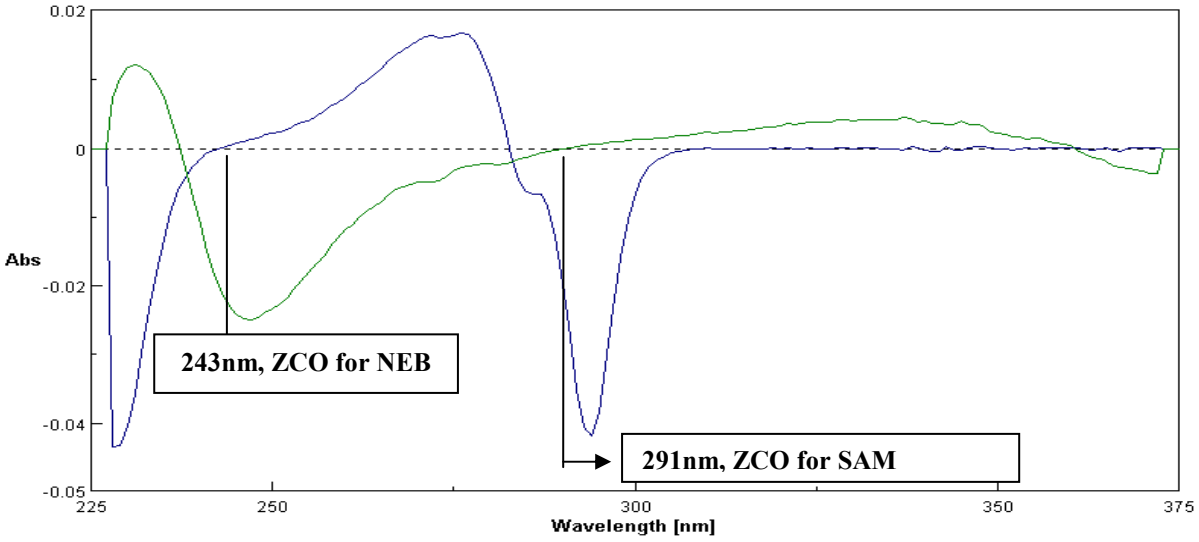


Figure 5: Overlaid first derivative spectra of NEB and SAM

Zero cross over (ZCO) point was defined as a particular wavelength at which one component has a response (positive or negative) while the response of other drug is zero. At ZCO we can measure the response of one component while the response of other remains zero, but for simultaneous determination of both drugs there must be two ZCOs at which one can be quantified while the response of the other would remain zero. In this case when two first derivative responses were overlapped following ZCO points were selected as shown in fig.5. The ZCO of both drugs were evaluated at various concentration levels of each drug. Wavelengths selected for quantitation were 291 nm for NEB (ZCO for SAM) and 243 nm for SAM (ZCO for NEB).

The selection of ZCO was evaluated by preparing various solutions containing different concentrations of each component. The derivative response of each drug at ZCO point was derived and plotted against respective concentration. The regression equation and correlation coefficients were derived from this data. They suggest no interference of one drug at ZCO point of other drug at all concentration levels (Table 1). Series E was selected as a calibration tool for the UV spectrophotometer. The standard preparation was selected at a concentration level of 25 μg/ml for NEB and 12.5 μg/ml for SAM.

Validation

Method was validated according to ICH Guidelines. According to ICH Guidelines for the assay method was validated with respect to linearity, range, precision, accuracy, and specificity.

Linearity

Linearity of concentration versus first derivative response was plotted in various series (A-E). The stock solutions were diluted in the following concentration range, and data were evaluated by regression analysis. Data suggested no interference of one drug at ZCO point of other drug at all concentration levels. Data related to regression analysis are tabulated in table 1.

Table 1

Data related to various calibration curves for NEB and SAM.

Series	Concentration (μg/ml)		Regression Equation	Correlation coefficient
	NEB	SAM		
A	15-35	0	y = 0.0012x-0.0009 (NEB)	0.9995
B	0	7.5-17.5	y = 0.0017x+0.0003 (SAM)	0.9998
C	15-35	12.5	y = 0.0012x-0.0009 (NEB)	0.9995
D	25	7.5-17.5	y = 0.0017x-0.0004 (SAM)	0.9996
E	15-35	7.5-17.5	y = 0.0011x-0.0012 (NEB)	0.9995
			y = 0.0017x+0.0002 (SAM)	0.9999

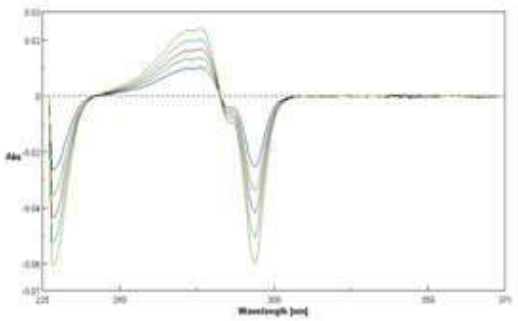


Figure 6: Series - A (NEB)

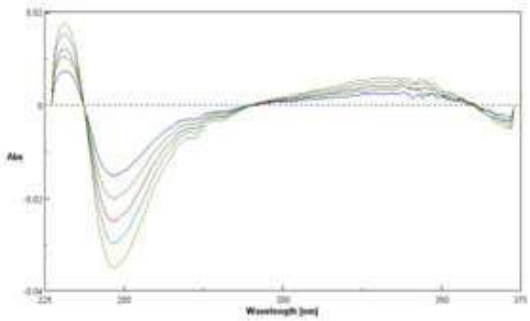


Figure 7: Series - B (SAM)

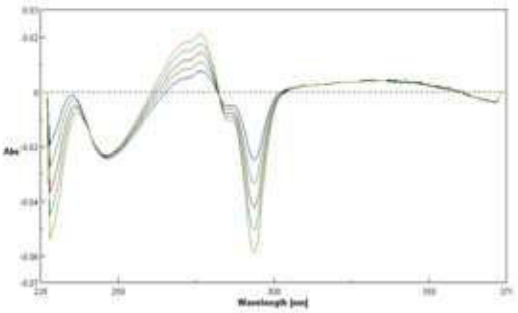


Figure 8: Series-C (Linearity of NEB, where SAM is constant)

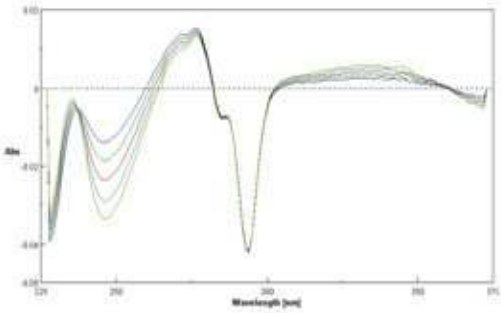


Figure 9: Series-D (Linearity of SAM, where NEB is constant)

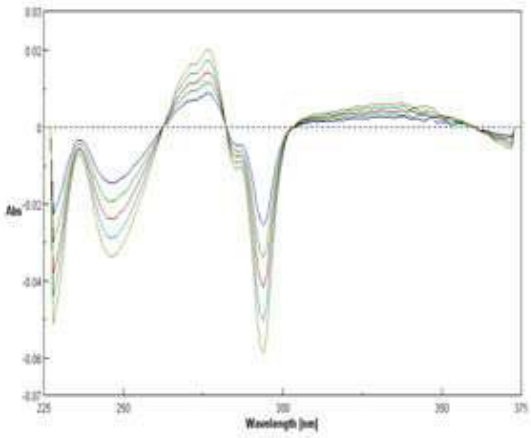


Figure 10: Series-E (Linearity of NEB and SAM)

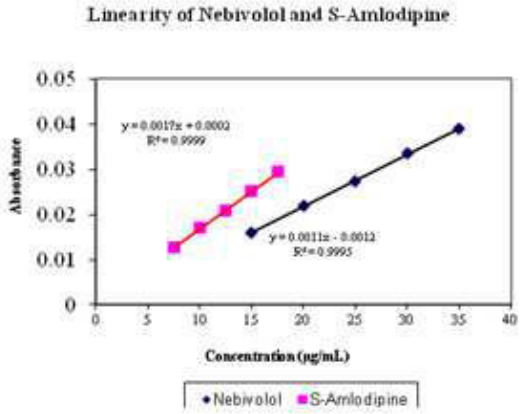


Figure 11: Linearity graph of NEB and SAM

The regression analysis of various series suggested that the FDS response was linear for both components in various concentration ranges. Fig.11 indicates the linearity graph of series E suggesting, R^2 of 0.999 for NEB and 0.999 for SAM.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of same homogeneous sample under the prescribed conditions.

System Precision

System precision was evaluated by analyzing the series E for three times. All the data were compared and % relative standard deviations were calculated.

Table 2

System precision data for NEB and SAM (n=3)

NEB (µg/ml)	Mean Absorbance for NEB	%RSD	SAM (µg/ml)	Mean Absorbance for SAM	%RSD
15	0.01621	0.76	7.5	0.01277	0.71
20	0.02128	0.14	10	0.01702	0.89
25	0.02718	0.15	12.5	0.02100	0.61
30	0.03346	0.12	15	0.02526	0.64
35	0.03891	0.35	17.5	0.02939	0.19

Method Precision

It was evaluated by analyzing the sample of same batch for six times. Data suggests high degree of method precision.

Table 3
Method precision data for NEB and SAM

No.	NEB	SAM
1	99.9	101.8
2	100.5	101.7
3	99.9	101.2
4	99.8	101.8
5	100.1	101.4
6	100.6	101.2
Mean	**100.1**	**101.5**
%RSD	**0.34**	**0.28**

Intermediate Precision

It was evaluated by analyzing the sample of by different analyst, on different day and using different instruments.

Table 4
Intermediate precision data for NEB and SAM

Analyst Change		
	%Assay	
	NEB	**SAM**
Analyst-I	100.9	101.0
Analyst-II	101.2	100.7
Mean	**101.1**	**100.9**
%RSD	**0.21**	**0.21**
Day Change		
	%Assay	
	NEB	**SAM**
Day-I	101.2	100.6
Day-II	100.8	100.4
Mean	**101.0**	**100.5**
%RSD	**0.28**	**0.14**
System Change		
	%Assay	
	NEB	**SAM**
System-I	101.7	100.4
System-II	101.0	99.8
Mean	**101.4**	**100.1**
%RSD	**0.49**	**0.42**

Specificity

Specificity of the method was evaluated by checking the interference of placebo. Placebo weight in the particular tablet was derived, by subtracting the weight of drugs from the average weight of the tablet and treated as per the procedure followed for the test sample. No absorbance observed at the wavelength for NEB and SAM.

Accuracy

To ensure the reliability and accuracy of method, the recovery studies were carried out by adding known quantity of drug with sample preparation. Accuracy of the method was determined at 3 different concentration levels of each component by the standard addition method.

Table 5
Accuracy data for NEB and SAM

	Parameter	Accuracy		
	Drug	**NEB**		
No.	**Level of Recovery**	**Amount of Drug Added (µg/ml)**	**Amount of Drug Found (µg/ml)**	**%Recovery**
1.	Sample Preparation (80%)	20.19	20.15	99.8
2.	Sample Preparation (100%)	25.24	25.04	99.2
3.	Sample Preparation (120%)	30.29	30.82	101.7
	Drug	**SAM**		
No.	**Level of Recovery**	**Amount of Drug Added (µg/ml)**	**Amount of Drug Found (µg/ml)**	**%Recovery**
1.	Sample Preparation (80%)	9.98	10.11	101.3
2.	Sample Preparation (100%)	12.48	12.48	100.0
3.	Sample Preparation (120%)	14.98	14.99	100.1

Assay

Preparation of Standard Solutions

Stock solution of Nebivolol hydrochloride

27.25 mg of Nebivolol hydrochloride equivalent to about 25 NEB was weighed accurately and transferred to 100ml volumetric flask. 70 ml of methanol was added and sonicated to dissolve. The volume was made up to the mark with methanol (250 µg/ml of NEB).

Stock solution of S-amlodipine besylate

34.67 mg of S-amlodipine besylate equivalent to about 25 mg of SAM was weighed accurately and transferred to 200ml volumetric flask. 150 ml of methanol was added and sonicated to dissolve. The volume was made up to the mark with methanol (125 µg/ml of SAM).

Working standard solution

5 ml of each stock solution were transfered in 50 ml volumetric flask and volume made to 50 ml with methanol (25 µg/ml of NEB and 12.5 µg/ml of SAM).

Preparation of Sample Solution

Twenty tablets of the combined dosage form were weighed and average weight was calculated. Five tablets (equivalent to 25.0 mg of NEB and 12.5 mg of SAM respectively) were weighed and transferred in to 500 ml volumetric flask, 350 ml of methanol was added and sonicated for 60 minutes. After cooling, the volume was made up to the mark with methanol and 10 ml was filtered through 0.45µ Millipore filter. 5ml of filtrate was diluted to 10ml with methanol to get the final concentration of 25 µg/ml of NEB and 12.5 µg/ml of SAM. This solution was used to get the first derivative test spectra.

Calculation for Assay

$$\%\ \text{Assay}_{(\text{Nebivolol})} = \frac{(\text{Test ABS} \times \text{Standard Concentration} \times \text{Assay of Standard})}{(\text{Standard ABS} \times \text{Sample Concentration})}$$

$$\%\ \text{Assay}_{(\text{S-Amlodipine})} = \frac{(\text{Test ABS} \times \text{Standard Concentration} \times \text{Assay of Standard})}{(\text{Standard ABS} \times \text{Sample Concentration})}$$

Convert standard concentration equivalent to Nebivolol and S-amlodipine using equivalency.

Equivalency: 441.94mg of Nebivolol hydrochloride ≈ 405.48mg of Nebivolol
567.1mg of S-amlodipine besylate ≈ 408.9mg of S-amlodipine

Convert standard concentration equivalent to Nebivolol and S-amlodipine using equivalency.

Where Test ABS and Std. ABS represent absorbance of Nebivolol hydrochloride at 291 nm, and S-amlodipine besylate at 243 nm, in test and standard preparation.

RESULTS AND DISCUSSION

In this study a simple, precise, accurate and convenient first derivative spectroscopic method was developed and validated for its application in the simultaneous determination of NEB and SAM in their combined dosage form. In first order derivative spectroscopy, wavelengths selected for quantitation were 291 nm for NEB (ZCO for SAM) and 243 nm for SAM (ZCO for NEB).Both drugs follow linearity with the concentration range (NEB: 15-35 µg/ml , SAM: 7.5-17.5 µg/ml) with R^2 value of 0.999 for NEB and 0.999 for SAM (Table 1). The percentage RSD was found the range of 0.12-0.76 for NEB and 0.19-0.89 for SAM in system precision (Table 2). The percentage RSD was found 0.34 for NEB and 0.28 for SAM in method precision (Table 3). The percentage RSD was found the range of 0.21-0.49 for NEB and 0.14-0.42 for SAM in intermediate precision (Table 4). The mean % recovery found to be 100.2% for NEB and 100.5% for SAM (Table 5).Method is specific as placebo gave no interference with the determination of both drugs. The mean % assay was found to be 100.1% for NEB and 101.5% for SAM. The overall results of various validation parameters were summarized in table 6.

Table 6
RESULT

Parameter	Nebivolol	S-amlodipine
Wavelength	291 nm	243 nm
Zero crossing point	243 nm	291 nm
Range (µg/ml)	15-35	7.5-17.5
Linearity (R^2)	0.9997	0.9999
Precision (% RSD)		
1)System precision	0.12-0.76	0.19-0.89
2)Method precision	0.34	0.28
3)Intermediate precision	0.21-0.49	0.14-0.42
Accuracy (% Recovery)	100.2	100.5

CONCLUSION

The developed method is novel, simple, accurate, sensitive, reproducible, economical which would be used for the determination of Nebivolol hydrochloride and S-amlodipine besylate in combined dosage form.

ACKNOWLEDGEMENT

The authors are highly thankful to Torrent Research Centre, Gandhinagar, India and Shree S.K.Patel college of Pharmaceutical education and research for providing samples and facilities to complete the work.

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