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DETERMINATION OF NEBIVOLOL AND INDAPAMIDE IN TABLET FORMULATIONS BY REVERSED-PHASE HPLC

CHAUDHARY AB, PRAJAPATI JK , SHAH CM, PATEL JA, YADAV ZG
Saraswati Institute of Pharmaceutical science, Dhanap, Gandhinagar, 382355, India.
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Abstract: The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of Nebivolol HCL (NEBI) and Indipamide (INDA) in tablet formulation. Chromatography was performed on a ODS Hypersil C18 (250 mm x 4.6 mm i.d., 5 µm particle size) column with mobile phase containing 0.07M Potassium dihydrogen phosphate (pH3.0±0.1 using orthophosphoric acid): Acetonitrile: Triethaylamine (40:60:0.1). The flow rate was 1.0 ml/min and the eluent was monitored at 282 nm. The selected chromatographic conditions were found to effectively separate Nebivolol HCL(RT-3.403min) and Indipamide (RT- 7.907 min). (Figure-3) Linearity for Nebivolol HCL and Indipamide were found in the range of 50-100µg/ml and 15-45µg/ml respectively. The proposed method was found to be fast, accurate, precise, and reproducible and can be used for simultaneous estimation of these drugs in tablet.

Keywords: Nebivolol HCL, Indapamide, Reversed-phase HPLC

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Corresponding Author: DR. ANKIT B. CHAUDHARY

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INTRODUCTION

Nevivolol HCL of α, α-[Iminobis (methyalene)] bis [6-flouro-3,4- dihydro-2H -1- Bnzopyran-2-methanol hydrochloric acid] , represents the class of lipophilic Beta-blocker a employed in the management of essential hypertension [1-3] .Nebivolol exerts its action by exhibiting a high selectivity for adrenergic receptors and also by reducing the peripheral vascular resistance. The individual determination of Nebivolol HCL has been carried out in tablets by UV spectrophotometer [4-5]RP-HPLC[6-7] in bulk and solid dosage forms. Indipamide of 4- chloro-N-(2- methyl-2,3- dihydro -1 H – ino-1-yl)-3- sulfamoyalbenzamide belongs to antihypertensive agent.[8-10]. Literature survey revealed that few analytical methods have been reported for the estimation of indipamide included RP-HPLC [11-15], UV spectrophotometry[16]. The analytical methods for simultaneous determination of Nebivolol HCL with other combination has been reported by RP-HPLC[17-20], HPTLC [21-23], UV Spectrophotometry[24-28].The analytical methods for simultaneous determination of indipamide with other combination has been reported by RP-HPLC[29-34], UV spectrophotometry [35-40], HPTLC [41-43].

The present work describes a validated reverse phase HPLC method for simultaneous determination of these drugs in tablets.

EXPERIMENTAL WORK:

Apparatus

A RP-HPLC instrument (YL-Instrument) equipped with UV-Visible detector (Shimadzu, model1800), manual injector with 20 µl loop, and C18 column (250 mm × 4.6 mm id, 5 µm particle size) and YL-Clarity software were used. Analytical weight balance (Mettler Toledo, schwerzenbach, Switzerland), and ultra sonic cleaner (Branson ultrasonic corporation)), pH meter (Systonic) used during the study.

Reagents and Materials

Nebivolol HCL and Indipamide were received as a gift sample from Vaibhav Analytical Services. , Acetonitrile of HPLC grade, Triethylamine of AR grade, Water of HPLC grade. The water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai,India).

Chromatographic Conditions

Phenomenex C18 column (250 mm x 4.6 mm i.d., 5 µm particle size) was used at ambient temperature. The mobile phase was consist of 0.074M Potassium dihydrogen phosphate (Ph adjusted to 3.0±0.1 using orthophosphoric acid): Acetonitrile : Triethylamine (40:60:0.1) at a

flow rate of 1.0 ml/min. The mobile phase was filtered through a nylon 0.45 µm-47 mm membrane filter and degassed before use. The elution was monitored at 282nm, and the injection volume was 20 µl.

Preparation of stock solution (Nebivolol 1000 µg/ml and Indipamide 300 µg/ml)

An accurately weighed quantity of standard Nebivolol (50 mg) and Indipamide (15 mg)

Were transferred to 50 ml volumetric flasks and volumes were made up to mark with mobile phase to mark with mobile phase to get 1000 µg/ml of Nebivolol and 300 µg /ml of Indipamide.

Mobile phase:

0.074M Potassium dihydrogen phosphate (pH adjusted to 3.0+ 0.1 using orthophosphoric acid), Acetonitril, Triethylamine(40:60:0.1) was used, sonicated and filtered (Milipore) through 0.45 µm filter.

NEBI and INDA Standard Stock Solutions

For HPLC analysis 50 mg of NEBI and 15 mg INDA powder was weighed accurately using sartorius precision balance (readability 0.01 mg) and transferred in to 50 ml volumetric flask, dissolved and diluted to 50 ml with mobile phase to produce stock solution containing 1000 µg/ ml of NEBI and 300µg/ml INDA respectively.

Sample Solution

20 tablets were taken and powdered. Weighed accurately a quantity of the powder equivalent to about 50 mg of NEBI and 15 mg of INDA into 50 ml volumetric flask and diluted to 100 ml with mobile phase. This solution was sonicated for 15 minutes. The solution was filtered through whatman filter paper No. 41. 5 ml of solution was transferred into 50 ml volumetric flask and diluted to the mark with mobile phase. Again 5 ml of solution was transferred into 10 ml volumetric flask and diluted to the mark with mobile phase to get a final concentration 100 µg /ml of NEBI and 30µg/ml of INDA.

Determination of wavelength of maximum absorbance

10 µg/ml solutions of Nebivolol HCL and Indipamide were separately prepared in mobile phase. Each solution was scanned between 200-400 nm in double beam UV-visible spectrophotometer (Shimadzu, model 1800). Wavelength was selected from the overlay spectra of NEBI and INDA .both the components show reasonably good response at 282nm.

Method Validation [44]:

Calibration curve (Linearity)

0.5, 0.75 , 1.0 , 1.25 , 1.5 , ml of Nebivolol HCL (NEBI) and indapamide (INDA) was transferred to 10 ml volumetric flasks from both 1000µg/ml of Nebivolol HCL and 300µg/ml of indapamide stock solution and volume was made up to mark with mobile phase to get final concentration of Nebivolol HCL (50,75,100,125,150µg/ml) and inadapamide (15,22.5,30,37.5,45,µg/ml). Plot the graph for area Vs time to get calibration curve.

A**curacy (% Recovery)**

Accuracy of the methods was assured by use of the standard addition technique, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (NEBI 50 µg/ml and INDA 15 µg/ml) with three different concentrations of standards (NEBI 25,50,75 µg/ml and INDA 7.5, 15, 22.5 µg/ml). The good recoveries with the standard addition method prove the good accuracy of the proposed methods. (Table-3)

Method Precision:

The precision of the method was demonstrated by inter - day and intra- day variation studies. In the intra-day studies, 5 repeated injections of standard solution were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, 5 injection of standard solution were made for 5 consecutive days and response of drugs peaks and percentage RSD were calculated. From the data obtained, the developed RP-HPLC method was found to be precise. (Table 4 & 5)

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, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per 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 calibration curve.

Robustness

Robustness was carried by varying three parameters from the optimized chromatographic conditions. No significant change was observed as per table 6.

Analysis NEBI and INDA in Combined Dosage Forms

Pharmaceutical formulation of NEBI and INDA was purchased from local pharmacy. The responses of formulations were measured at 282 nm for quantification of NEBI and INDA by using RP-HPLC. The amounts of NEBI and INDA present in sample solution were determined by fitting the responses into the regression equation for NEBI and INDA in both the methods. . Results are given in Table 2.

RESULT AND DISCUSSION:

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of 0.07M Potassium dihydrogen phosphate(pH3.0±0.1 using orthophosphoric acid) :Acetonitrile: Triethaylamine (40:60:0.1) and 1.0 ml/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 282 nm at which much better detector responses for both drugs were obtained. As it was shown in Fig. 3 the retention times were 3.403 min for NEBI and 7.907 min for INDA. The calibration graphs for NEBI and INDA were constructed by plotting the peak area versus their corresponding concentrations, good linearity for both was found over the range nebivolol range 50-100µg/ml and indapamide range 15-45µg/ml. Results obtained by applying the RP-HPLC method showed that the concentrations of NEBI and INDA can be simultaneously determined in prepared mixtures. The proposed method has been applied to the assay of NEBI and INDA in pharmaceutical dosage form. The validity of the method was further assessed by applying the standard addition technique. The results obtained indicate the additives. Present do not interfere with analysis of the studied mixtures. System suitability test parameters for NEBI and INDA for the RP-HPLC method are reported in Table 1. The optical and regression characteristics and validation parameters are reported in Table 2.

CONCLUSION:

Results are in good agreement with lable claim which indicates there is no interference of routinely used exicipients. The proposed method was accurate and precise. Therefore proposed method can be used for routine analysis of Nebivolol HCL and Indapamide in tablets.

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Figure 1 Structure of nebivolol HCL

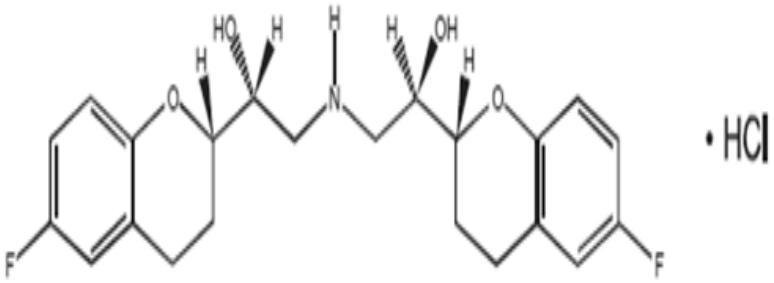

Figure 2 Structure of Indapamide

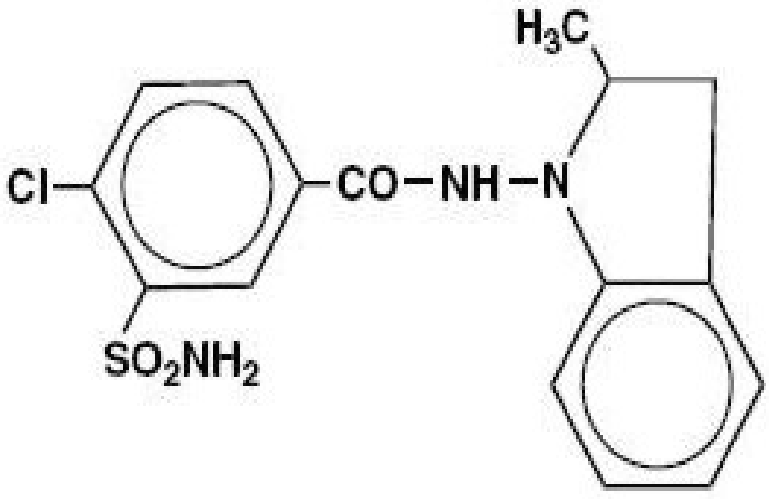

Figure 3 A typical RP-HPLC chromatogram of NEBI (100µg/ml) and INDA (30µg/ml) with corresponding retention time.

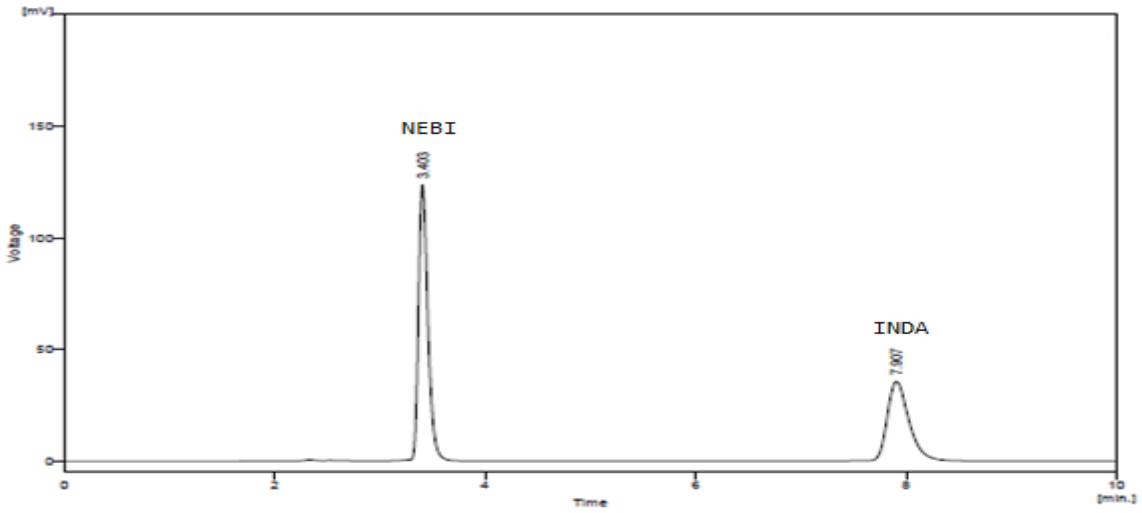

Table 1: Statistical analysis of parameters required for system suitability testing of the HPLC method

System suitability parameter	NEBI	INDA
Retention Time	3.403	7.907
Tailing Factor	1.4	1.5
Resolution	16.73	
Theoretical plate	6867	7156

Table 2: Optical and Regression characteristics and validation parameters of HPLC method for analysis of NEBI and INDA

Parameter	Nebivolol HCL	Inadapamide
Calibration Range(µg/ml)	50-150	15-45
Regression Equation	Y= 8.674x – 104.0	Y=16.31x + 26.66
Slope (m)	8.674	16.31
Intercept (c)	104.0	26.66
Correlation co-efficient (r)	0.9989	0.9989
Inter Day (%RSD, n=5)	0.29-1.09	0.63-1.41
Intra Day (%RSD, n=5)	0.63-1.25	0.67-1.44
Detection Limit(µg/ml)	1.431	0.770
Quantitation limit(µg/ml)	4.338	2.334

Table 3: Data of recovery study for NEBI and INDA by HPLC method

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=3)
NEBI	50	25	73.2	97.33±1.1274
	50	50	98.48	98.48±1.0424
	50	75	123.36	98.68±0.8678
INDA	15	7.5	21.92	97.42±1.0610
	15	15	29.24	97.46±0.9888
	15	22.5	36.86	98.29±1.0084

Table 4 Precision study of NEBI

Drug	Concentration (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± S.D (n=5)	%RSD	Mean ± S.D (n=5)	%RSD
NEBI	50	346.91±3.79	1.09	352.82±4.42	1.25
	75	538.95±2.75	0.51	541.79±4.49	0.82
	100	765.51±6.60	0.86	766.31±4.89	0.63
	125	970.65±2.82	0.29	957.23±8.84	0.92
	150	1221.37±4.02	0.32	1201.48±11.87	0.98

Table 5 Precision study of INDA

Drug	Concentration (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ±S.D (n=5)	%RSD	Mean ±S.D (n=5)	%RSD
INDA	15	257.174±3.63	1.41	264.93±3.83	1.44
	22.5	402±62±4.92	1.22	395.27±3.11	0.78
	30	512.38±4.43	0.86	506.32±4.37	0.86
	37.5	619.39±3.92	0.63	626.08±4.22	0.67
	45	761.23±5.73	0.75	757.18±6.84	0.90

Table 6 Robustness

Parameter	Value	Area	
		NEBI	INDA
pH	2.9	755.230	505.214
	3.0	762.653	512.392
	3.1	771.654	514.787
	Mean ±SD	763.17±8.2246	510.797±4.98
	%RSD	1.07	0.97
Flow rate	0.9	772.470	523.695
	1	762.653	512.392
	1.1	754.691	510.555
	Mean ±SD	763.271±8.905	515.547±7.11
	%RSD	1.16	1.38
Mobile phase	42:58 (Buffer :ACN)	776.376	528.106
	40:60(Buffer: ACN)	762.653	512.392
	38:62(Buffer :ACN)	760.150	515.198
	Mean ±SD	766.393±873	518.563±8.38
	%RSD	1.13	1.62

Table 7 Application of the proposed method to the pharmaceutical dosage forms

Formulation	NEBI			INDA		
	Amount labeled (mg)	Amount found (mg)	% Amount Found S.D. (n=3)	Amount labeled (mg)	Amount found (mg)	% Amount Found S.D. (n=3)
Brand I	5	5.029	100.59± 0.605	1.5	1.51	100.714 ± 0.536

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