ISSN: 2347-6567

International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

IJAMSCR |Vol. 14 | Issue 3| Jul- Sep- 2026

www.ijamscr.com

DOI : https://doi.org/10.61096/ijamscr.v14.iss3.2026.1141-1154

Analytical Method Development and Validation for Estimation of Nadolol and Bendroflumethiazide in Bulk and Tablet Dosage Form by RP-HPLC

G. Renuka[*1], Govindula Sai Shresta[1], Guduru Aishwarya[1], J Bharath[1], Jakku Shivani[1], Jena Bingnesh[1], Dr. Kantlam

[1]*Department of Pharmaceutical Analysis, Brilliant Grammar School Educational Society's Group of Institutions (Engineering and Pharmacy), Abdullapur (V), Abdullapurmet (M), Rangareddy (D), Hyderabad - 501 505.*

*Corresponding Author: **G.Renuka***
Email: Renu1291@gmail.com

Published on: 06.07.2026
Published by: Futuristic Publications
2026| All rights reserved.

Creative Commons Attribution 4.0 International License.

Abstract: A simple, rapid, precise, and accurate reverse phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Nadolol and Bendroflumethiazide in bulk drug substances and tablet dosage forms. Chromatographic separation was achieved using a C18 column with an optimized mobile phase consisting of an appropriate mixture of organic solvent and aqueous buffer at a constant flow rate. Detection was carried out at a selected wavelength where both drugs exhibited adequate absorbance.

The proposed method showed good resolution with well-defined and symmetrical peaks for both Nadolol and Bendroflumethiazide. Method validation was performed in accordance with ICH guidelines, evaluating parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability. The method demonstrated excellent linearity over the selected concentration ranges with correlation coefficients close to unity. Accuracy studies showed satisfactory percentage recovery of both drugs, while precision studies revealed low %RSD values, confirming the reproducibility of the method. Robustness studies indicated that minor deliberate variations in chromatographic conditions did not significantly affect the analytical results.

The validated RP-HPLC method was successfully applied to the quantitative analysis of Nadolol and Bendroflumethiazide in tablet dosage forms, with assay results within acceptable limits. The developed method is suitable for routine quality control analysis of Nadolol and Bendroflumethiazide in bulk and pharmaceutical formulations.

Keywords: Nadolol; Bendroflumethiazide; RP-HPLC; Method Development; Method Validation; Tablet Dosage Form; ICH Guidelines.

1. INTRODUCTION

Analytical chemistry is a branch of chemistry focused on the qualitative and quantitative determination of the chemical components in substances. It plays a vital role in scientific research, pharmaceuticals, environmental monitoring, food safety, and many industrial processes. Analytical chemistry is broadly divided into qualitative analysis, which identifies what substances are present, and quantitative analysis, which measures how much of each substance is present. To achieve this, a wide range of techniques is employed, each suited to specific types of analysis and sample conditions.

Among the most widely used techniques is spectroscopy, which involves the interaction of light with matter. Common forms include UV-Visible spectroscopy, used for analyzing compounds that absorb

ultraviolet or visible light; Infrared (IR) spectroscopy, useful for identifying functional groups in organic molecules; and Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma (ICP) for detecting metal ions in trace amounts. Mass spectrometry (MS) is another powerful technique used to determine the molecular weight and structure of compounds by measuring the mass-to-charge ratio of ions.

Chromatographic techniques, such as Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC), are extensively used for separating mixtures into their individual components before identification and quantification. These methods are crucial in drug testing, food analysis, and forensic investigations. Electroanalytical methods like potentiometry, voltammetry, and conductometry are used to study chemical properties through their electrical behavior, particularly in redox reactions and ionic compounds.

Other notable techniques include titration, a classical method used in volumetric analysis to determine concentration, and thermal analysis, such as Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC), which study changes in physical and chemical properties as a function of temperature. Overall, analytical chemistry combines precision, accuracy, and advanced instrumentation to provide essential information about chemical substances in various applications.

The qualitative and quantitative composition of the substance examined is determined by analytical chemistry. To interpret the sample material, all aspects are necessary. Analytical chemistry is classified into two types: quantitative and qualitative. A qualitative analysis tells us about the nature of the sample by determining the presence or absence of certain elements.

The conventional and Physicochemical properties are used for the study of the sample in non-instrumental. The instrumental methods of analysis are focused on measurements to determine a chemical composition of certain physical properties of the substance using the apparatus. Analytical procedures created using advanced instruments such as spectrophotometers, HPLC, GC, and HPTLC therefore have broad variety of applications to assure raw and finished materials quality and quantity.

1.1 Chromatography

Chromatography represents for two words: “chromo”-color and “graphy” –writing.[1] Chromatography is a process used to isolate the mixture components by continuous distribution between two stages of the component. One stage pass (Mobile phase) continuously over the other phase (stationary phase).

1.1.1 Type of chromatography

- **Absorption chromatography**

If the phase is solid and mobile, it is considered an Adsorption chromatography. If the phase is liquid or gaseous.

Examples: Thin layer chromatography, column chromatography, gas-solid chromatography.

- **Partition chromatography**

The partition chromatography is the stationary and mobile phases are liquid.

Example: Paper partition chromatography, Gas-liquid chromatography.

1.1.2 Theory of chromatography

- **The plate theory**

According to the original martin and Synge model. The plate model suggests that the chromatographic column has a high number of distinct layers, which are referred to as "Theoretical Plates." The separate equilibrations of the sample between the stationary phase and mobile phase occur in the plates. The analyte moves down the column by transfer of equilibrated mobile phase from one plate to next plate.

- **The rate theory**

A most realistic description of the processes at work inside a column takes. The time it takes for the solute to reach equilibrium between the stationary and mobile phases. The pace of elution therefore influences the band shape of a chromatographic peak. It is also affected by the different path available to solute molecule as they travel between particles of stationary phase.

1.1.3 Phase of chromatography

A) Based on modes of chromatography

- • Normal phase chromatography

The mobile phase in normal phase chromatography is non-polar, whereas the stationary phase is polar. Hence, the station phase retains the polar analyte. The polarity of solute molecules increases their adsorption capability, due to longer elution time. This is because of lower affinity between the non-polar compounds and they stationary phase. Polar compound in the mixture being passing through the column. These techniques can be used to separate, identity and it is not generally used for pharmaceutical applications.[2]

• Reverse phase chromatography

In the reverse phase column, chemical compounds are commonly used in many fields such as pharmaceuticals, the petroleum industry, forensics, and clinical science to isolate chemical compounds into their individual parts for purification. Non-polar hydrophobic packing with the functional group octyl or octa decyl silane or C18, C8, C4. The stationary phase and the mobile phase is polar solvent. In this mode, the polar compound gets eluted first and non-polar for a longer time, compounds are kept. Since most drugs and pharmaceuticals are polar in nature, they are not retained for longer periods and are therefore eluted more quickly.[3]

b) Based on principle of chromatography

- **Ion exchange chromatography**

Stationary stage Contains ion groups like NR3+, SO3 which is communicating with the ionic groups of the molecules in the study. This is sufficient for the separation of charged molecules. Shift in pH and the concentration of salt will modulate the retention.

- **Ion pair chromatography**

The techniques are often referred to reverse phase ion pair chromatography or soap chromatography. It can be used for the isolation of the ion compounds and this process can also replace ion exchange in chromatography. Strong acid and basic compounds can be distinguished by reverse phase mode by from of ion pairs (columbic association species shaped between two ions with opposite electrical charge) adequate counter ions.

- **Affinity chromatography**

This technique is very much used relevant biochemical separation interactions. This is stationary step comprises unique group of molecules. The sample can be absorbed if any steric and charged. The necessary conditions have been achieved. This technique can be used isolate proteins, enzymes and complex antibodies blends.

- **Size exclusion chromatography**

The molecules are distinguished by their molecular masses. The biggest molecules are first eluted and the smallest last. This approach is commonly used where a combination of involves at least 10 % molecular mass difference. [4,6]

c) Based on scale of operation

Analytical HPLC: Where only the samples are evaluated. Sample recovery is not done because only very small samples are used.

Preparative HPLC: Where the individual compound fractions can be collected by means of a fractional collector. Samples gathered shall be reused.

d) Based on type of analysis

Qualitative analysis: The compound is identified, the presence of impurities is detected, the number of components is determined, etc. The retention time values are performed.

Quantitative analysis: The quantity of individuals or multiple components in a mixture is determined. The peak area of standard and sample is compared for this purpose.

EXPERIMENTAL WORK

INSTRUMENTS USED

HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector.
pH meter	LabIndia
Weighing machine	Sartorius

Volumetric flasks	Borosil
Pipettes and Burettes	Borosil
Beakers	Borosil
Digital ultra sonicator	Labman

CHEMICALS USED:

Nadolol	Corgard®
Bendroflumethiazide	Aprinox®
Water and Methanol for HPLC	LICHROSOLV (MERCK)
Acetonitrile for HPLC	Merck
Triethylamine	Trolamine

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Nadolol and Bendroflumethiazide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.15ml of the Nadolol and 2.81ml of the Bendroflumethiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Methanol: TEA Buffer with varying proportions. Finally, the mobile phase was optimized to Phosphate buffer (pH 3.0): Methanol (70:30 v/v) respectively.

Optimization of Column:

The method was performed with various columns like Symmetry and Phenomenex. Gemini C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	40ºC
Column	:	Hypersil C18 (4.6×250mm) 5µ
Mobile phase	:	Phosphate buffer (pH 3.0): Methanol (70:30 v/v)
Flow rate	:	1ml/min
Wavelength	:	260 nm
Injection volume	:	10 µl
Run time	:	10 min

VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 700 ml (70%) of phosphate buffer (pH. 3), 300ml of methanol (30%) were mixed and degassed in digital ultra sonicated for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Phosphate buffer (pH 3.0): Methanol (70:30 v/v)
Column : Hypersil C18 (4.6×250mm) 5µ
Column temperature : 40ºC
Wavelength : 260nm
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 8minutes

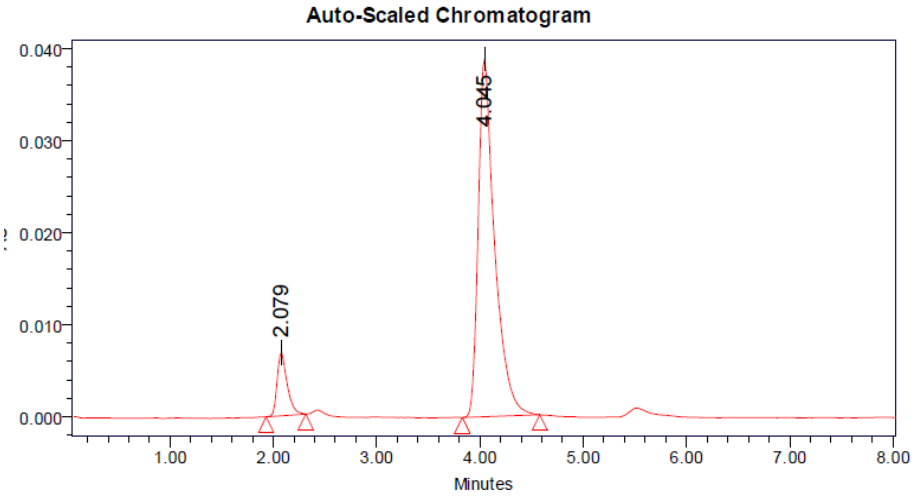

Fig.18: Optimized Chromatogram (Standard)

Table 18: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Nadolol	2.079	46168	6841	1.33	4251
2	Bendroflumethiazide	4.045	429069	38885	1.59	5224

Optimized Chromatogram (Sample)

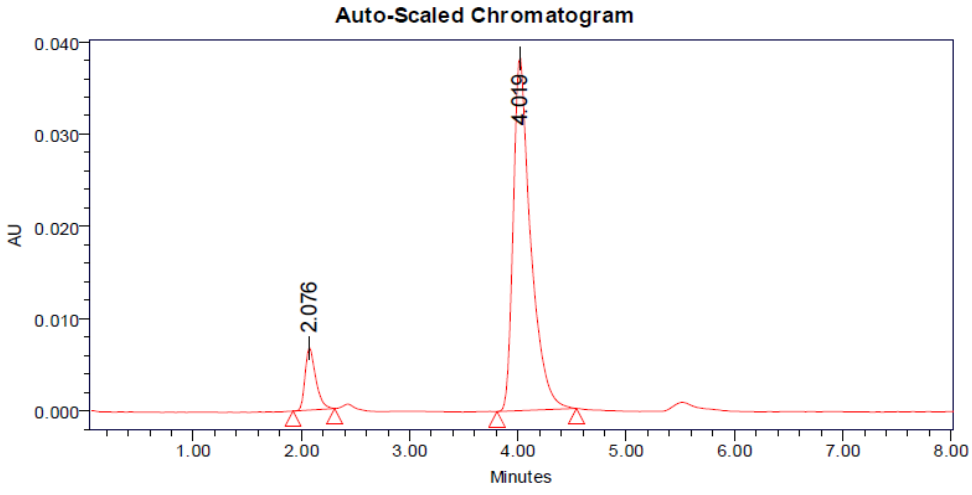

Fig.19: Optimized Chromatogram (Sample)

Table 19: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USPTailing	USPPlate Count
1	Nadolol	2.076	46150	6766	1.36	5152
2	Bendroflumethiazide	4.019	427826	38246	1.58	6071

Acceptance criteria:

- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION

Blank:

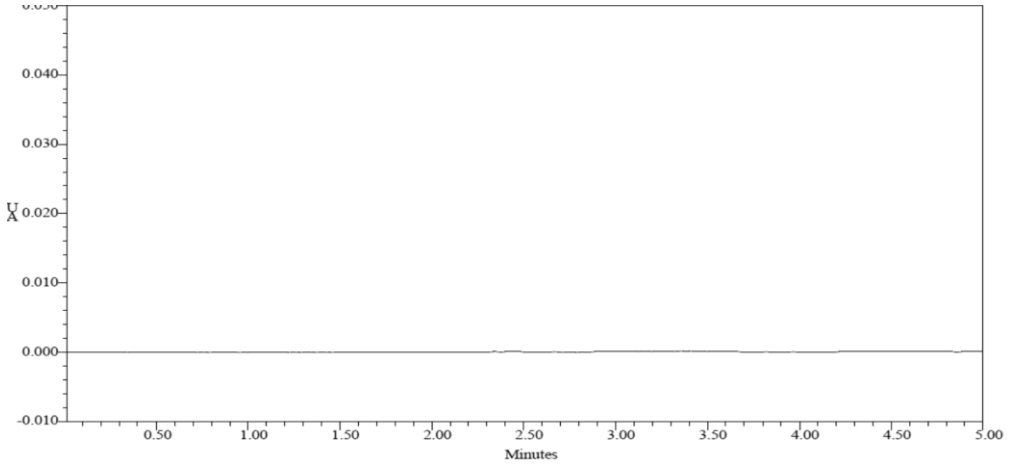

Fig.20: Chromatogram showing blank (mobile phase preparation)

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantities Nadolol and Bendroflumethiazide in drug product.

Assay (Standard):

Table 20: Peak results for assay standard of Nadolol

S. No	PeakName	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Nadolol	2.078	49569	6811	6945	1.51
2	Nadolol	2.080	49649	6999	6149	1.57
3	Nadolol	2.078	49731	6972	6473	1.49
4	Nadolol	2.079	49479	6971	6190	1.49
5	Nadolol	2.082	49684	6841	6294	1.49
Mean			49607			
Std.Dev.			107.963			
%RSD			0.217637			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table 21: Peak results for assay standard of Bendroflumethiazide

S. No	PeakName	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Bendroflumethiazide	4.041	423328	44147	7672	1.35
2	Bendroflumethiazide	4.033	423805	44538	7786	1.13
3	Bendroflumethiazide	4.050	423229	44964	5772	1.34
4	Bendroflumethiazide	4.045	423876	44959	5191	1.35
5	Bendroflumethiazide	4.032	423575	38885	5137	1.35
Mean			423559.5			
Std.Dev.			328.2606			
%RSD			0.0775			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.
-

Assay (Sample):

Table 22: Peak results for Assay sample of Nadolol

S. No	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Nadolol	2.078	46684	6918	1.34	5217	1
2	Nadolol	2.079	46168	6841	1.33	5251	2
3	Nadolol	2.077	46088	6851	1.37	7127	3

Table 23: Peak results for Assay sample of Bendroflumethiazide

S. No	Name	RT	Area	Height	USPTailing	USPPlateCount
1	Bendroflumethiazide	4.050	430575	39127	1.60	6197
2	Bendroflumethiazide	4.045	429069	38885	1.59	6224
3	Bendroflumethiazide	4.037	429543	38892	1.58	8203

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=429729/423559.5*10/120*120/0.199*99.7/100*1.1944/600*100

The % purity of Nadolol and Bendroflumethiazidein pharmaceutical dosage form was found to be 100.1522%

LINEARITY

Table 25: Chromatographic Data for Linearity Study for Nadolol:

Concentration µg/ml	Average Peak Area
5	15065
10	31009
15	46166
20	60569
25	76862

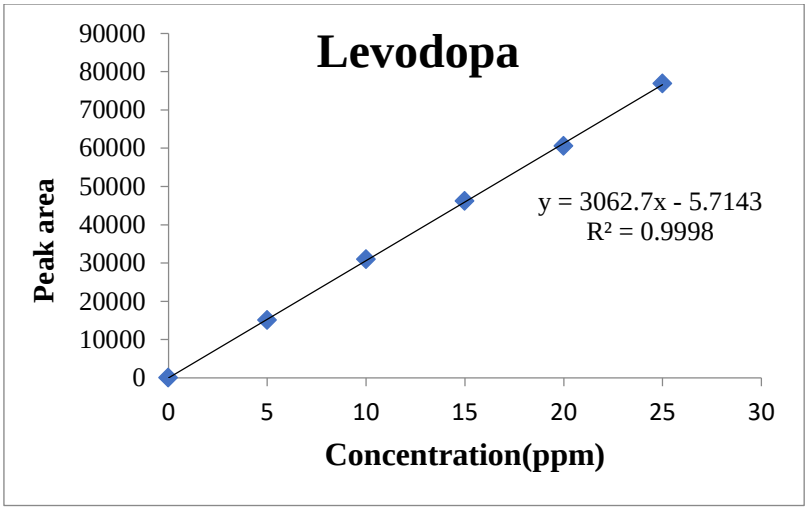

Fig.39: Chromatogram showing linearity level

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Nadolol is a straight line.

Y = mx + c

Slope (m) =3062

Intercept (c) = 5.7143

Correlation Coefficient (r) = 0.9998

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 5.7143. These values meet the validation criteria.

Table 26: Chromatographic Data for Linearity Study for Bendroflumethiazide:

Concentration µg/ml	Average Peak Area
100	131289
200	284775
300	427559
400	555861
500	712514

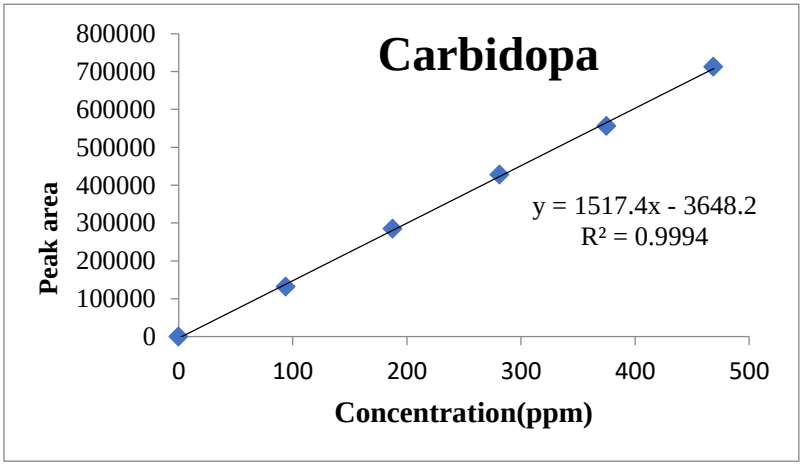

Fig 40: Chromatogram showing linearity level

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Bendroflumethiazide is a straight line.

Y = mx + c

Slope (m) =1517

Intercept (c) = 3648

Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 3650.1. These values meet the validation criteria.

Precision:

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

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 27: Results of repeatability for Nadolol:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Nadolol	2.077	46054	6784	4208	1.32
2	Nadolol	2.076	46803	6867	6088	1.34
3	Nadolol	2.076	46150	6766	4152	1.36
4	Nadolol	2.077	46056	6715	4184	1.32
5	Nadolol	2.074	46247	6746	4065	1.33
Mean			46262			
Std.dev			312.7099			
%RSD			0.675954			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 28: Results of repeatability for Bendroflumethiazide:

S. No	**Peak name**	**Retention time**	**Area (µV*sec)**	**Height (µV)**	**USP Plate Count**	**USP Tailing**
1	Bendroflumethiazide	4.031	427962	38634	5158	1.57
2	Bendroflumethiazide	4.024	429623	38673	5092	1.58
3	Bendroflumethiazide	4.019	427826	38246	5071	1.58
4	Bendroflumethiazide	4.016	427829	38310	5046	1.58
5	Bendroflumethiazide	4.014	429559	38181	5036	1.58
Mean			428559.8			
Std.dev			943.2246			
%RSD			0.220092			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Day 1:

Table 29: Results of Intermediate precision day1 for Nadolol

S. No	PeakName	RT	Area (µV*sec)	Height (µV)	USPPlate count	USPTailing
1	Nadolol	2.075	46204	6673	5117	1.33
2	Nadolol	2.074	46300	6735	5043	1.36
3	Nadolol	2.075	46259	6652	5087	1.28
4	Nadolol	2.075	46223	6667	5134	1.31
5	Nadolol	2.075	46205	6674	5151	1.32
6	Nadolol	2.074	46189	6703	5157	1.33
Mean			46230			
Std.Dev.			41.88556			
%RSD			0.090603			

Acceptance criteria:

%RSD of Six different sample solutions should not more than 2

Table 30: Results of Intermediate precision day1 for Bendroflumethiazide

S.No	PeakName	RT	Area (µV*sec)	Height (µV)	USPPlatecountt	USPTailing
1	Bendroflumethiazide	4.013	428922	38004	7038	1.58
2	Bendroflumethiazide	4.011	428524	37935	7999	1.57
3	Bendroflumethiazide	4.010	427239	37850	7003	1.57
4	Bendroflumethiazide	4.008	427667	37780	7982	1.57
5	Bendroflumethiazide	4.006	427826	37824	7983	1.57
6	Bendroflumethiazide	4.006	427093	37970	7042	1.58
Mean			427878.5			
Std.Dev.			718.1952			
%RSD			0.16785			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

Day 2:

Table 31: Results of Intermediate precision Day 2 for Nadolol

S. No	PeakName	RT	Area (µV*sec)	Height (µV)	USPPlate count	USPTailing
1	Nadolol	2.076	46803	6867	5149	1.57
2	Nadolol	2.076	46056	6715	5190	1.13
3	Nadolol	2.077	46252	6652	6088	1.58
4	Nadolol	2.075	46205	6674	5184	1.58
5	Nadolol	2.075	46940	7249	5087	1.57
6	Nadolol	2.072	46727	6983	5151	1.57
Mean			46497.17			
Std.Dev.			369.4739			
%RSD			0.794616			

Acceptance criteria:

% RSD of Six different sample solutions should not more than 2 Table:

Table 32: Results of Intermediate precision Day 2 for Bendroflumethiazide

S. No	PeakName	RT	Area (µV*sec)	Height (µV)	USPPlate count	USPTailing
1	Bendroflumethiazide	4.024	429623	38673	6789	1.49
2	Bendroflumethiazide	4.024	427829	38310	5772	1.34
3	Bendroflumethiazide	4.016	427263	37850	5092	1.32
4	Bendroflumethiazide	4.010	427826	37824	6046	1.28
5	Bendroflumethiazide	4.006	421284	40752	6003	1.32
6	Bendroflumethiazide	4.008	421832	40281	6983	1.33
Mean			425942.8			
Std.Dev.			3492.681			
%RSD			0.819988			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2

6.3.4: ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 36: The accuracy results for Nadolol

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	22938.33	7.5	7.3	99.88	100.166
100%	45426	15	14.7	98.89	
150%	70096.67	22.5	22.2	101	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table 37: The accuracy results for Bendroflumethiazide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	209357	140.6	140.2	99.7%	99%
100%	420697.7	281.25	281.1	99%	
150%	631550.7	421.8	421.4	99%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION FOR NADOLOL AND BENDROFLUMETHIAZIDE

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Nadolol

=3.3×520.9/3062
=0.56µg/ml

Bendroflumethiazide

=3.3×7922.4/1517
=17.2µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ=10\times\sigma/S$$

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Result:

Nadolol

=10×520.9/3062
=1.7µg/ml

Bendroflumethiazide

=10×7922.4/1517
=52.2µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Nadolol and Bendroflumethiazide. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Nadolol and Bendroflumethiazide were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

Table 38: Results for Robustness - Nadolol

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	46168	2.079	4251	1.33
Less Flow rate of 0.8mL/min	51177	2.29	5269	1.38
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	42190	1.890	5126	1.32
Less organic phase (about 5 % decrease in organic phase)	42402	1.885	5126	1.19
More organic phase (about 5 % Increase in organic phase)	42112	1.908	5854	1.36

Table 39: Results for Robustness-Bendroflumethiazide

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	429069	4.045	5224	1.59
Less Flow rate of 0.8mL/min	472673	4.450	6328	1.58
More Flow rate of 1.0mL/min	392497	3.660	6217	1.54

Less organic phase (about 5 % decrease in organic phase)	391379	4.251	6996	1.61
More organic phase (about 5 % Increase in organic phase)	391703	3.239	6120	1.50

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION

Summary

A simple, rapid, precise, and accurate reverse phase high-performance liquid chromatographic (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Nadolol and Bendroflumethiazide in bulk drug substances and tablet dosage forms. The method development involved systematic optimization of chromatographic conditions, including selection of a suitable stationary phase, mobile phase composition, flow rate, and detection wavelength, to achieve good resolution and symmetrical peak shapes for both analytes.

Chromatographic separation was achieved using a C18 column with an optimized mobile phase consisting of an appropriate ratio of organic solvent and aqueous buffer, delivered at a constant flow rate. Detection was carried out at a selected wavelength where both drugs showed adequate absorbance. Under the optimized conditions, Nadolol and Bendroflumethiazide were well separated with acceptable retention times, resolution, and theoretical plate counts.

The developed RP-HPLC method was validated according to ICH guidelines for parameters such as system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and assay of marketed formulation. Linearity was observed over the selected concentration ranges for both drugs with correlation coefficients close to unity. Accuracy studies demonstrated satisfactory recovery of both analytes, indicating the absence of interference from excipients present in the tablet formulation. Precision studies confirmed that the method is reproducible with low %RSD values. Robustness testing showed that minor deliberate variations in chromatographic conditions did not significantly affect the results.

The validated method was successfully applied to the quantitative analysis of Nadolol and Bendroflumethiazide in tablet dosage forms, and the assay results were found to be within acceptable limits, confirming the suitability of the method for routine quality control analysis.

Conclusion

The developed RP-HPLC method for the simultaneous estimation of Nadolol and Bendroflumethiazide is simple, sensitive, accurate, and reproducible. The method provides good resolution between the two drugs with short analysis time, making it economical and time-efficient. Validation results confirmed that the method meets all ICH requirements and is reliable for quantitative analysis.

The absence of interference from tablet excipients, along with excellent accuracy and precision, demonstrates the specificity and robustness of the method. Therefore, the proposed RP-HPLC method can be successfully employed for routine analysis, quality control testing, and stability studies of Nadolol and Bendroflumethiazide in bulk drugs as well as in pharmaceutical tablet dosage forms.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Brilliant Grammar School Educational Society's Group of Institutions (Engineering and Pharmacy), Abdullapur (V), Abdullapurmet (M), Rangareddy (D), Hyderabad., for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Pharma Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

BIBLIOGRAPHY

1. Azim Md, Sabir, Mitra Moloy, Bhasin Parminder S. HPLC Method development and Validation: A Review. In t. Res. J. Pharm, 2013; 4(4): 39-46.
2. Snyder L.R., Kirkland J.J and Glajch J. L., Prac-tical HPLC Method Development, John Wiley and Sons Inc. Canada, 1997; (2): 233-291.

3. Amesham Biosciences of Reversed Phase Chromatography, Principles and Methods, Int J Pharm Sci Res, 2012; 6-8.
4. Breaux J, Jones K, Boulas P., Understanding and implementing efficient analytical methods development and validation. Pharm Technol Anal Chem Test, 2003; (5) 6-13.
5. Sethi PD, HPLC: Quantitative Analysis of Pharmaceutical Formulation, New Delhi, CBS Publishers and Distributors, 1996; 113-202.
6. Sankar SR, Textbook of Pharmaceutical Analysis. Rx publications, Tirunelveli, 2006; 5(13): 1-2.
7. Manasa Padma Meduri*; Pooja Agarwal; G. Vimala; Nafiza Banu. The development and validation studies of RP-HPLC method – A review. World J Pharm Sci, 2016; 4(1): 85-92.
8. Validation of Chromatographic Methods.Development and validation of a simple reversed phase HPLC-UV method for determination of oleuropein in olive leaves, Fuad Al Rimawi*Elsevier, science direct journal of food and drug analysis, 2014; 22: 28.
9. Reversed phase HPLC Buffers: High Quality Buffers (solutions, solids or concentrates): available from: ccc.chem.pitt.edu/wipf/web/ HPLC_RP_buffers.pdf. Accessed April 05, 2013.
10. Effective HPLC method development. Available from http://www.cvg.ca/images/HPLC_Method_Development.pdf.Accessed April 05, 2013.
11. Changhe Wen, Designing HPLC Methods for Stability Indication and Forced Degradation Samples for API, Collected from American Pharmaceutical Review at American pharmaceutical review. com. Accessed April, 2013; 05.
12. Sabir AM, Molony M, Parminder SB. HPLC Method Development and validation: A Review. International research Journal of pharmacy, 2013; 4(4): 39-46.
13. Noman A, Bukhaiti ALWedad Q, Alfarga A, AbedSherif M, Mahdi AA. And Waleed AA. HPLC technique used in food analysis-Review. International Journal of Agriculture Innovations and Research, 2016; 5(2): 181-188.
14. Lindholm J, Development and Validation of HPLC Method for Analytical and Preparative Purpose, Acta Universities Upsaliensis Uppsala, 2004; 13-14.
15. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. 2nd edition. New York. John wiley, 1997; 233-291. http://dx.doi.org/10.1002/9781118592014
16. B. Prathap, G.H.S. Rao, G. Devdass, A. Dey, N. Harikrishnan, Review on Stability Indicating HPLC Method Development, International Journal of Innovative Pharmaceutical Research, 2012; 3(3): 229-237.
17. Sowjanya P., Subashini D., Rakesh S., A Review on Method Development and validation by HPLC, RRJPPS, 2015; 4(1).
18. Donald D. Hong and Mumtaz Shah, Development and validation of HPLC Stability indicating Assays, In: Sens T. Carstensen, C.T.Rhodes, editors Drug Stability - Principle & Practice. 3rd Edition. New York: Marcel Dekker Inc, 2008; 332.
19. LR Snyder, JL Glajch, JJ Kirkland. Practical HPLC Method Development. New York: John Wiley, 1988; 227-251.
20. P. D. Sethi; High Performance Liquid Chromatography, Quantitative Analysis of Pharmaceutical Formulations; 1stedition, New Delhi: CBS Publishers and Distributors, 2001; 1, 3-11: 116-120.