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METHDOD DEVELOPMENT AND VALIDATION OF INDAPAMIDE AND NEBIVOLOL HYDROCHLORIDE BY RP-HPLC METHOD

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ABSTRACT

An isocratic Simultaneous estimation by RP-HPLC Method was developed and validated for the quantification of Indapamide and Nebivolol Hydrochloride in tablet dosage form. Quantification was achieved by using the mobile phase (Ammonium acetate buffer pH4.5: Methanol) in the ratio of 45:55. A Kromosil C18 column (250*4.6, 3.5µm) was used as stationary phase. The flow rate was 1.0 ml/min. Measurements were made at a wavelength of 226nm. The average retention time for Indapamide and Nebivolol Hydrochloride were found to be 2.35 min and 3.49 min. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay methods were found to be linear from 9-21 µg/ml for Indapamide and 30-70 µg/ml for Nebivolol Hydrochloride. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of Indapamide and Nebivolol Hydrochloride in tablet dosage form.

KEYWORDS

Indapamide, Nebivolol Hydrochloride, RP-HPLC method, KROMOSIL C18 column, Methanol, Ortho phosphoric acid and Validation.

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INTRODUCTION

Nebivolol Hydrochloride

Nebivolol is a highly cardio selective vasodilatory beta1 receptor blocker used in treatment of hypertension. Nebivolol lowers blood pressure (BP) by reducing peripheral vascular resistance, and significantly increases stroke volume with preservation of cardiac output. The net hemodynamic effect of nebivolol 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 nebivolol (NB) 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[1-3].

Mechanism of action

Nebivolol is a selective β1-receptor antagonist. Activation of β1-receptors by epinephrine increases the heart rate and the blood pressure, and the heart consumes more oxygen. Nebivolol blocks these receptors which reverses the effects of epinephrine, lowering the heart rate and blood pressure. In addition, beta blockers prevent the release of renin, which is a hormone produced by the kidneys which leads to constriction of blood vessels. At high enough concentrations, this drug may also bind beta 2 receptors.

Medical uses

- Antihypertensive Agents
- Adrenergic beta-Antagonists
- Vasodilator Agents

Indapamide

Indapamide (IND) is a benzamide-sulfonamideindole. It is called as thiazide-like diuretic but structure is different enough (lacking the thiazo-ring). So it is not clear that the mechanism is comparable.

Categories: Antihypertensive Agents, Diuretics.

Mechanism of action

Indapamide blocks the slow component of delayed rectifier potassium current (IKs) without altering the rapid component (IKr) or the inward rectifier current. Specifically it blocks or antagonizes the action of proteins KCNQ1 and KCNE1. Indapamide is also thought to stimulate the synthesis of the vasodilatory hypotensive prostaglandin PGE2.

Pharmacodynamics

Indapamide is an antihypertensive and a diuretic. It contains both a polar sulfamoyl chlorobenzamide moiety and a lipid- soluble methylindoline moiety. Indapamide bears a structural similarity to the thiazide diuretics which are known to decrease vascular smooth muscle reactivity. However, it differs chemically from the thiazides in that it does not possess the thiazide ring system and contains only one sulfonamide group. Indapamide appears to cause vasodilation, probably by inhibiting the passage of calcium and other ions (sodium, potassium) across membranes. This same effect may cause hypocalcemia in susceptible individuals. Indapamide has also been shown to cause uterine myometrial relaxation in experimental animals. Overall, indapamide has an extra-renal antihypertensive action resulting in a decrease in vascular hyper reactivity and a reduction in peripheral and arteriolar resistance 4-6. Literature survey reveals that there are few methods for the estimation of nebivolol and indapamide in single or combination with other drugs. Hence, the present study describes a simple, accurate and precise RP-HPLC method for the simultaneous estimation of nebivolol and Indapamide in their combined tablet dosage form[7-16] .

MATERIALS AND METHOD

Instruments The chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software, reversed phase C18 column (Kromosil C18 column (250*4.6, 3.5µm)) as stationary phase, Electron corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY- 220, Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

Materials

Pharmaceutically pure sample of Indapamide and Nebivolol Hydrochloride were obtained as gift samples from Chandra lab, Prashanthinagar, Kukatpally, Hyderabad, India. The purity of the drug was evaluated by obtaining its melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification. HPLC-grade Methanol ware from standard reagents pvt Ltd. Ammonium acetate (AR grade) was from Merck. A tablet formulation of Indapamide and Nebivolol Hydrochloride (1.5mg

and 5mg label claims) were procured from local market (Nebula-D, Zydus Cadila Pharmaceutical Company, India).

Determination of Working Wavelength (λmax)

25 mg of the Indapamide standard drug is taken in a 25 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in UV between 200-400 nm using methanol as blank. The λ_{max} was found to be **206nm**.

25 mg of the Nebivolol Hydrochloride standard drug is taken in a 25 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in UV between 200-400 nm using methanol as blank. The λ_{max} was found to be **210m**. The Iso bestic Point Indapamide and Nebivolol Hydrochloride were found to be **226nm**.

Preparation of mobile phase

Buffer Preparation: Dissolved 3.85gm of ammonium acetate in 150ml of distilled water then diluted to 1000ml with water, adjusted the P^H 4.5 with orthophosphoric acid and filtered through 0.45µm nylon membrane filter and degassed. **Mobile phase:** Buffer and methanol were mixed in the ratio of 45:55 and sonicated to degas.

Analysis of formulation

Preparation of standard solution: Weighed accurately 50 mg of NB and 15 mg of IND and transferred to 100ml volumetric flask, added few ml of mobile phase to dissolve and made up the volume with mobile phase to 100ml.From the above solution 1.0ml was taken and transferred to 10ml volumetric flask and the volume was made up to the mark with the mobile phase. It Contains 50 ppm of NB and 15 ppm of IND.

Preparation of sample solution: Twenty tablets were accurately weighed and powdered. A quantity of powder equivalent to 50mg of NB and 15mg of IND was taken and transferred to 100ml volumetric flask and the volume was made up to the mark with the mobile phase, filtered. From the above solution 1.0ml was taken and transferred to 10ml volumetric flask and the volume was made up to the mark with the mobile phase. It Contains 50ppm of NB & 1 5PPM of IND. Calculation 5 replicates of each of sample and standard solutions were injected and their average peak areas were taken.

The amount of Indapamide and Nebivolol Hydrochloride present in the formulation by using the formula given below, and results shown in above table no.6.

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation
AT: Peak area due to assay preparation
WS: Weight of standard drug taken
WT: Weight of sample in assay preparation
DT: Dilution of assay preparation
DS: Dilution of standard preparation
AW: Average weight of 20 tablets
LC: Label claim
P: Purity of standard drug

METHOD VALIDATION

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 30-70 µg/ml for Nebivolol Hydrochloride and 9-21g/ml for Indapamide of the drug in Table No.1 and 1.1. From the primary stock solution 0.6ml, 0.8ml, 1.0ml, 1.2ml, 1.4 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 30µg/mL, 40µg/mL, 50µg/mL, 60µg/mL and 70 µg/mL of Nebivolol.

Hydrochloride and 9µg/mL, 12µg/Ml, 15µg/mL, 18µg/mL, 21µg/mL of Indapamide Figure No.1 and 1.1.

Calibration curve (Figure No.2 and 2.1) with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 50µg/ml of Nebivolol Hydrochloride and 15µg/ml of Indapamide without changing the parameter of the proposed chromatographic method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 different days over a period of 1 week for 50µg/ml and 15µg/ml concentrations of standard solutions of Indapamide and Nebivolol Hydrochloride. The result was reported in terms of relative standard deviation (% RSD).

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (2) and (3), respectively.

LOD = 3.3 δ/S ……. (3)

LOQ =10 δ/S …….. (4)

Where, σ = the standard deviation of the response

S = the slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Indapamide and Nebivolol Hydrochloride by the standard addition method. Known amounts of standard solutions of Indapamide and Nebivolol Hydrochloride were added at 20% concentration to pre quantified sample solutions of Nebivolol Hydrochloride (40, 50, 60 µg/ml) and Indapamide (12, 15, 18µg/ml). The amount of Indapamide and Nebivolol Hydrochloride recovered was estimated by using the following formulas.

$$\% \text{ Recovery} = \frac{\text{amount found}}{\text{Amount added}} \times 100$$

$$\text{Amount Found}(mcg/ml) = \frac{\text{Mean test area}}{\text{Mean standard area}} \times \text{Standard concentration}$$

Specificity

In an assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results are unaffected by the presence of these extraneous materials. There should be no interference of the diluents, placebo at retention time of drug substances [17-18].

Robustness: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in (Table No.4)

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The %RSD assay values between two analysts was calculated i.e., (limit <2 %). This indicates the method was rugged. The results were shown in Table No.5.

RESULTS AND DISCUSSION

In RP HPLC method, the primary requirement for developing a method for analysis is that the using different solvents and buffers and columns to get better retention time and theoretical plates, and

better cost effective and time saving method than the previously developed methods. The Iso bestic Point of Nebivolol Hydrochloride and Indapamide were found to be 226nm (Figure No.3-5) by scanning in UV region. The chromatographic method was optimized with mobile phase consisting of Ammonium acetate buffer: Methanol (45:55) and C18 KROMOSIL C18 column. All the validation parameters were studied at a the wavelength 226nm. Accuracy was determined by calculating the recovery (Table No.3) and the results were in acceptable range (limit 98-102%). The method was successfully used to determine the amount of Nebivolol Hydrochloride and Indapamide present in the Tablet. The results obtained were in good agreement with the corresponding labeled amount (Table No.3). The method was linear in the concentration range of 30 to 70 µg/ml for Nebivolol Hydrochloride and 9 to 91 µg/ml for Indapamide (Table No.1). Precision was calculated as repeatability and intra and inter day variations (% RSD) for the drug (Table No.7). Robustness and ruggedness results were in acceptable range (Table No.4 and Table No.2). Summary of all validation parameters for method is given in Table No.8. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis Nebivolol Hydrochloride and Indapamide in tablet dosage form

Table No.1 and 1.1: Linearity

S.No	**NEBIVOLOL HYDROCHLORIDE**	
1	mcg	Area
2	30	505.316
3	40	724.182
4	50	923.441
5	60	1112.171
6	70	1327.336

S.No	**INDAPAMIDE**	
1	mcg	Area
2	9	74.614
3	12	98.914
4	15	119.075
5	18	144.005
6	21	162.939

Table No.2, 2.1 and 2.2: LOD and LOQ values from calibration curve

S.No	Parameter	IND	NB
1	Slope	7.39	20.32
2	S.D	4.7	15.81
3	LOD	2.12	2.57
4	LOQ	6.42	7.78

Table No.3.1 and 3.2: Recovery data

% Standard	Amount of IND present (µg/ml)	Amount of IND added(µg/ml)	Avg. area of 3 recoveries	%Recovery
80	12	3	120.12	99.45
100	15	3	140.32	99.95
120	18	3	170.25	101.25
			MEAN	100.25

% Standard	Amount of NB present (µg/ml)	Amount of NB added(µg/ml)	Avg. area of 3 recoveries	%Recovery
80	40	10	905.01	99.25
100	50	10	1097.309	100.23
120	60	10	1304.25	99.21
			MEAN	100.01

Table No.4: Results of Robustness study

Chromatographic change		Retention time		Asy	mmetry
		IND	NB	IND	NB
Flow rate	1ml/min	2.363	3.510	1.286	1.324
	0.8ml/min	2.987	4.450	1.294	1.319
	1.2ml/min	1.900	2.830	1.292	1.273
Wavelength	226nm	2.363	3.510	1.286	1.324
	224nm	2.347	3.490	1.286	1.316
	228nm	2.350	3.500	1.333	1.324

Table No.5: Results of Ruggedness

		Std Area	Spl Area	%Assay	%RSD
Analyst-1	INDAPAMIDE	134.510	135.240	99.96%	0.12%
Analyst-2		133.510	136.473	99.85%	
Analyst-1	NEBIVOLOL HYDROCHLORIDE	1024.510	1042.320	99.52%	1.23%
Analyst-2		1028.952	1026.325	99.65%	

Table No.6: Assay Results

Area	Preparation	IND	NB
Standard Area	1	132.925	1026.595
	2	145.802	1020.325
	3	137.749	1015.592
	4	135.217	1035.171
	5	141.308	1023.211
Average		138.600	1024.179
Sample area	1	140.97	1027.058
	2	135.989	1015.619
	3	135.114	1005.062
	4	149.17	1023.142
	5	135.898	1021.145
Average		138.600	1018.405
Tablet average weight		100.4mg	100.4mg
Standard weight		7.5mg	25mg
Sample weight		502mg	502mg
Label amount		1.5mg	5mg
std.purity		99.8	99.8
Cal.:		1.50mg	4.96mg
% Assay		99.80	99.24

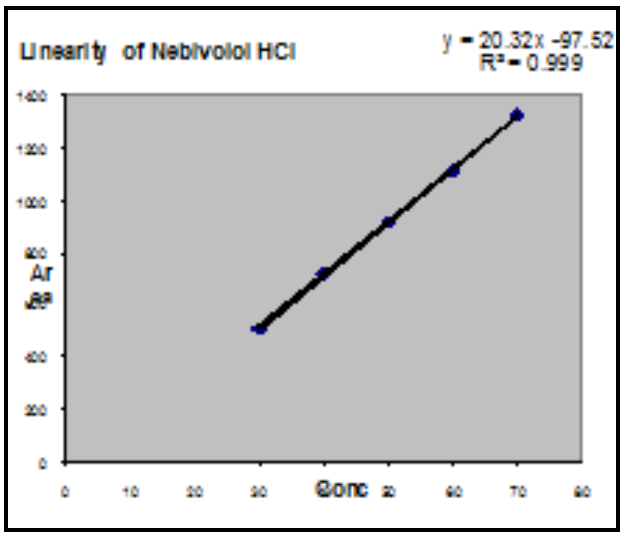
Table No.7: Method Precision (Repeatability)

Injection	IND		NB	
	Retention Time	**Area**	**Retention Time**	**Area**
1	2.363	132.773	3.510	1023.181
2	2.35	131.826	3.500	1034.039
3	2.360	132.826	3.510	1023.73
4	2.363	133.075	3.513	1023.366
5	2.32	135.357	3.470	1041.225
6	2.357	133.526	3.503	1021.136
AVG	2.3522	133.231	3.499	1027.780
SD	0.0165	1.181	0.017	8.014
% RSD	0.70	0.89	0.49	0.78

Table No.8: Validation parameters of evaluated method

S.No	Parameter	Value Obtained of INDAPAMIDE	Value Obtained NEBIVOLOL HYDROCHLORIDE
1	ACCURACY(%Recovery)	99.45-101.25%	99.25-100.23%
2	Linearity concentrations Range(µ g/mL) Regression coefficient (R2 value)	9-21 µ g/mL 0.998	30-70 µ g/mL 0.999
3	LOD	2.12	2.57
4	LOQ	6.42	7.78
3	Precision (% RSD) od precision(Repeatability) (%RSD, n = 6)	0.70-0.89	0.49-0.78%
4	Robustness(%assay)	99.96%	99.52%
5	Ruggedness(%RSD analyst to analyst variation)	0.12%	1.23%

a.SD=Standard deviation, b. LOD = Limit of detection, c. LOQ = Limit of quantification, d. RSD = Relative standard deviation



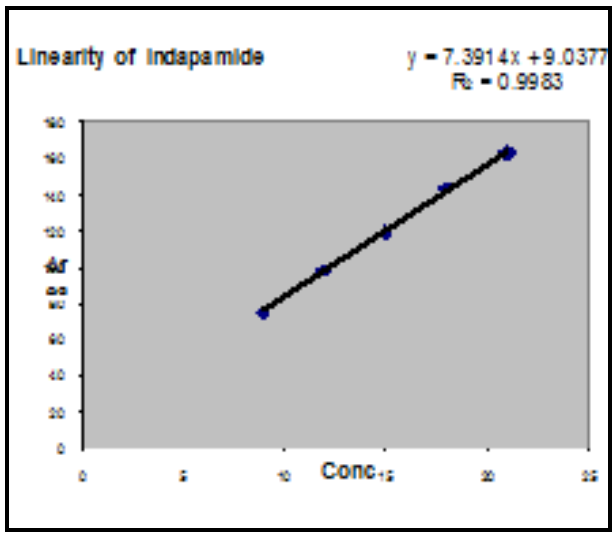


Figure No.1 and 1.1: Linearity (calibration) curve of Nebivolol HydrochlorideandIndapamide

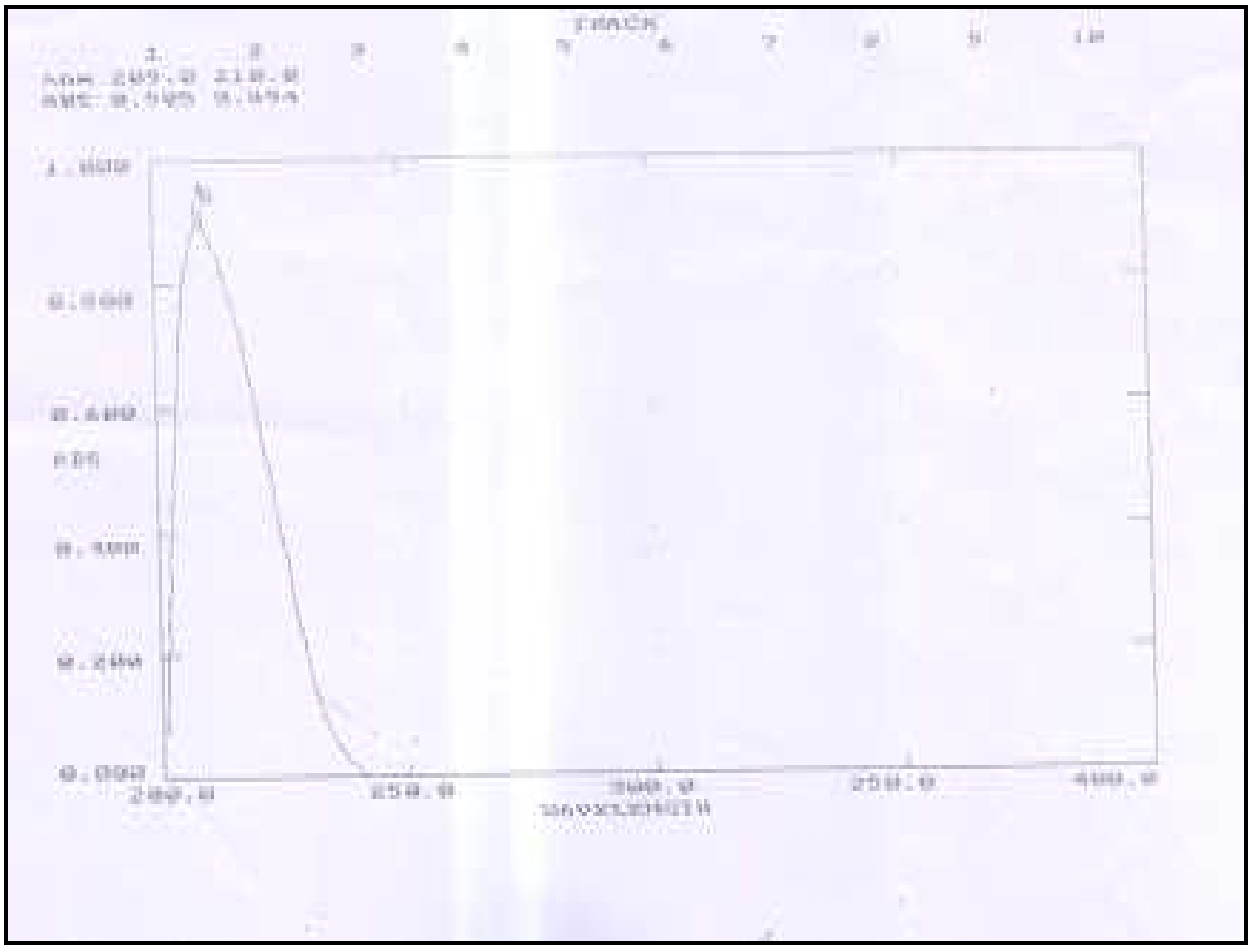


Figure No.2: Determination of Working Wavelength (λmax) of Nebivolol Hydrochloride

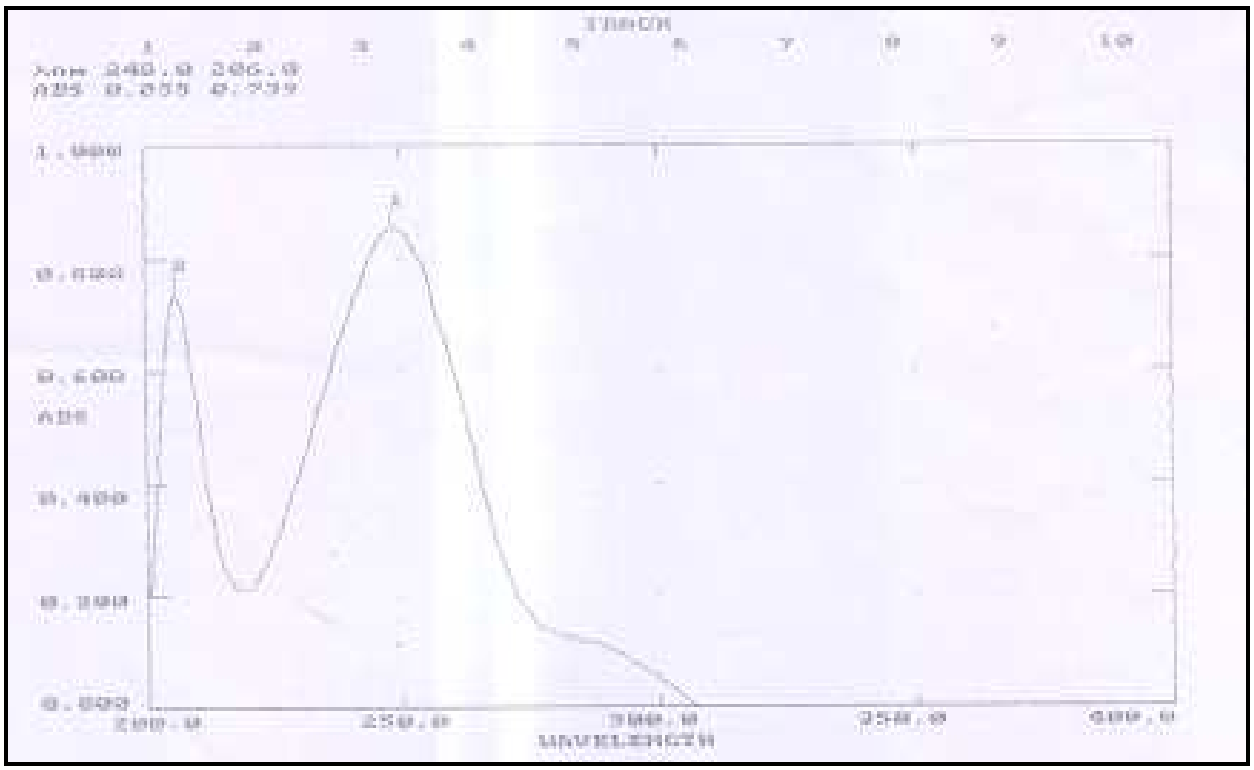


Figure No.2.1: Determination of Working Wavelength (λmax) of Indapamide

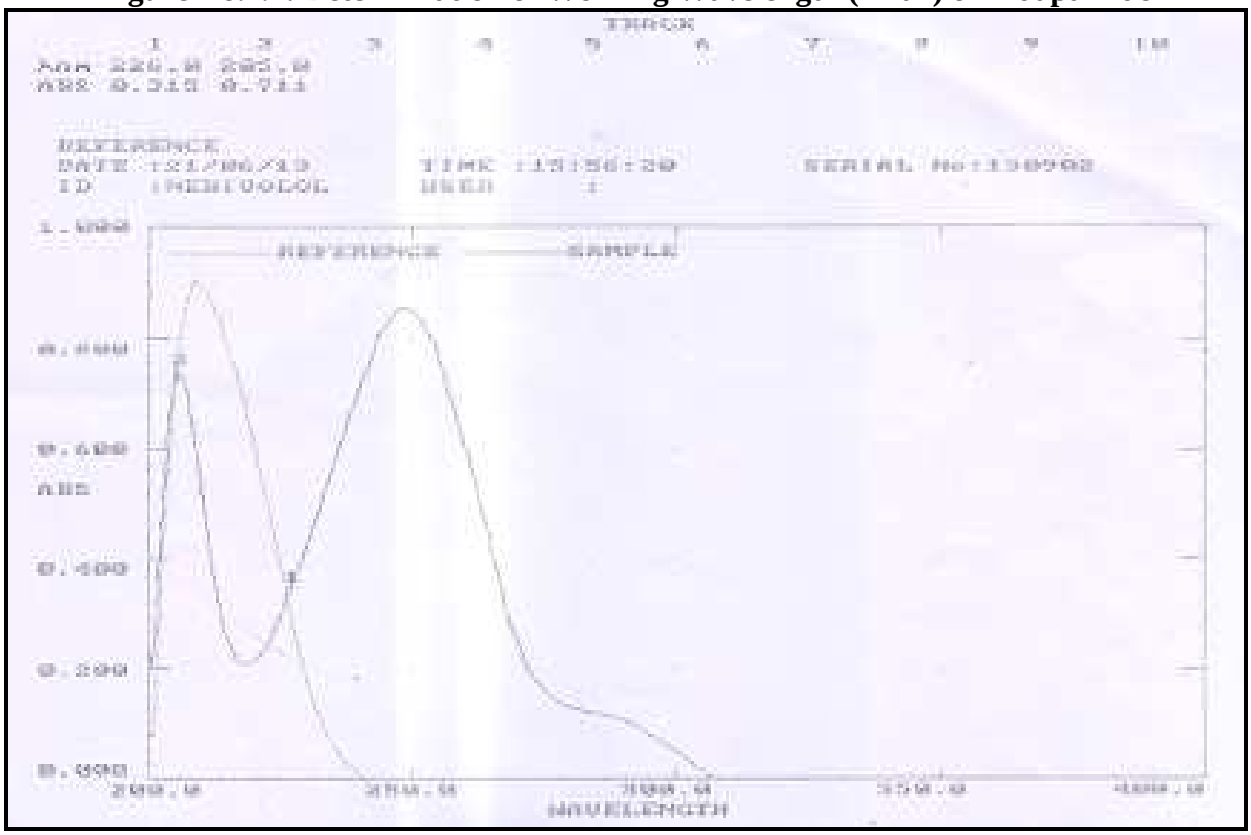


Figure No.2.2: Determination of Isosbestic Point

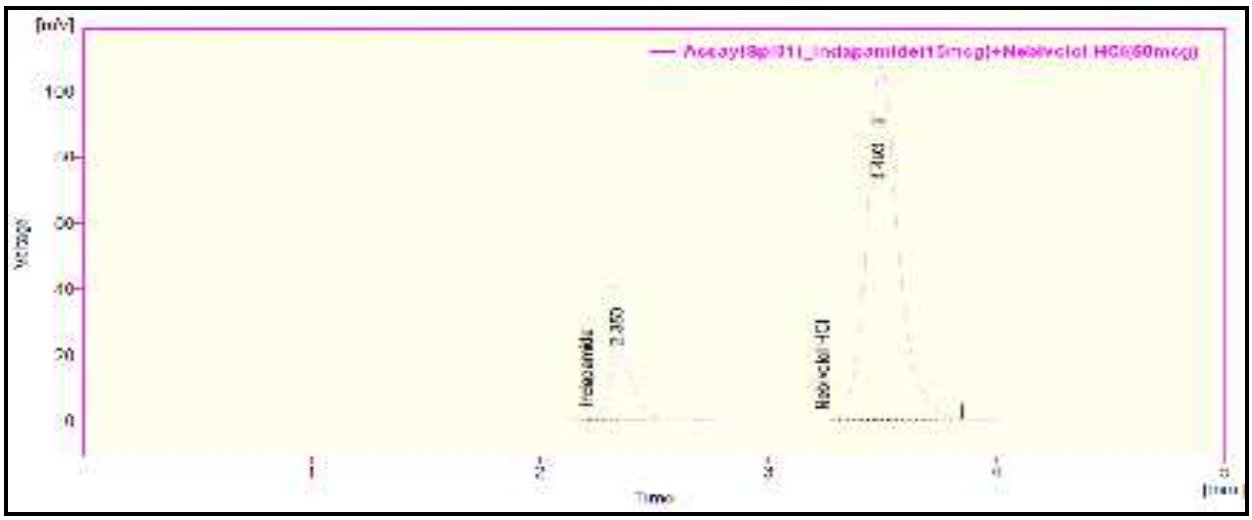


Figure No.3: Chromatogram of Assay sample preparation

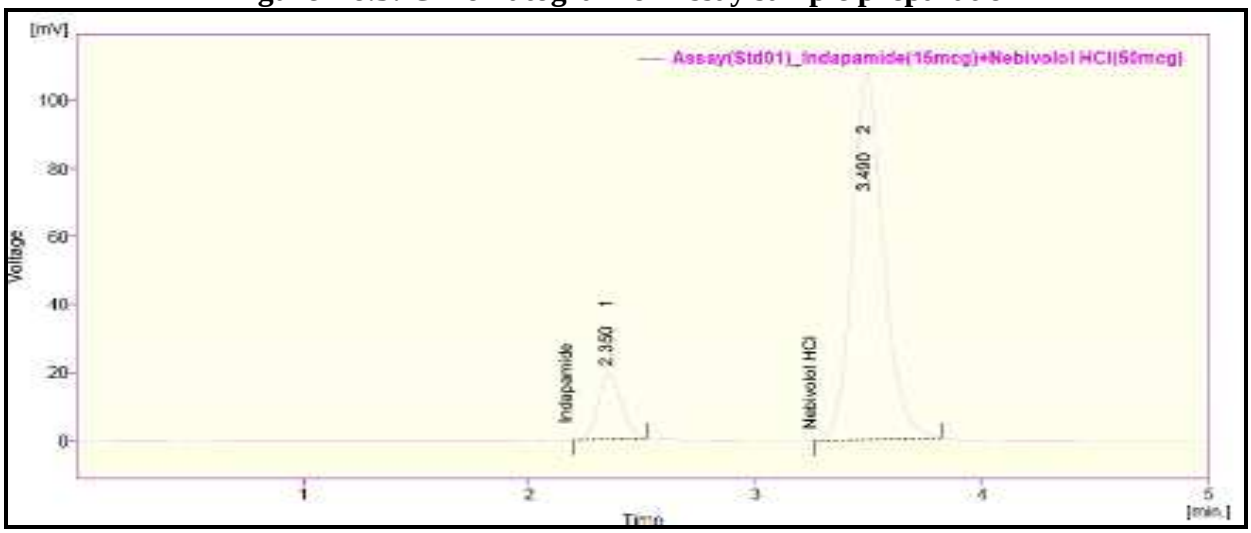


Figure No.4: Chromatogram of Assay standard preparation

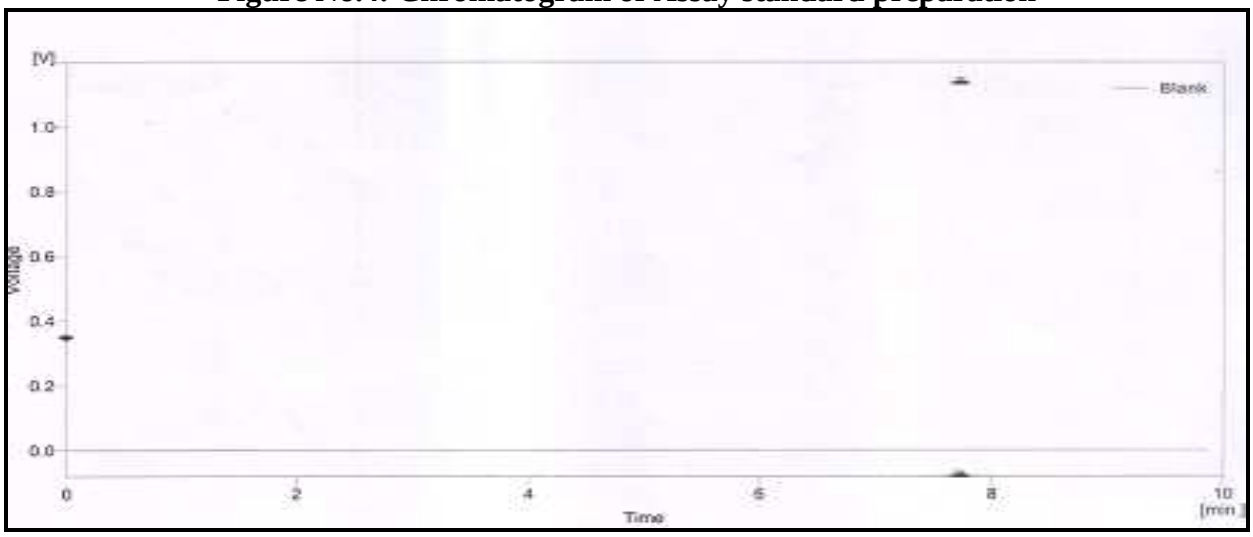


Figure No.5: Chromatograms of Specificity (placebo)

CONCLUSION

The proposed Simultaneous Estimation of nebivolol hydrochloride and Indapamide by RP-HPLC method was found to be simple, sensitive, accurate and precise for determination of Nebivolol Hydrochloride and Indapamide in tablet. The method utilizes easily available and cheap solvent for analysis of Nebivolol Hydrochloride and Indapamide hence the method was also economic for estimation of Nebivolol Hydrochloride and Indapamide from Tablet .The common excipients and other additives are usually present in the tablet mixture do not interfere in the analysis of Nebivolol Hydrochloride and Indapamide, hence it can be conveniently adopted for routine quality control analysis of the drug in pharmaceutical formulation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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