# DEVELOPMENT OF STABILITY INDICATING RP-HPLC METHOD AND VALIDATION FOR THE ESTIMATION OF NADOLOL AND BENDROFLUMETHIAZIDE IN PURE FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM

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***ABSTRACT: A Novel Analytical simple, reproducible and efficient RP-HPLC method was developed for simultaneous estimation of Nadolol and Bendroflumethiazide in pure form and marketed combined pharmaceutical dosage forms. A column having Develosil ODS HG-5 RP C18, 15cmx4.6mm, i.d. Column in isocratic mode with mobile phase containing Methanol: Acetonitrile in the ratio of 85:15% v/v was used. The flow rate was 1.0 ml/min and effluent was monitored at 258nm. The retention times and linearity range for Nadolol and Bendroflumethiazide was found to be (2.217, 5861min) and (0-14, 0-28), respectively. The method has been validated for linearity, accuracy and precision, robustness and limit of detection and limit of quantitation. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.08µg/ml and 0.24µg/ml for Nadolol and 0.1µg/ml 0.3µg/ml for Bendroflumethiazide respectively. The proposed method was found to be accurate, precise and selective for simultaneous estimation of Nadolol and Bendroflumethiazide in pure form and marketed combined pharmaceutical dosage forms.***
***Keywords: Nadolol and Bendroflumethiazide, RP-HPLC, Validation, Accuracy, Precision.***

## I. INTRODUCTION

Nadolol is a non-selective beta-adrenergic antagonist with antihypertensive and antiarrhythmic activities. Nadolol[1] competitively blocks beta-1 adrenergic receptors located in the heart and vascular smooth muscle, inhibiting the activities of the catecholamines epinephrine and norepinephrine and producing negative inotropic and chronotropic effects. This agent exhibits antiarrhythmic activity via the impairment of atrioventricular (AV) node conduction and a corresponding reduction in sinus rate. In the kidney, inhibition of the beta-2 receptor within the juxtaglomerular apparatus results in the inhibition of renin production and a subsequent reduction in angiotensin II and aldosterone levels, thus inhibiting angiotensin II-dependent vasoconstriction and aldosterone-dependent water retention. Nadolol[2] is a nonselective beta adrenal receptor blocker that is used to lower blood pressure. It has a long duration of action as it is usually taken once daily and a wide therapeutic index as patients start at doses of 40mg daily but may be increased to doses as high as 240mg daily. Patients taking Nadolol[3] should not aburptly stop taking it as this may lead to exacerbation of ischemic heart disease. The IUPAC Name of Nadolol is (2R, 3S)-5-[3-(tert-butyl amino)-2-hydroxy propoxy]-1, 2, 3, 4-tetrahydro naphthalene-2, 3-diol. The Chemical Structure of Nadolol is as following

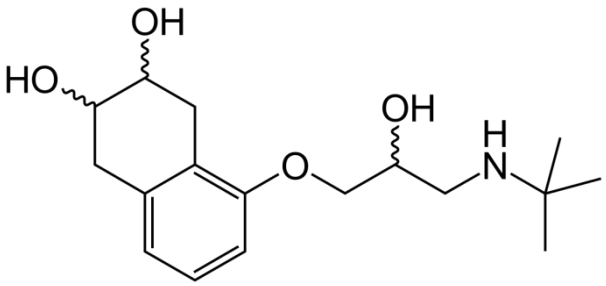


Fig.1. Chemical Structure of Nadolol

Bendroflumethiazide is a long-acting agent, also known as bendrofluazide, belonging to the class of thiazide diuretics with antihypertensive activity. A thiazide diuretic with actions and uses similar to those of hydrochlorothiazide. It has been used in the treatment of familial hyperkalemia, hypertension, edema, and urinary tract disorders. Bendroflumethiazide[4] is a thiazide diuretic which works by inhibiting sodium reabsorption at the

beginning of the distal convoluted tubule (DCT). Water is lost as a result of more sodium reaching the collecting ducts. Bendroflumethiazide has a role in the treatment of mild heart failure although loop diuretics are better for reducing overload. The main use of Bendroflumethiazide[5] currently is in hypertension (part of the effect is due to vasodilation). Bendroflumethiazide, a thiazide diuretic, removes excess water from the body by increasing how often you urinate (pass water) and also widens the blood vessels which help to reduce blood pressure. It inhibits Na+/Cl- reabsorption from the distal convoluted tubules in the kidneys. Thiazides also cause loss of potassium and an increase in serum uric acid. Thiazides are often used to treat hypertension, but their hypotensive effects are not necessarily due to their diuretic activity. Thiazides have been shown to prevent hypertension-related morbidity and mortality although the mechanism is not fully understood. Thiazides cause vasodilation by activating calcium-activated potassium channels (large conductance) in vascular smooth muscles and inhibiting various carbonic anhydrases in vascular tissue. The IUPAC Name of Bendroflumethiazide[6] is 3-benzyl-1, 1-dioxo-6-(trifluoro methyl)-3,4-dihydro-2H-1λ6,2,4-benzothiadiazine-7-sulfonamide. The Chemical Structure of Bendroflumethiazide is as follows

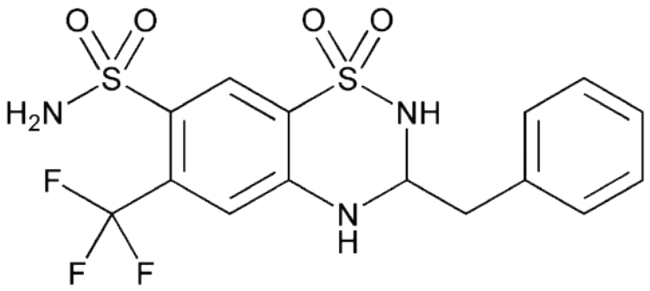


Fig.2. Chemical Structure of Bendroflumethiazide

Literature survey[36-40] reveals that few analytical methods are available for simultaneous estimation of Nadolol and Bendroflumethiazide. But there are some limitations are observed for the available methods, hence, the present work describes the development of a simple, precise, accurate and reproducible RP-HPLC method for the simultaneous estimation of Nadolol and Bendroflumethiazide.

## II. MATERIALS AND METHODS

**Table-1: List of Instrument used**

| S. No. | Instruments/Equipment/Apparatus |
|---|---|
| 1. | HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters). |
| 2. | T60-LAB INDIA UV – Vis spectrophotometer |
| 3. | Electronic Balance (SHIMADZU ATY224) |
| 4. | Ultra Sonicator(Wensar wuc-2L) |
| 5. | Thermal Oven |
| 6. | Symmetry ODS RP $C_{18}$,5□m, 15mm x 4.6mm i.d. |
| 7. | $P^H$ Analyzer (ELICO) |
| 8. | Vacuum filtration kit (BOROSIL) |

**Table-2: List of Chemicals used**

| S.No. | Name | Specifications | | Manufacturer/Supplier |
|---|---|---|---|---|
| | | Purity | Grade | |
| 1. | Doubled distilled water | 99.9% | HPLC | Sd fine-Chem ltd; Mumbai |
| 2. | HPLC Grade Water | 99.9% | HPLC | Sd fine-Chem ltd; Mumbai |
| 3. | Methanol | 99.9% | HPLC | Loba Chem; Mumbai. |
| 4. | Hydrochloric Acid | 99.9 | A.R. | Sd fine-Chem ltd; Mumbai |
| 5. | Acetonitrile | 99.9% | HPLC | Loba Chem; Mumbai. |
| 6. | Sodium Hydroxide | 99.9 | A.R. | Sd fine-Chem ltd; Mumbai |

**Nadolol Standard Solution Preparation**

Weigh accurately 10 mg of standard Nadolol and it transferred into a clean & dry 100 ml of volumetric flask. Add 10ml mobile phase and further do sonication in order to dissolve. Finally make up to the volume up to mark with the mobile phase. The final resulted solution contained about 100 µg/ml of Nadolol.

**Bendroflumethiazide Standard Solution Preparation**

Weigh accurately about 10 mg of standard Bendroflumethiazide and transferred into a clean and dry 100 ml volumetric flask. Add 10ml mobile phase and further do sonication in order to dissolve. Finally make up the volume with the same mobile phase i.e. same solvent system. The volume was made up to the mark with same solvent. The final solution contained about 100µg/ml of Bendroflumethiazide.

**Initialization of the HPLC Instrument**

First switched on the HPLC instrument. The selected column[7] was washed with the HPLC grade water for 45 minutes. Then selected column was saturated with the mobile phase for 45 minutes. Then keep the mobile phase for stabilization[8]. The mobile phase was run to obtain the peaks. After completion of stabilization. After 20 minutes the standard drug solution was injected in HPL

**OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS:**

The different HPLC chromatographic conditions were used to find out the optimum chromatographic condition for best elution of drugs.

**Table-3: Different Chromatographic used and their Optimizations**

| S.No. | Column Used | Mobile Phase | Flow Rate | Wave length | Observation | Result |
|---|---|---|---|---|---|---|
| 1 | Symmetry C i8, 5pni, 25emx4.6mm i.d. | water: Methanol =30:70 | 1.0 ml/min | 258nm | Peaks didn't Separate | Method rejected |
| 2 | Waters C18, 5pm, 25cmx4.6inini.d. | Water : ACN = 55:45 | 1.0 ml/min | 258nm | Early elution of peak | Method rejected |
| 3 | Waters C18, 5pm, 25cmx4.6inini.d. | ACN: methanol= 60: 40 | 1.0 ml/min | 258nm | Low resolution peak | Method rejected |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | Develosil ODS HG-5 RP C18, 5pin,15emx4.6mm i.d. | ACN: methanol 90:10 | 1.0 ml/ min | 258nm | Resolution increases but Peak shapes not good | Method rejected |
| 5 | Develosil ODS HG-5 RP C18, 5pni, l5emx4.6mm i.d. | Methanol : Acetonitrile = 85:15 | 1.0 ml/min | 258nm | Nice and Good Peaks | Method Accepted |

**Method Validation**

The present method of analysis was conducted to obtain a new, cost effective, convenient method for HPLC determination of Nadolol and Bendroflumethiazide in bulk and pharmaceutical formulation. The experimental method was validated according to the recommendations of ICH[35]-1996 and USP-30 for the parameters like specificity, system suitability, accuracy, linearity, precision, robustness.

**Specificity**

The specificity of the method was evaluated to ensure that there is no interference of excipients, diluting solution in the chromatogram of Nadolol and Bendroflumethiazide. The specificity[9] was studied by injecting the excipients, diluting solution and standard solution of Nadolol and Bendroflumethiazide.

**System Suitability**

System suitability[10-11] was performed by injecting six replicates of standards and two replicates of sample preparation at a 100% level to verify the accuracy and precision of the chromatographic system. This method was evaluated by analyzing the repeatability of retention time, tailing factor, theoretical plates of the column.

**Precision**

The precision of an analytical method is the degree of agreement among individual test results where the method is applied repeatedly to multiple samplings. Precision[12] of the assay was assessed with respect to repeatability, reproducibility and intermediate precision by estimating the assay for six different sample preparations of same batch. Results of analysis for repeatability, intermediate precision, and reproducibility[13] are given in the table 4.

**Robustness**

The robustness[14] is the ability of method to remain unaffected by small changes in parameters. The robustness of the method was determined by purposely altering experimental conditions and % assay of Nadolol and Bendroflumethiazide, peak tailing, theoretical plates, % RSD[15] were calculated.

**Accuracy**

Recovery[16] assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 80%, 100%and 120% to the pre analyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate. The results were tabulated in Table 3.

**Linearity**

Calibration graphs[17] were constructed by plotting peak area vs concentration of Nadolol and Bendroflumethiazide and regression equations were calculated. The calibration graphs were plotted and over 5 different linear concentrations in the range[18] of 5.

**Limit of Detection:** Detection Limit as far as possible (DL) or breaking point of identification (LOD) of an individual method is the most minimal measure of analyte in an example that can be recognized however not really quantitated as a n correct esteem. In investigative strategies that display standard commotion, the LOD[19] can be founded on a flag to-clamor (S/N) proportion (3:1), which is generally communicated as the centralization of analyte in the example.

**Limit of Quantification:** Quantization Limit is the breaking point of Quantization (LOQ) or Quantization cutoff of an individual butt-centric typical system is the most reduced measure of analyte in an example that can be quantitatively decided with appropriate exactness and precision. For diagnostic al strategies, for example, HPLC that display gauge commotion, the LOQ[20] is for the most part evaluated from an assurance of S/N proportion (10:1) and is typically affirmed by infusing guidelines which give this S/N proportion and have a worthy percent relative standard deviation too.

## III. RESULTS AND DISCUSSION

**Method Development**

**Selection of Wavelength**

Selectivity[21] of HPLC method that uses UV detector depends on proper selection of Wavelength. A wavelength which gives good response for the drug to be detected is to be selected. From the UV spectra 284 nm was selected as the wavelength for study. The λ max of this method can be determined as 284 nm.

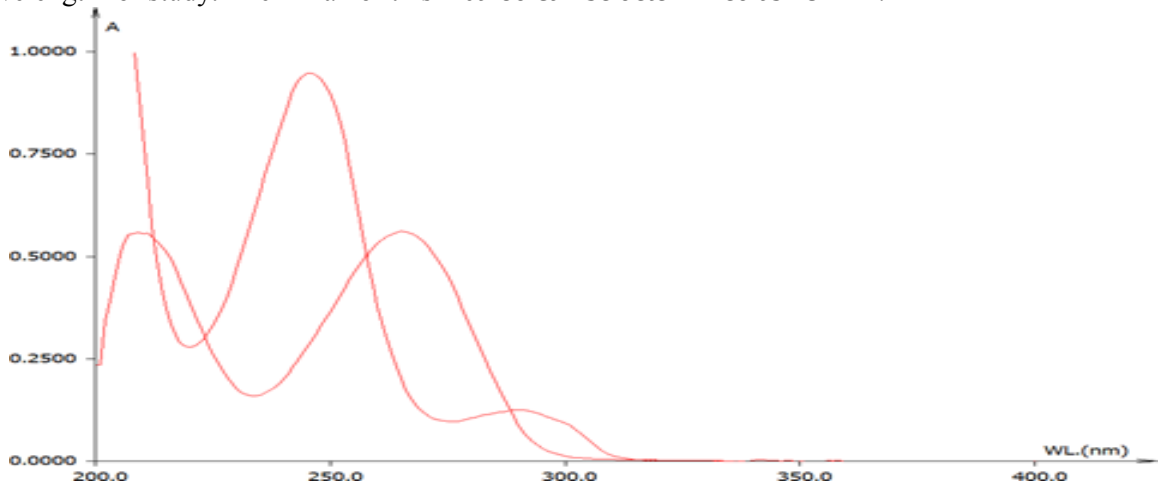


**Fig-3: Isobestic point Nadolol and Bendroflumethiazide (258nm)**

**Optimized Chromatographic Method:**

**Table-4: Summary of Optimized Chromatographic Conditions**

| | |
|---|---|
| Mobile phase | Methanol: Acetonitrile 85:15% v/v |
| Column | Develosil ODS HG-5 RP $C_{18}$, 15cmx4.6mm, i.d. Column. |
| Column Temperature | Ambient |
| Detection Wavelength | 258 nm |
| Flow rate | 1.0 ml/ min. |
| Run time | 15 min. |
| Temperature of Auto sampler | Ambient |
| Diluent | Mobile Phase |
| Injection Volume | 10µl |
| Type of Elution | Isocratic |

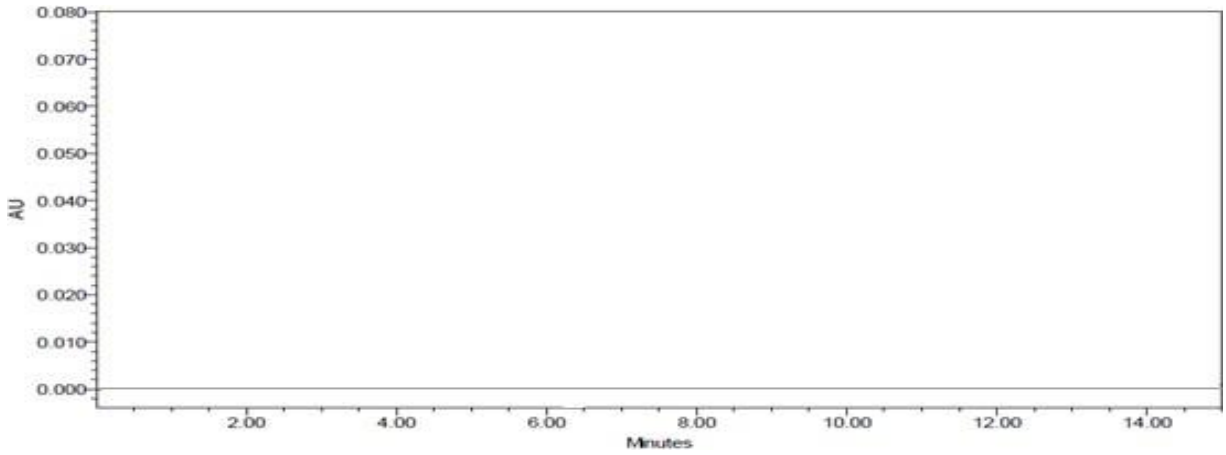


Fig. 4. HPLC Spectrum of Nadolol and Bendroflumethiazide (Blank Solution)

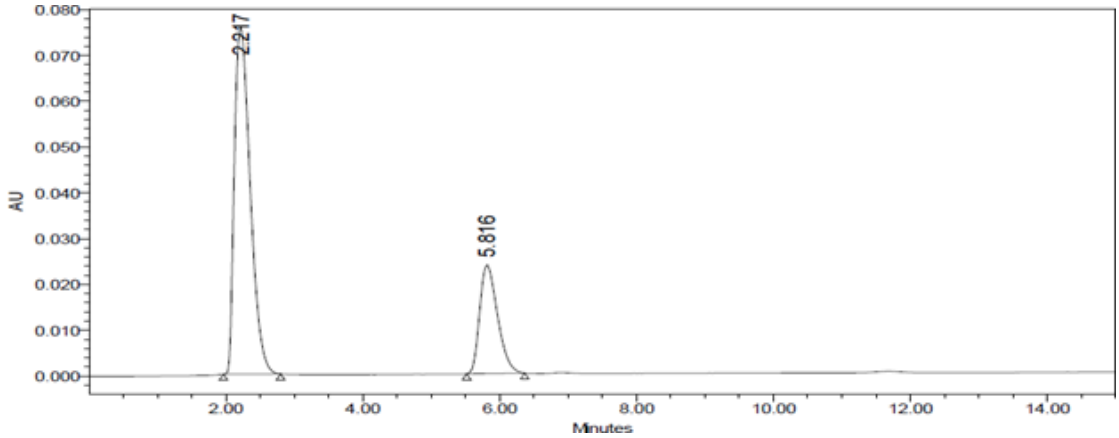


Fig.5. Chromatogram of Nadolol and Bendroflumethiazide in Optimized Chromatographic Condition

**Validation of Method**

The present method of analysis was conducted to obtain a new, cost effective, convenient method for HPLC[22] determination of Nadolol and Bendroflumethiazide in bulk form and marketed pharmaceutical dosage form. The experimental method was validated according to the recommendations of ICH-1996 and USP-30 for the parameters like specificity, system suitability, accuracy, linearity[23], precision, robustness.

**1. Linearity and Range:**

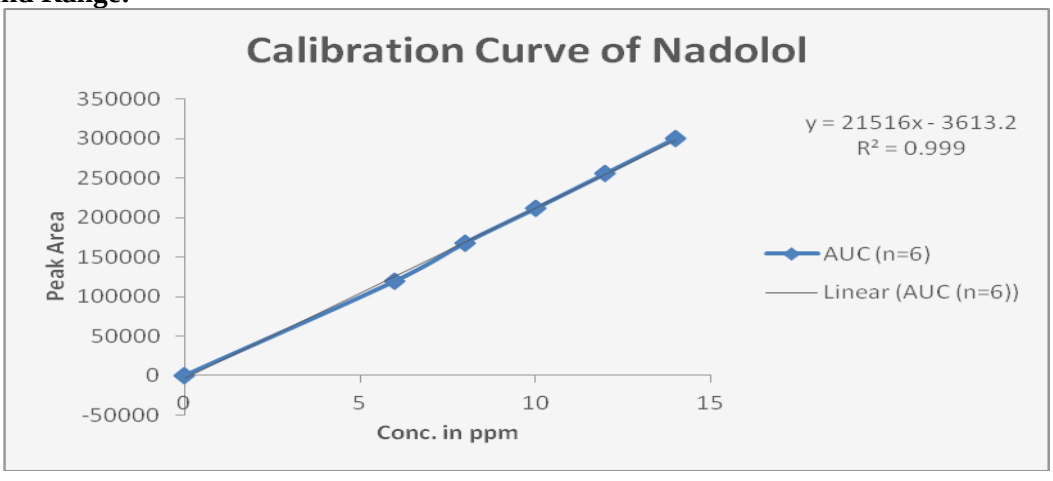


Fig.6. Standard curve for Nadolol

**Table-5: Linearity Results for Nadolol**

| CONC. (µg/ml) | AUC (n=6) |
|---|---|
| 0 | 0 |
| 6 | 119571 |
| 8 | 167873 |
| 10 | 211264 |
| 12 | 255428 |
| 14 | 299987 |

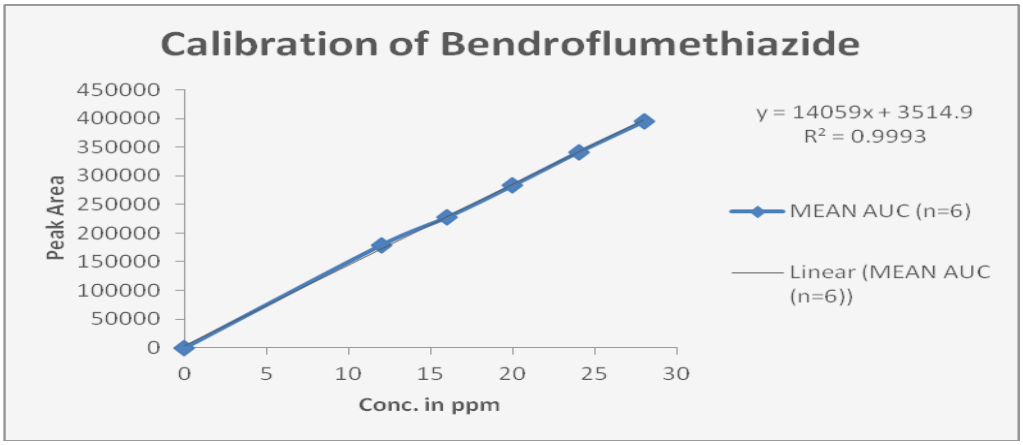


Fig.7.Standard Curve for Bendroflumethiazide

**Table-6: Linearity Results for Bendroflumethiazide**

| CONC.(µg/ml) | MEAN AUC (n=6) |
|---|---|
| 0 | 0 |
| 12 | 179371 |
| 16 | 227893 |
| 20 | 283264 |
| 24 | 341428 |
| 28 | 394987 |

**2. Accuracy:**

**Table-7: Accuracy Readings for Nadolol**

| Sample ID | Concentration (µg/ml) | | | %Recovery of Pure drug | Statistical Analysis |
|---|---|---|---|---|---|
| | Conc. Found | Conc. Recovered | Peak Area | | |
| $S_1$ : 80 % | 8 | 7.997368 | 115949 | 99.9671 | Mean= 100.7003% |
| $S_2$ : 80 % | 8 | 8.106622 | 117485 | 101.3328 | S.D. = 0.6884036 |
| $S_3$ : 80 % | 8 | 8.064087 | 116887 | 100.8011 | % R.S.D.= 0.683616% |
| $S_4$ : 100 % | 10 | 9.904901 | 142767 | 99.04901 | Mean= 100.36157% |
| $S_5$ : 100 % | 10 | 10.02966 | 144521 | 100.2966 | S.D. = 1.346221 |
| $S_6$ : 100 % | 10 | 10.17391 | 146549 | 101.7391 | R.S.D.= 1.3413706% |
| $S_7$ : 120 % | 12 | 12.01807 | 172476 | 100.1506 | Mean= 100.183756% |
| $S_8$ : 120 % | 12 | 11.88079 | 170546 | 99.00657 | S.D. = 1.19411 |
| $S_9$ : 120 % | 12 | 12.16729 | 174574 | 101.3941 | % R.S.D. = 1.19191% |

***Recovery study:*** Bendroflumethiazide

*Table-8: Accuracy Results for Bendroflumethiazide*

| Sample ID | Concentration (µg/ml) | | | %Recovery of Pure drug | Statistical Analysis |
|---|---|---|---|---|---|
| | Conc. Found | Conc. Recovered | Peak Area | | |
| $S_1$ : 80 % | 16 | 16.08685 | 229679 | 100.5428 | Mean= 100.54488% |
| $S_2$ : 80 % | 16 | 15.93079 | 227485 | 99.56745 | S.D. = 0.97847% |
| $S_3$ : 80 % | 16 | 16.2439 | 231887 | 101.5244 | R.S.D.= 0.9731% |
| $S_4$ : 100 % | 20 | 20.07632 | 285767 | 100.3816 | Mean= 99.97095% |
| $S_5$ : 100 % | 20 | 19.98769 | 284521 | 99.93847 | S.D. = 0.395406 |

| | | | | | |
|---|---|---|---|---|---|
| $S_6$ : 100 % | 20 | 19.91856 | 283549 | 99.59279 | % R.S.D.= 0.39552% |
| $S_7$ : 120 % | 24 | 23.75432 | 337476 | 98.97634 | Mean= 100.27718% |
| $S_8$ : 120 % | 24 | 24.11494 | 342546 | 100.4789 | S.D. = 1.21262<br>% R.S.D. = 1.20927% |
| $S_9$ : 120 % | 24 | 24.33032 | 345574 | 101.3763 | |

**Precision:**

**Repeatability**

**Table-9: Data showing repeatability analysis for Nadolol & Bendroflumethiazide**

| HPLC Injection Replicates | AUC for Nadolol | AUC for Bendroflumethiazide |
|---|---|---|
| Replicate – 1 | 113568 | 241022 |
| Replicate – 2 | 113241 | 240137 |
| Replicate – 3 | 115408 | 242911 |
| Replicate – 4 | 117412 | 245245 |
| Replicate – 5 | 112541 | 241941 |
| Replicate – 6 | 112546 | 240444 |
| **Average** | **114119.3333** | **241356.6667** |
| **Standard Deviation** | **1925.83838** | **1416.95812** |
| **% RSD** | **1.68756** | **0.58708** |

**Intermediate Precision:**

**Intra-Assay & Inter-Assay:**

The intra & inter day variation[24] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Nadolol and Bendroflumethiazide revealed that the proposed method is precise.

**Table-10: Results of Intra-Assay & Inter-Assay**

| Conc. of Nadolol (API) (µg/ml) | Observed Conc. of Nadolol (µg/ml) by the proposed method | | | |
|---|---|---|---|---|
| | Intra-Day | | Inter-Day | |
| | Mean (n=6) | % RSD | Mean (n=6) | % RSD |
| 8 | 8.09 | 0.97 | 8.03 | 0.96 |

| | | | | |
|---|---|---|---|---|
| 10 | 10.05 | 0.45 | 10.04 | 0.47 |
| 12 | 11.98 | 0.37 | 11.90 | 0.12 |

**Table-11: Data for Bendroflumethiazide Intra-Assay & Inter-Assay Analysis**

| Conc. of Bendroflumethiazide (API) (µg/ml) | Observed Conc. of Bendroflumethiazide (µg/ml) by the proposed method | | | |
|---|---|---|---|---|
| | Intra-Day | | Inter-Day | |
| | Mean (n=6) | % RSD | Mean (n=6) | % RSD |
| 8 | 7.97 | 0.27 | 8.09 | 0.59 |
| 10 | 10.14 | 1.29 | 9.95 | 0.64 |
| 12 | 12.08 | 0.61 | 11.94 | 0.26 |

**Result and Discussion:**

The Intraday and interday related studies shows that the % RSD was found to be within limit i.e. (≤2%). So it is indicated that the developed is within the limits. Hence finally we concluded that the developed method[25] was found to be precise.

**5. Limit of Detection (LOD) & Limit of Quantification (LOQ):**

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

$$L.O.D. = 3.3 (SD/S).$$

$$L.O.Q. = 10 (SD/S)$$

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

**Result & Discussion**

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.08 & 0.24 µg/ml respectively for Nadolol.

The LOD was found to be 0.1 µg/ml and LOQ was found to be 0.3 µg/ml for Bendroflumethiazide which represents that sensitivity[26] of the method is high.

**Method Robustness:**

Influence of small changes in chromatographic conditions[27] such as change in flow rate (± 0.1ml/min), Temperature (±2$^0$C), Wavelength[28] of detection (±2nm) & acetonitrile content in mobile phase[29] (±2%) studied to determine the robustness of the method are also in favour of (Table-4, % RSD < 2%) the developed RP-HPLC method for the analysis of Nadolol (API).

**Table-12: Result of Method Robustness Test**

| Change in parameter | % RSD |
|---|---|
| Flow (1.1 ml/min) | 1.05 |
| Flow (0.9 ml/min) | 0.67 |
| Temperature (27$^0$C) | 0.58 |
| Temperature (23$^0$C) | 0.61 |
| Wavelength of Detection (280 nm) | 0.38 |

| | |
|---|---|
| Wavelength of detection (270 nm) | 0.17 |

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2$^0$C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness[30] of the method are also in favour of (Table-4, % RSD < 2%) the developed RP-HPLC method for the analysis of Bendroflumethiazide (API).

**Table-13: Result of Method Robustness Test**

| Change in parameter | % RSD |
|---|---|
| Flow (1.1 ml/min) | 0.09 |
| Flow (0.9 ml/min) | 0.07 |
| Temperature (27$^0$C) | 0.28 |
| Temperature (23$^0$C) | 0.74 |
| Wavelength of Detection (235 nm) | 0.86 |
| Wavelength of detection (240 nm) | 0.67 |

**System Suitability Parameter:** It is an integral part of so many analytical procedures. The parameters are based on the idea that the equipment, electronics, analytical operations and the samples to be analyzed constitute as an integral system which can be examined. Finally system suitability test parameters[31-32] is established. The obtained data is shown in the following table-16.

**Table-14: Data of System Suitability Parameter**

| S.No. | Parameter | Limit | Result |
|---|---|---|---|
| 1 | Resolution | Rs ± 2 | 3.65 |
| 2 | Asymmetry | T ≤ 2 | Nadolol = 0.35<br>Bendroflumethiazide = 0.23 |
| 3 | Theoretical plates | N < 2000 | Nadolol = 3771<br>Bendroflumethiazide = 2437 |

**Estimation of Nadolol and Bendroflumethiazide in Pharmaceutical Dosage Form**

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Finally the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra sonicator, Then the final volume was make up to the mark with the mobile phase. The final solution was filtered through a selected membrane filter (0.45 µm) and in order to sonicate to degas the mobile phase (Solvent system[33]). From this above stock solution (1 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system (Mobile phase).

The prepared solutions were injected in five replicates into the HPLC system and the observations were recorded. A duplicate injection (Blank Solution) of the standard solution also injected into the HPLC system and the chromatograms and peak areas were recorded and calculated. The obtained data are shown in Table 40.

**ASSAY:**

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

Where:

AT = Test Preparation Peak Area
AS = Standard preparation Peak Area
WS = Working standard weight taken in mg
WT = Sample weight taken in mg
DS = Standard solution dilution
DT = Sample solution dilution
P = Working standard percentage purity

The assay was performed as explained in the previous chapter (Above). The results which are obtained are following:

**Table-15: Recovery Data for estimation Nadolol and Bendroflumethiazide in Corzide**

| Brand name of Nadolol and Bendroflumethiazide | Labelled amount of Drug (mg) | Mean (± SD) amount (mg) found by the proposed method (n=6) | Assay % (± SD) |
|---|---|---|---|
| Corzide Tablets (Pfizer Pharmaceutical Co., Ltd.) | 80mg/5mg | 79.687 (±0.598)/4.898 | 99.875 (±0.598)/99.698 (± 0.467) |

**Result & Discussion**: The %purity of Nadolol & Bendroflumethiazide for Tablets was found to be 99.875% and 99.698% respectively.

**Stability Studies**

**Results of Degradation Studies:**

The results of the stress studies indicated the **Specificity** of the method that has been developed. Nadolol and Bendroflumethiazide were stable only in photolytic stress conditions and little bit in thermal stress conditions[34]. The results of forced degradation studies are given in the following Table-18.

*Table-16: Results of Forced Degradation Studies of Nadolol and Bendroflumethiazide*

| Stress Condition | Time (hours) | Assay of active substance | Assay of degraded products | Mass Balance (%) |
|---|---|---|---|---|
| Acid Hydrolysis (0.1N HCl) | 24Hrs. | 95.62 | 4.38 | 100.00 |
| Basic Hydrolysis (0.IN NaOH) | 24Hrs. | 97.13 | 2.87 | 100.00 |

| | | | | |
|---|---|---|---|---|
| Thermal Degradation (60 $^0$C) | 24Hrs. | 96.24 | 3.76 | 100.00 |
| UV (254nm) | 24Hrs. | 95.43 | 4.57 | 100.00 |
| 3% Hydrogen peroxide | 24Hrs. | 96.16 | 3.84 | 100.00 |

## III. SUMMARY AND CONCLUSION

Isocratic elution is easy, needs only one pump & flat standard splitting up for easy and also reproducible results. So, it was preferred for the present research over gradient elution.

In case of RP-HPLC various columns are offered, however below Develosil ODS HG-5 RP C18, 5µm, l5cmx4.6mm i.d. column was preferred since using this column top shape, resolution as well as absorbance were great.

Mobile stage & diluent for preparation of different examples were completed after researching the solubility of API in various solvents of our disposal (methanol, Acetonitrile, water, 0.1 N NaOH, 0.1 N HCl).

Discovery wavelength was picked after checking the basic remedy of drug over 200 to 400nm. From the U.V spectrum of Nadolol and also Bendroflumethiazide it is evident that the majority of the HPLC work can be achieved in the wavelength variety of 200-300 nm easily. Even more, a circulation rate of 1 ml/min & an injection volume of 10µl were found to be the best evaluation.

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