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Full Length Original Research Paper

Analytical method development and Validation of Amlodipine Besylate and Nebivolol in their Dosage Form

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Abstract:

This work is concerned with application of simple, economical, precise, accurate and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Nebivolol hydrochloride(NBH) and S-Amlodipine besylate (AMB) on thermo Hypersil C18 (Thermo Electron Corporation) (250 x 4.6 mm, 5 µm) using Acetonitrile and Phosphate buffer (pH 2.5 ± 0.1) in ratio of 60:40 as mobile phase. pH of buffer adjusted to 2.5 ± 0.1 with O-phosphoric acid. The flow rate was adjusted at 1.0 ml/min and the detection wavelength was 271 nm. The retention time for AMB and NBH was found to be 6.39 and 7.54 min, respectively. Proposed method was validated for precision, accuracy, linearity range, robustness and ruggedness.

Keywords: Nibivolol, S- Amlodipine besylate, Reverse Phase High Performance Liquid Chromatography

Introduction

Nebivolol hydrochloride[1-2] (NBH) is chemically , a,a -(imino bis (methy- lene)) bis(6-fluoro-3,4-dihydro-2 H -1-benzopyran-2- methanol) hydrochloride which is a highly selective β1 receptor antagonist without partial agonist activity. It is official in Martindale, the extra pharmacopeia. Amlodipine besylate[3] (AMB) is chemically R,S-2-((2- aminoethoxy) methyl)-4- (2-chloroethyl)-3- ethoxy carbonyl-5- methoxy carbonyl- 6-methyl- 1,4- dihydro pyridine benzene sulphonate used in the treatment of hypertension and congestive heart failure. It is official in British pharmacopoeia [4]. Many methods have been described in the literature for the determination of NBH and AMB individually and in combination with other drugs. [5-18] So far, Fixed dose combination containing NBH (5 mg) and AMB (2.5 mg) is available in the tablet form in the market. The aim of this work was to develop an RP-HPLC method with ultraviolet detection for the simultaneous determination of NBH and AMB in pharmaceutical dosage forms. The present study describes a precise, accurate, specific and sensitive RP-HPLC method as per ICH guidelines for the simultaneous estimation of NBH and AMB in tablets.[19]

Materials and Methods:

Chemicals and Reagents

Acetonitrile HPLC grade was procured from Finar chemicals limited, Ahmedabad India. Working standard of NBH and AMB was provided by Drakt Pharmaceutical Ltd, Vadodara. Potassium di hydrogen phosphate, triethyl amine and o-phosphoric acid were A.R grade from Finar chemicals limited, Ahmedabad India.

Water HPLC grade was obtained from a Milli-Q RO water purification system. Tablets of two different brands, NEBICARD-SM and NEBISTAR-SA having combination of NBH (5 mg) and AMB (2.5 mg) were used.

Instrumentation

A Gradient HPLC system is used of LC-2010 CHT Shimadzu Corporation 2000, JAPAN. The HPLC system was equipped with LC solution software for data processing.

Selection of Chromatographic Condition

The mobile phase containing Acetonitrile and Phosphate buffer (60:40), phosphate buffer was prepared by 2.72 g of KH_2PO_4 was accurately weighed on electronic weighing balance and dissolved in some quantity of Milli-Q water in a 1000 mL volumetric flask, and then volume was made up to mark with water. Add 2 mL triethylamine per liter of buffer and adjust the pH to 2.5 ± 0.1 with orthophosphoric acid using pH-meter. Acetonitrile and buffer was filtered separately through nylon 0.45µm membrane filter and filled in separate bottle, the mobile phase was delivered to the column using two pumps.

The flow rate was set to 1.0mL/min. Both drugs showed good absorbance at 271 nm, which was selected as wavelength for further analysis. All determinations were performed at constant column temperature (25 ± 2ºC).In order to optimize the LC separation of NBH and AMB, initially, wavelength of 271nm was selected for the UV detection because at this wavelength there was maximum overlap of the spectra of NBH and AMB. Nylon filter 0.45µm was selected for sample filtering as very good sample condition was observed against other filters. The buffer solution of pH 2.5 ± 0.1 and mobile phase composition of Acetonitrile and Phosphate buffer (60:40) was found most appropriate for separation of NBH and AMB on Thermo Hypersil C18 (250*4.6) mm, 5µm column. Flow rate of 1.0mL min-1 selected based on capacity factor and column efficiency. NBH and AMB were well resolved in reasonable time of 10 minutes. The retention times of AMB and NBH were 6.39 min and 7.54 min, respectively.

Preparation of Stock Solutions

Accurately weighed NBH (50 mg) and AMB (25 mg) were transferred to a 100 mL volumetric flask and dissolved in mobile phase and then diluted to the mark with mobile phase to obtain a standard stock solution of NBH (500µg/mL) and AMB (250µg/mL). Appropriate aliquots from standard NBH and AMB stock solutions were transferred to series volumetric flasks of 100 mL capacity. The volume was adjusted to the mark with mobile phase to give a solution containing 5, 10, 15, 20 and 25 µg/mL NBH and 2.5, 5, 7.5, 10 and 12.5µg/mL AMB, respectively. The mixed standard solutions were prepared in the ratio of 2:1 for NBH and AMB as marketed formulations are available in this ratio.

Sample Preparation

To determine the content of NBH and AMB simultaneously in conventional tablets (label claim: 5mg Nebivolol hydrochloride and 2.5 mg S-Amlodipine besylate per tablet, combination tablet containing both analytes), the twenty tablets were weighed, their mean weight determined and they were finely powdered and powder equivalent to 25 mg NBH and 12.5 mg AMB was weighed. Then equivalent weight of the drug was transferred into a 50 ml volumetric flask containing 25 ml mobile phase, sonicated for 15 min and diluted to 50 ml with mobile phase to

obtain solution of NBH (500µg/mL) and AMB (250µg/mL). The mixture was filtered using 0.45 µm nylon membrane filter. A 3 mL aliquot of test solution was transferred to a 100 mL volumetric flask, and the contents of the flask were diluted to volume with mobile phase to obtain concentrations of 15 µg/mL NBH and 7.5 µg/mL AMB.

The solution was analyzed under optimized chromatographic conditions and chromatogram is depicted in figure no.2.

Method Validation

The proposed HPLC method was validated as per ICH guidelines.

Specificity

The specificity of NBH and AMB was noticed in presence of tablet excipients. In addition there was no any interference at the retention time of NBH and AMB in the chromatogram of placebo solution.

The specificity (selectivity) of the method was checked by a comparison of the chromatograms obtained from samples and the corresponding placebo. Additives in tablets are practically insoluble in methanol or the mobile phase, whereas the active constituents are freely soluble. No interference from additives was obtained.

Result is shown in figure 1, 2.

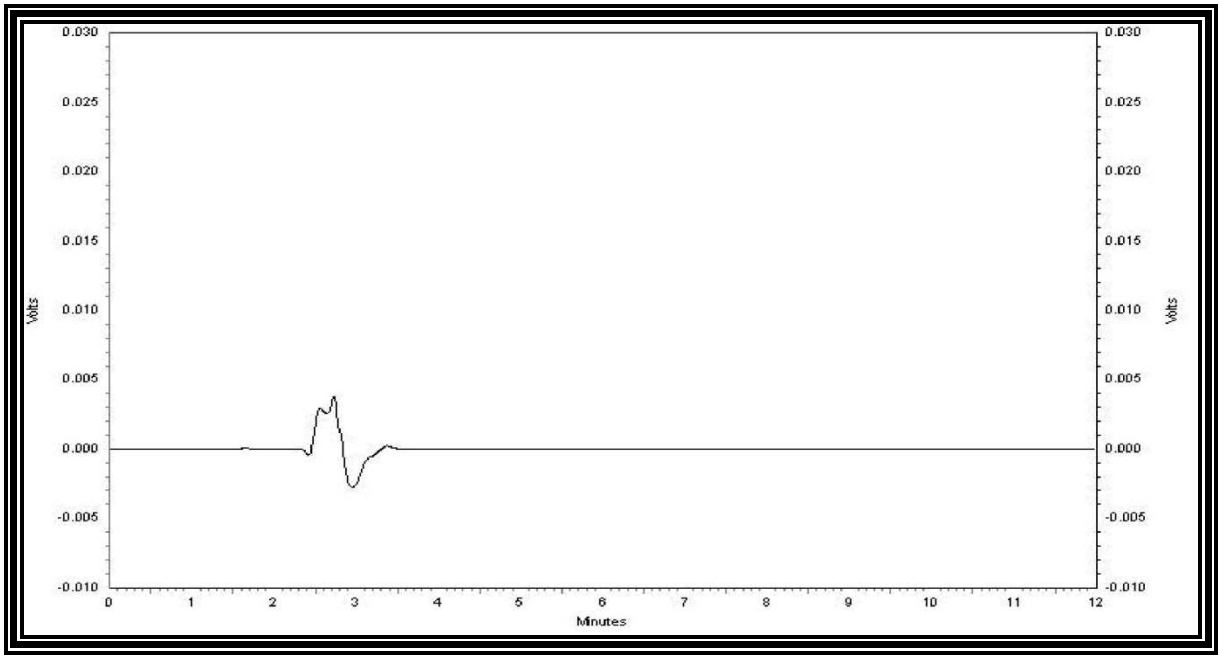

Figure 1: Chromatogram of placebo

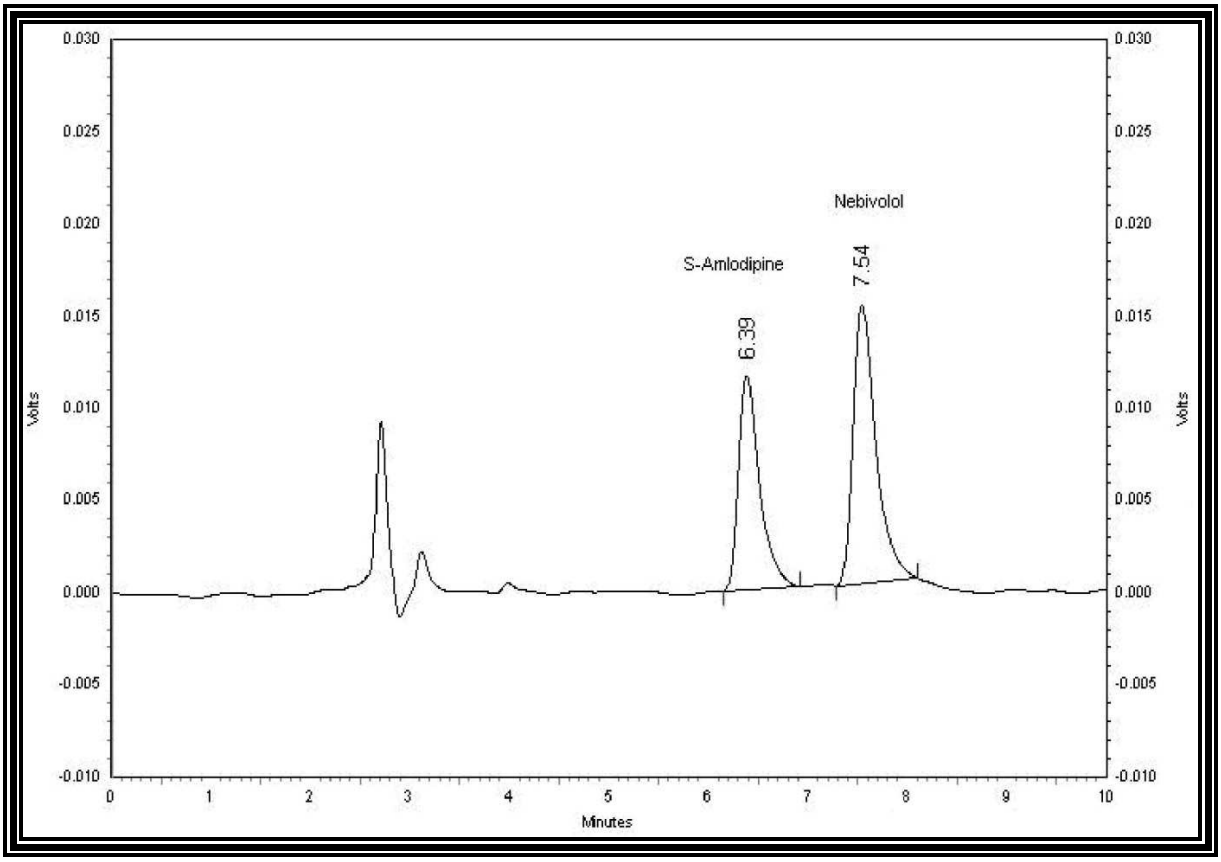

Figure 2: A typical chromatogram of a tablet sample solution containing 15µg/ml of NBH and 7.5 µg/ml of AMB.

Linearity and Range

Calibration curves were plotted over a concentration range of 5-25 µg/mL and 2.5-12.5 µg/mL for NBH and AMB, respectively. Accurately measured standard working solutions of NBH and AMB (1.0, 2.0, 3.0, 4.0, and 5.0 mL) were transferred into a series of 100 mL volumetric flasks and diluted to the mark with the mobile phase. 20 µL of each solution was injected under operating conditions previously described.

Calibration plots were constructed by plotting peak area against the corresponding concentration of each drug. Each reading was the average of 3 determinations.

Results are shown in Table 1, 2, 3 and figure 3, 4.

Table 1: Result of calibration curve for NBH

Concentration (µg/mL)	Peak area Mean ± Std. Deviation (n=3)
5	158335 ± 4940.24
10	281664 ± 5446.35
15	397323 ± 4996.22
20	508170 ± 5118.55
25	624291 ± 5818.68

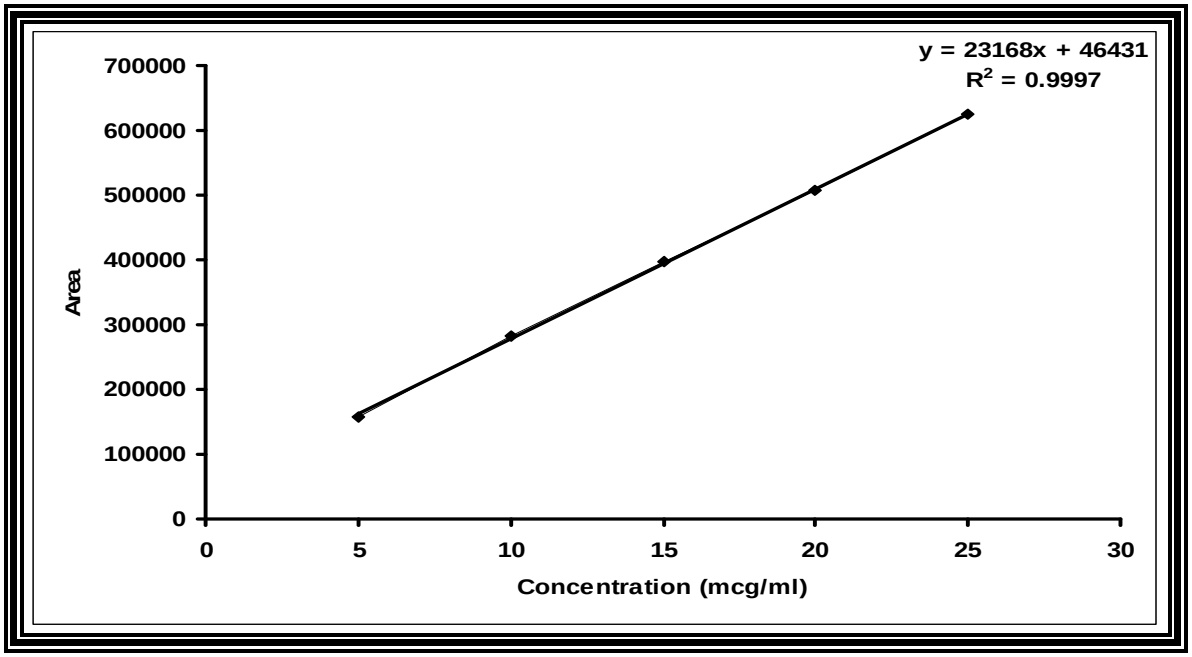

Figure 3: Calibration curve of NBH

Table 2: Result of calibration curve for AMB

Concentration (µg/mL)	Peak Area Mean ± Std. Deviation (n=3)
2.5	95196 ± 576.77
5	181285 ± 5437.59
7.5	274019 ± 5123.36
10	359449 ± 2982.74
12.5	460984 ± 4111.77

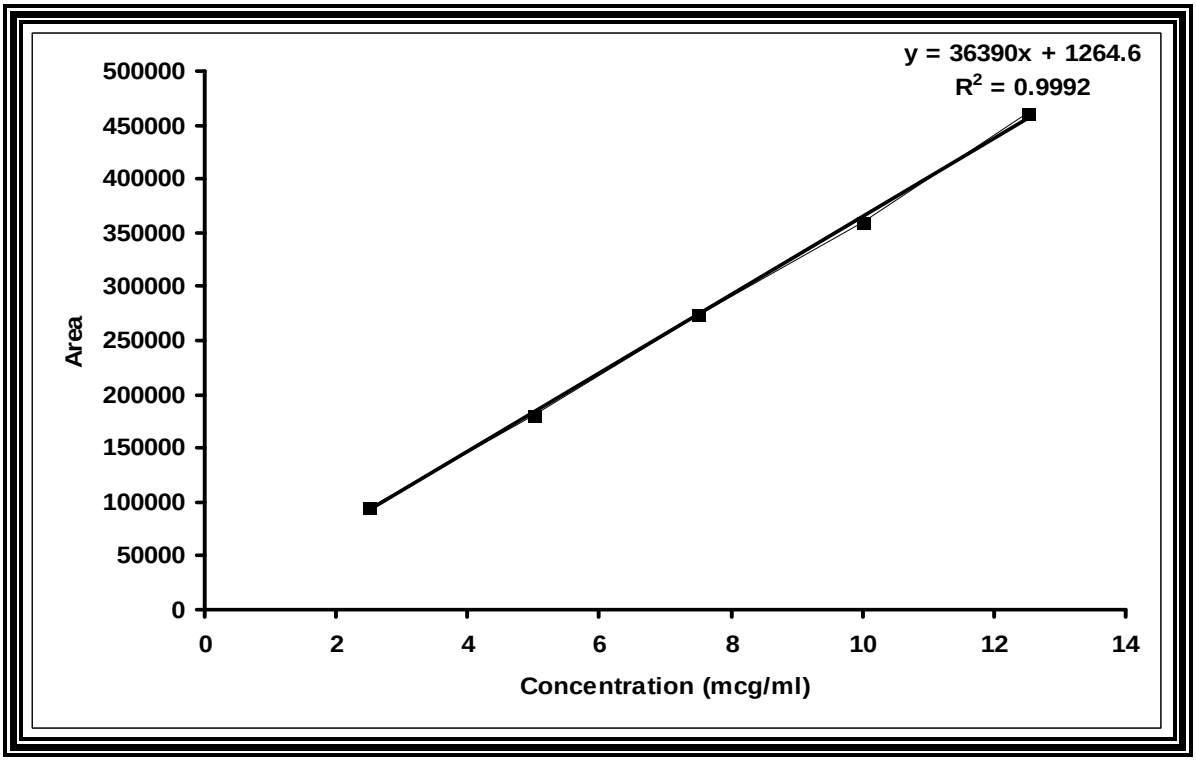

Figure 4: Calibration curve of AMB

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Table 3: Statistical data of NBH and AMB by RP-HPLC method

Parameter	NBH	AMB
Linear Range (µg/mL)	5-25	2.5-12.5
Slope	23168	36390
Intercept	46431	1264.4
Standard deviation of slope	193.58	245.19
Standard deviation of intercept	1176.35	2675.25
Limit of Detection (µg/mL)	0.74	0.33
Limit of Quantification (µg/mL)	2.27	1

Precision

System precision was evaluated by analyzing the standard solution 5 times and method precision (repeatability) was evaluated by assaying 6 sample sets of test solution on the same day (intraday). System precision and method precision were also evaluated by performing the same procedure on a different day (interday) under the same experimental conditions (intermediate precision).

Results are shown in Table 4.

Table 4: System Precision data for NBH and AMB (n=6)

NBH (15 µg/mL)			AMB(7.5 µg/mL)		
Parameter	Intra day	Inter day	Parameter	Intra day	Inter day
Average area	393154	393641	Average area	273063	272965
% Assay	99.77	99.91	% Assay	99.59	99.55
S.D	2805.72	2706.08	S.D	2432.15	2242.40
R.S.D	0.71	0.64	R.S.D	0.89	0.82

Accuracy (Recovery studies)

The accuracy of the method was determined by use of standard additions at three different levels, i.e. multiple level recovery studies. Sample stock solution of tablet formulation containing 10 µg/mL and 5 µg/mL for NBH and AMB was prepared. This solution was spiked with 50%, 100%, and 150% of the standard drug solutions and percentage recoveries were determined.

Results are shown in Table 5.

Table 5: Determination of Accuracy for marketed formulations

Component	Initial amount (µg)	Amount added (%)	Amount recovered (µg)	Recovery (%)	%R.S.D
NBH	10	50	4.98	99.75	0.59
	10	100	10.01	100.06	0.48
	10	150	15.03	100.17	0.96
AMB	5	50	2.50	100.13	1.08
	5	100	4.97	99.42	0.82
	5	150	7.50	99.97	1.02

Robustness of method

Robustness of the method was evaluated by introducing small deliberate changes assaying test solutions under slight but deliberate changes in analytical conditions, such as a change in flow rate (±0.1 mL/min), a change in buffer pH (± 0.2 unit), a change in the proportions of the components of the mobile phase, i.e., buffer: acetonitrile (61: 39 and 59: 41, v/v), the use of different lots of LC columns, and increasing the temperature of the column by 5°C.

Results of robustness studies are shown in Table 6, 7.

Table 6: Data obtained for AMB in the robustness study

System suitability parameter			
Robust condition	Assay, %	No. of Theoretical plates	Asymmetry
Flow, 0.9 mL/min	99.80	4571	1.18
Flow, 1.1 mL/min	99.94	4628	1.15
Buffer, pH 2.48	100.01	4753	1.17
Buffer, pH 2.52	99.85	4719	1.20
Buffer: Acetonitrile (61:39, v/v)	99.38	4460	1.18
Buffer: Acetonitrile (59:41, v/v)	99.72	4751	1.15
Oven temperature, 30°C	99.86	4693	1.20

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Table 7: Data obtained for NBH in the robustness study

System suitability parameter			
Robust condition	Assay, %	No. of Theoretical plates	Asymmetry
Flow,0.9 mL/min	99.69	4571	1.18
Flow,1.1 mL/min	99.84	4628	1.17
Buffer, pH 2.48	100.33	4753	1.18
Buffer, pH 2.52	99.64	4719	1.22
Buffer: Acetonitrile (61:39, v/v)	99.07	4460	1.21
Buffer: Acetonitrile (59:41, v/v)	100.30	4751	1.26
Oven temperature, 30°C	99.95	4693	1.19

Results and Discussion

To develop a precise, accurate and suitable RPHPLC method for the simultaneous estimation of NBH and AMB, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The results obtained by the validation of the combined formulation are summarized in Table 8. System suitability tests were carried out as per ICH guidelines are summarized in Table.9.

Table 8: Determination of Accuracy for marketed formulations

Component	Initial amount (µg)	Amount added (%)	Amount recovered (µg)	Recovery (%)	%R.S.D
NBH	10	50	4.98	99.75	0.59
	10	100	10.01	100.06	0.48
	10	150	15.03	100.17	0.96
AMB	5	50	2.50	100.13	1.08
	5	100	4.97	99.42	0.82
	5	150	7.50	99.97	1.02

Table 9: System suitability data for NBH and AMB

NBH		AMB	
Concentration (µg/mL)	Area	Concentration (µg/mL)	Area
15	396857	7.5	274124
15	400456	7.5	271378
15	395769	7.5	270755
15	404121	7.5	274534
15	394892	7.5	276005
15	398112	7.5	275634
Average	398368	Average	273738
S.D.	3424.70	S.D.	2190.19
R.S.D	0.87	R.S.D	0.79

Conclusions

The proposed method is simple, sensitive and reproducible and hence the method can be used in routine for simultaneous determination of NBH and AMB in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of NBH and AMB in multi component pharmaceutical preparation.

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