Analytical Method Development and Validation for Estimation of Cardio Protective Agents in Formulated Dosage Form By RP-HPLC Method

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***Abstract*—A simple, precise, accurate, and stability-indicating Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Indapamide and Nebivolol Hydrochloride in bulk drugs and pharmaceutical dosage forms. Method development was carried out using a Waters X-Bridge BEH C18 column (150 × 4.6 mm, 3.5 µm). Various chromatographic conditions were investigated to obtain satisfactory peak resolution, theoretical plate count, and tailing factor. The optimized chromatographic conditions consisted of Methanol: Phosphate Buffer (70:30 v/v, pH 4 adjusted with orthophosphoric acid) as mobile phase, flow rate of 0.5 mL/min, detection wavelength of 239 nm, and injection volume of 20 µL. Under optimized conditions, Indapamide and Nebivolol Hydrochloride were eluted at retention times of 3.278 min and 6.404 min respectively. The method was validated according to ICH Q2(R1) guidelines with respect to specificity, linearity, accuracy, precision, robustness, LOD, and LOQ. The developed method demonstrated excellent analytical performance and was found suitable for routine quality control analysis of combined tablet dosage forms.**

***Index Terms*—RP-HPLC, Indapamide, Nebivolol Hydrochloride, Method Validation, Stability-Indicating Method, ICH Guidelines, Pharmaceutical Analysis.**

I. INTRODUCTION

Hypertension remains one of the most prevalent cardiovascular disorders worldwide and is a major risk factor for stroke, myocardial infarction, heart failure, and renal disease. Combination therapy involving antihypertensive agents with complementary mechanisms of action has become a preferred strategy for achieving optimal blood pressure control. Among the various fixed-dose combinations available, Indapamide and Nebivolol Hydrochloride offer significant therapeutic benefits in the management of hypertension.

Indapamide is a thiazide-like diuretic chemically known as Benzamide, 3-(aminosulfonyl)-4-chloro-N-(2-methyl-1-indolinyl)-3-sulfamoylbenzamide. It acts primarily by inhibiting sodium and chloride reabsorption in the distal convoluted tubule of the nephron, thereby promoting diuresis and reducing blood pressure. Additionally, Indapamide exerts direct vasodilatory effects on vascular smooth muscle, contributing to its antihypertensive activity.

Nebivolol Hydrochloride is a highly selective β1-adrenergic receptor antagonist possessing vasodilatory properties mediated through nitric oxide release. The drug reduces heart rate, myocardial contractility, and peripheral vascular resistance, resulting in effective blood pressure reduction. Nebivolol is distinguished from conventional beta-blockers by its favorable hemodynamic profile and enhanced tolerability.

The simultaneous determination of these drugs in combined dosage forms requires a reliable analytical method capable of providing accurate, precise, and reproducible results. High Performance Liquid Chromatography (HPLC) is one of the most powerful analytical techniques used for pharmaceutical analysis due to its high sensitivity, selectivity, and reproducibility. Therefore, the present study aimed to develop and validate a stability-indicating RP-HPLC method for simultaneous estimation of Indapamide and Nebivolol Hydrochloride according to ICH guidelines.

II. DRUG PROFILE

Table 1: Physicochemical Properties of Indapamide

Parameter	Description
Molecular Formula	C16H16ClN3O3S
Molecular Weight	365.83 g/mol
Category	Thiazide-like Diuretic
Appearance	White to Off-white Powder
Solubility	Soluble in methanol, ethanol, acetic acid
Melting Point	160 ± 2°C
Storage	Store at Room Temperature

Table 2: Physicochemical Properties of Nebivolol Hydrochloride

Parameter	Description
Molecular Formula	C22H25F2NO4·HCl
Molecular Weight	441.9 g/mol
Category	β-Adrenergic Blocking Agent
Appearance	White to Off-white Powder
Solubility	Soluble in Methanol and DMSO
Melting Point	220 ± 2°C
Storage	Store at Room Temperature

III. MATERIALS AND METHODS

3.1 Chemicals and Reagents

Indapamide and Nebivolol Hydrochloride reference standards were obtained from Angle Biopharma Pvt. Ltd., Ahmedabad, India. Methanol, acetonitrile, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, orthophosphoric acid, hydrochloric acid, sodium hydroxide, and HPLC-grade water were utilized during the study.

3.2 Instrumentation

The chromatographic analysis was performed using Waters Alliance e2695 HPLC equipped with UV detector and Waters X-Bridge BEH C18 column (150 × 4.6 mm, 3.5 µm).

Table 3: Instrumentation Details

Instrument	Manufacturer
HPLC System	Waters Alliance e2695
Column	Waters X-Bridge BEH C18
UV Spectrophotometer	PG Instruments T60
Sonicator	Enertech SE60US
Analytical Balance	Denver

IV. METHOD DEVELOPMENT

Several chromatographic trials were performed by varying mobile phase composition, pH, and flow rate to obtain satisfactory chromatographic performance. Eight different chromatographic conditions were investigated before arriving at the optimized method.

Table 4: Summary of Method Development Trials

Trial	Mobile Phase	Flow Rate (mL/min)	Observation
I	Methanol:Buffer (60:40)	0.8	Poor plate count
II	Methanol:Buffer (60:40)	0.8	High retention
III	Methanol:Buffer (50:50)	0.8	High tailing
IV	Methanol:Buffer (40:60)	0.8	Poor efficiency
V	Methanol:Buffer (60:40)	0.6	Moderate performance
VI	Methanol:Buffer (65:35)	0.6	Improved resolution
VII	Methanol:Buffer (70:30)	0.6	Long retention
VIII	Methanol:Buffer (70:30)	0.5	Optimized

V. OPTIMIZED CHROMATOGRAPHIC CONDITIONS

The optimized chromatographic method was selected based on acceptable system suitability parameters.

Table 5: Optimized Chromatographic Parameters

Parameter	Condition
Column	Waters X-Bridge BEH C18 (150 × 4.6 mm, 3.5 µm)
Mobile Phase	Methanol: Phosphate Buffer (70:30)
Buffer pH	4.0
Flow Rate	0.5 mL/min
Detection Wavelength	239 nm

Injection Volume	20 µL
Run Time	20 min
Column Temperature	Ambient
Retention Time (IND)	3.278 min
Retention Time (NEB)	6.404 min

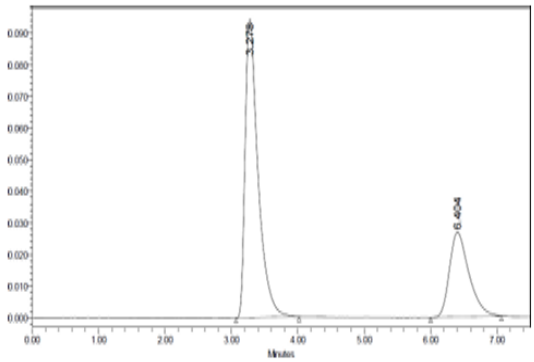

Figure 1 : Optimized Chromatogram

VI. ASSAY PROCEDURE

Standard and sample solutions were prepared by dissolving accurately weighed quantities of Indapamide and Nebivolol Hydrochloride in methanol followed by dilution with mobile phase. Tablets equivalent to the label claim were powdered, extracted, filtered, and diluted appropriately before chromatographic analysis.

Table 6: Assay Results

Drug	Standard Area	Sample Area	Assay (%)
Indapamide	332.072	331.203	99.74
Nebivolol HCl	Within Limits	Within Limits	99–101

VII. METHOD VALIDATION

The developed method was validated according to ICH Q2(R1) guidelines. Validation parameters included specificity, linearity, accuracy, precision, robustness, LOD, and LOQ.

7.1 Specificity

Specificity was evaluated by analyzing blank, standard, and sample solutions. No interference from excipients or diluents was observed at the retention times of the analytes. The method was also subjected to stress degradation studies under acidic, alkaline, and thermal conditions.

7.2 Linearity

Linearity was established using concentrations ranging from:

Table 7. Linearity Concentration Range

Drug	Concentration Range (µg/mL)
Indapamide	3 – 18
Nebivolol HCl	5 – 30

Excellent linear relationships were obtained between concentration and peak area.

7.3 Accuracy

Recovery studies were performed at 80%, 100%, and 120% concentration levels.

Table 8: Accuracy Study Design

Level	Indapamide (µg/mL)	Nebivolol HCl (µg/mL)
80%	12	20
100%	15	25
120%	18	30

The percentage recoveries were found within acceptable limits of 98–102%.

7.4 Precision

Precision was evaluated as system precision, method precision, and intermediate precision.

Table 9: Precision Acceptance Criteria

Parameter	Acceptance Limit
%RSD of Peak Area	≤ 2.0
Repeatability	Complies
Intermediate Precision	Complies

7.5 Robustness

Robustness studies were conducted by introducing deliberate variations in flow rate and chromatographic conditions. The method showed no significant changes in chromatographic performance, confirming robustness.

7.6 LOD and LOQ

LOD and LOQ were calculated using the standard deviation of response and slope method.

Table 10: Equations Used

Parameter	Formula
LOD	3.3σ/S
LOQ	10σ/S

Where σ = standard deviation and S = slope of calibration curve.

VIII. RESULTS AND DISCUSSION

System suitability studies demonstrated that Trial VIII produced satisfactory chromatographic performance with acceptable retention times, theoretical plate counts, and tailing factors. The optimized chromatographic conditions provided adequate separation between Indapamide and Nebivolol Hydrochloride. Retention times of 3.278 min and 6.404 min enabled efficient quantification within a short analysis period.

Validation studies confirmed excellent specificity, linearity, precision, and accuracy. The method was found to be robust under slight variations in chromatographic conditions and suitable for routine quality control applications. Stress degradation studies indicated that degradation products did not interfere with the determination of analytes, demonstrating the stability-indicating nature of the method.

IX. CONCLUSION

A simple, economical, accurate, precise, robust, and stability-indicating RP-HPLC method was successfully developed and validated for the simultaneous estimation of Indapamide and Nebivolol Hydrochloride in pharmaceutical dosage forms. The optimized chromatographic conditions employed a Waters X-Bridge BEH C18 column with Methanol: Phosphate Buffer (70:30, pH 4) at a flow rate of 0.5 mL/min and detection at 239 nm. The developed method met all ICH validation requirements and can be effectively employed for routine quality control analysis and stability studies of combined dosage formulations.

REFERENCES

[1] ICH. Validation of Analytical Procedures: Text and Methodology Q2(R1). Geneva: International Conference on Harmonisation; 2005.

[2] Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. New York: Wiley; 2010.

[3] Sethi PD. HPLC: Quantitative Analysis of Pharmaceutical Formulations. New Delhi: CBS Publishers; 2011.

[4] Legorburu MJ, et al. Quantitative determination of indapamide in pharmaceuticals and urine by HPLC with amperometric detection. J Pharm Biomed Anal. 1999;20(1):45-52.

[5] Patel LJ, et al. RP-HPLC and HPTLC methods for estimation of Nebivolol Hydrochloride in tablet dosage form. Indian J Pharm Sci. 2007;69(4):564-568.

[6] Doshi AS, et al. Determination of Nebivolol and Valsartan in capsule formulations by liquid chromatography. J Chromatogr Sci. 2008;46(7):612-617.

[7] Shah DA, et al. Stability-indicating RP-HPLC method for determination of Nebivolol Hydrochloride in tablet dosage forms. J AOAC Int. 2008;91(5):1028-1034.

[8] Sahoo MK, et al. RP-HPLC method for estimation of Nebivolol in tablet dosage form. Indian Drugs. 2009;46(8):58-63.

[9] Gupta Y, et al. Simultaneous estimation of Nebivolol Hydrochloride and Hydrochlorothiazide by RP-HPLC. Int J Pharm Sci. 2009;71(4):425-430.

[10] Patel PU, et al. Spectrophotometric methods for simultaneous estimation of Indapamide and Nebivolol Hydrochloride. Indian J Pharm Educ Res. 2011;45(2):176-182.

[11] Shetty ASK, Pai JB, et al. RP-HPLC method for quantitative estimation of Indapamide in bulk and dosage forms. Int J Pharm Sci Res. 2011;2(9):2335-2340.

[12] Joshi H, et al. HPLC method development and validation for Atenolol and Indapamide in tablets. Int J Pharm Anal. 2011;3(4):115-120.

[13] Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. London: CBS Publishers; 2007.