CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://zenodo.org/records/13151294

Avalable online at: http://www.iajps.com *Research Article*

ESTIMATION OF NADOLOL AND BENDROFLUMETHIAZIDE IN API AND ITS DOSAGE FORM BY RP HPLC

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Article Received: September 2020 **Accepted:** October 2020 **Published: November** 2020

Abstract:

We have developed a simple, general, and reliable HPLC Method and validation for the simultaneous estimation of nadolol and bendroflumethiazide drugs according to (ICH) guidelines. Nadolol and bendroflumethiazide peaks have been observed at a retention time of 1.757, and 3.208 minutes, respectively, and they have kept 5 minutes as a total run time. As per linearity results, the average correlation coefficient of nadolol and bendroflumethiazide is 0.999 which indicates they have good linearity, robustness, and stability. Keywords: LC-MS/MS, RP-HPLC, Nadolol, Bendroflumethiazide.

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QR code

Please cite this article in press Mohammod Yakub Pasha et al, ***Estimation Of Nadolol And Bendroflumethiazide In Api And Its Dosage Form By RP-HPLC****., Indo Am. J. P. Sci, 2020; 07(11).*

INTRODUCTION:

ANALYTICAL CHEMISTRY

Analytical chemistry is a branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter.

It is mainly involved in the qualitative analysis or detection of compounds and quantitative analysis of the compounds. A qualitative method yields information about the identity of atomic or molecular species or functional groups in the sample. A quantitative method, in contrast provides numerical information as to the relative amount of one or more of these components. [13]

CLASSIFICATION OF ANALYTICAL METHODS

Analytical methods are classified into two categories; they are classical methods and instrumental methods.

Classical methods

Analysis of substances was carried out by separating the components of interest in a sample by precipitation, extraction or distillation. For qualitative analysis, the separated components were then treated with reagents that yielded products that could be recognized by their colors, their boiling or melting points, their solubilities in a series of solvents, their optical activities, their odors or their refractive indexes. For quantitative analysis, the amount of analyte was determined by gravimetric or by volumetric measurements.

In gravimetric measurements, the mass of the analyte or some compound produced from the analyte was determined. In volumetric measurements, also called titrimetric analysis, the volume or mass of a standard reagent required to react completely with the analyte is measured.

Instrumental methods

Measurement of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, fluorescence, mass-to-ratio began to be used for quantitative analysis of various inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation forthe separation of components of complex mixtures prior to their qualitative and quantitative determination. These newer methods for separating and determining chemical species are collectively known as instrumental methods of analysis. Most of the instrumental methods fit into one of the following three categories viz.., spectroscopy, electrochemistry and chromatography.

Advantages of instrumental methods

Small samples can be used
High sensitivity is obtained
Measurements are reliable
Determination is very fast
Complex samples can be analysed

Limitations of instrumental methods:

An initial or continuous calibration is required
Sensitivity and accuracy depend on the instrument
Cost of equipment is large
Concentration range is limited
Specialized training is needed[1]

CHROMATOGRAPHY

Chromatography is relatively a new technique which was first invented by M.Tswett, a botanist in 1906. Chromatography was derived from Greek words chroma and graphos meaning “colour” and “writing” respectively. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases. Differences in compounds partition coefficient results in differential retention on the stationary phase and thus changing the separation. [12]

Chromatography may be defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phases. Chromatography may be preparative or analytical. The purpose of preparative Chromatography is to separate the components of a mixture for further use (and is thus a form of purification). Analytical Chromatography is done normally with smaller amounts of material and is for measuring the relative proportion of analytes in a mixture.

The chromatographic method of separation, in general, involves the following steps:

1. Adsorption or retention of substance on the stationary phase.
2. Separation of adsorbed substances by mobile phase.
3. Recovery of the separated substances by continuous flow of the mobile phase; the method being called elution.
4. Qualitative and quantitative analysis of the eluted substances.[11]

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

A variety of methods are available for analyzing pharmaceutical compounds. High Performance/Pressure Liquid Chromatography (HPLC) is one of the best methods of choice for analysing a variety of natural and synthetic compounds. It is because it offers high performance over ambient pressure. [3] The phenomenal growth in chromatography is largely due to the introduction of the technique called high-pressure liquid chromatography, which is frequently called high-performance liquid chromatography (both are abbreviated as HPLC). It allows separations of a large variety of compounds by offering some major improvements over the classical column chromatography, TLC, GC; and it presents some significant advantages over more recent techniques such as supercritical fluid chromatography (SFC), capillary electrophoresis (CE), and electro kinetic chromatography. [2]

Effective and fast method development is of paramount importance throughout this drug development life cycle. This requires a thorough understanding of HPLC principles and theory which lay a solid foundation for appreciating the many variables that are optimized during fast and effective HPLC method development and optimization. Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components.

High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface.

Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference thepresence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention.[10]
The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of Chromatographic methods .
The four main types of HPLC techniques are

1. Normal-Phase Chromatography.
2. Reversed-Phase Chromatography.
3. Ion-Exchange Chromatography.
4. Size-Exclusion Chromatography.

Normal-Phase Chromatography

The term "normal phase" is used to denote a chromatographic system in which a polar stationary phase is employed and a less polar or non-polar mobile phase is used for elution of the analytes. In the normal-phase mode, neutral solutes in solution are separated on the basis of their polarity; the more polar the solute, the greater is its retention on the column. Since the mobile phase is less polar than the stationary phase, by increasing the polarity of the mobile phase results in decreased solute retention. Normal-Phase chromatography is most commonly applied to the analysis of samples that are soluble in non-polar solvents and it is particularly well suited to the separation of isomers and to class separations.

Although the separation mode has occasionally been misidentified as reversed phase, it is normal phase by virtue of the fact that increased aqueous levels of the mobile phase reduce carbohydrate retention, and elution order follows carbohydrate polarity.

Normal-phase separations have occasionally been combined off-line with Reversed-phase chromatography to separate a wider range of species than could be accomplished by either technique alone. The feasibility of such a system, however, is contingent on the compatibility of the normal-phase eluent with that of the reversed-phase column.

Reversed-Phase Chromatography

Reversed-Phase Chromatography, the most widely used chromatographic mode, is used to separate neutral molecules in solution on the basis of their hydrophobicity. As the namesuggests, Reversed-Phase Chromatography is the reverse of Normal-Phase Chromatography in the sense that it involves the use of a non-polar stationary phase and a polar mobile phase. As a result, a decrease in the polarity of the mobile phase results in a decrease in solute retention. Modern Reversed-Phase Chromatography typically refers to the use of chemically bonded stationary phases, where a functional group is bonded to silica, for this reason, Reversed-Phase Chromatography is often referred to in the literature as Bonded-Phase Chromatography. Occasionally, however, polymeric stationary phases such as polymethacrylate or polystyrene, or solid stationary phases such as porous graphitic carbon, are used. Weak acids and weak bases, for which ionization can be suppressed, may be separated on reversed-phase

columns by the technique known as ion suppression. In this technique a buffer of appropriate pH is added to the mobile phase to render the analyte neutral or only partially charged. Acidic buffers such as acetic acid are used for the separation of weak acids, and alkaline buffers are used for the separation of weak bases.

The analysis of strong acids or strong bases using reversed-phase columns is typically accomplished by the technique known as ion-pair chromatography (also commonly called paired-ion or ion-interaction chromatography). In this technique, the pH of the eluent is adjusted in order to encourage ionization of the sample; for acids pH 7.5 is used, and for bases pH 3.5 is common.

Reversed-Phase Chromatography is the most popular mode for the separation of low molecular weight (<3000), neutral species that are soluble in water or other polar solvents. It is widely used in the pharmaceutical industry for separation of species such as steroids, vitamins, and β-blockers. Because of the mobile phase in Reversed-Phase Chromatography is polar, Reversed-Phase Chromatography is suited to the separation of polar molecules that either are insoluble in organic solvents or bind too strongly to the polar, normal-phase materials.[3]

Ion-Exchange Chromatography

In Ion-Exchange Chromatography (IEC), species are separated on the basis of differences in electric charge. The primary mechanism of retention is the electrostatic attraction of ionic solutes in solution to "fixed ions" of opposite charge on the stationary phase support. The stationary phase or ion exchange is classified as an anion-exchange material when the fixed ion carries a positive charge and as a cation exchanger when it carries a negative charge.A specialized form of IEC is ion chromatography (IC), which is the name applied to theanalysis of inorganic anions, cations, and low molecular weight, water-soluble organic acids and bases. Although any HPLC technique used to separate the above species can be termed ion chromatography, in general IC involves the use of ion-exchange columns and a conductivity detector. Ion chromatography itself can be sub classified. Suppressed IC involves the use of a membrane device, known as a suppressor, between the column and the detector to lower the response of the eluent and thereby enhance the signal from the solute; non suppressed or "single-column" IC does not contain a suppressor.[9]

Size-Exclusion Chromatography

Size-Exclusion Chromatography (SEC) is a convenient and highly predictable method for separating simple mixtures whose components are sufficiently different in molecular weight. For small molecules, a size difference of more than about 10% is required for acceptable resolution; for macromolecules a twofold difference in molecular weight is necessary [15]. Size-Exclusion Chromatography can be used to indicate the complexity of a sample mixture and to provide approximate molecular weight values for the components. It is an easy technique to understand, and SEC can be applied to the separation of delicate bio macromolecules as well as to the separation of synthetic organic polymers.

Because SEC is a gentle technique, rarely resulting in loss of sample or reaction, it has become a popular choice for the separation of biologically active molecules. Each solute is retained as a relatively narrow band, which facilitates solute detection with detectors of only moderate sensitivity. One of the major applications of SEC is polymer characterization.[8]

INSTRUMENTATION OF HPLC

The individual components HPLC and their working functions are described below.

- Mobile phase and reservoir
- Solvent degassing system
- Pump
- Injector
- Column
- Detector
- Data system

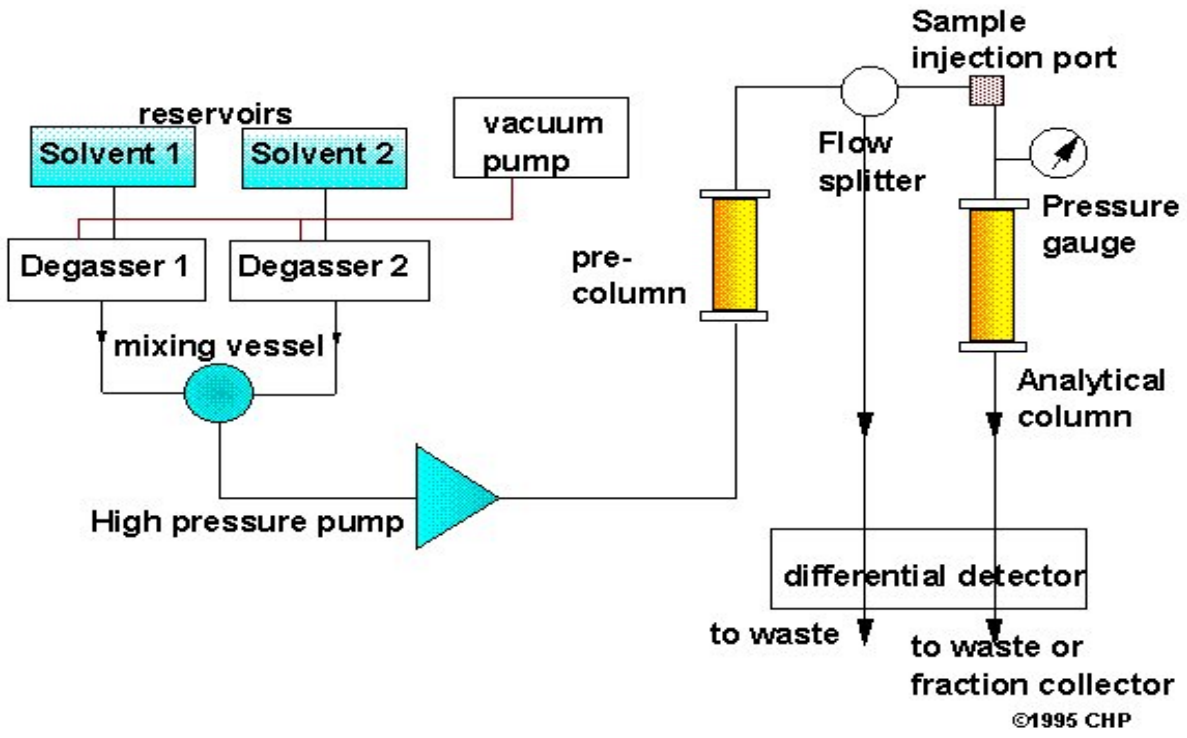

Figure Schematic Diagram of HPLC

MOBILE PHASE AND RESERVOIR

The most common type of solvent reservoir is a glass bottle. Most of the manufacturers supply these bottles with special caps Teflon tubing and filters to connect to the pump inlet and to the purge gas (helium) used to remove dissolved air. The mobile phase is pumped under pressure from one or several reservoirs and flows through the column at a constant rate. With micro particulate packing there is a high-pressure drop across a chromatography column. Eluting power of the mobile phase is determined by its overall polarity the polarity of the stationary phase and the nature of the sample components. For normal phase separations elute power increases with increasing polarity of the solvent but for reversed phase separations eluting power decreases with increasing solvent polarity. Optimum separating conditions can be achieved by making use of mixture of two solvents. Some other properties of the solvents, which need to be considered for a successful separation, are boiling point, viscosity, detector compatibility, flammability and toxicity.

Mobile phases used for HPLC typically are mixtures of organic solvents and water or aqueous buffers. Isocratic methods are preferable to gradient methods. Gradient methods will sometimes be required when the molecules being separated have vastly different partitioning properties. When a gradient elution method is used care must be taken to ensure that all solvents are miscible.[4]

AIM OF THE THIS WORK

Literature review reveals that there is no analytical method reported for the analysis of Nadololand and Bendroflumethiazide by simultaneous estimation by RP-HPLC. Spectrophotometer, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Nadolol and and Bendroflumethiazide in pharmaceutical dosage form. Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Nadolol and Bendroflumethiazide. The developed method will be validated according to ICH guidelines.[7]

OBJECTIVES OF THE WORK

The analytical method for the simultaneous estimation of Nadolol and Bendroflumethiazide will be developed by RP-HPLC method by optimizing the chromatographic conditions.

The developed method is validated according to ICH guidelines for various parameters specified in ICH guidelines, Q2 (R1).

Literature review reveals that there is no analytical method reported for the analysis of Nadololand and Bendroflumethiazide by simultaneous estimation by RP-HPLC. Spectrophotometer, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Nadolol and and Bendroflumethiazide in pharmaceutical dosage form. Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Nadolol and Bendroflumethiazide. The developed method will be validated according to ICH guidelines.

MATERIALS

The list of instruments used in the course of experimental work is as follows:

Table List of Instruments

S.No.	Instrument	Model No.	Software	Manufacturer's name
1	HPLC Alliance	Waters 2695	Empower	Waters
2	UV double beam spectrophotometer	UV 3000	UV Win 5	Lab India
3	Digital weighing balance	BSA224SCW	-	Satorius
4	pH meter	AD102U	-	Lab India
5	Ultra sonicator	SE60US	-	-
6	Suction pump	VE115N	-	-

The experimental work involves several chemicals. Chemicals used presently are listed bel [5]

Table List of Chemicals

S.No.	Chemical	Manufacturer	Grade
1	Water	Merck	HPLC Grade
2	Methanol	Merck	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade
4	Potassium dihydrogen orthophosphate	Merck	A.R
5	Nadolol & Bendroflumethiazide	-	-

Trial 1

Mobile phase : Water: Methanol (50:50%v/v)
Column : Thermosil C18 (4.6*150mm) 5µm
Flow rate : 1.0 ml/min
Wavelength : 260 nm
Column temp : Ambient
Sample Temp : Ambient
Injection Volume : 10 µl

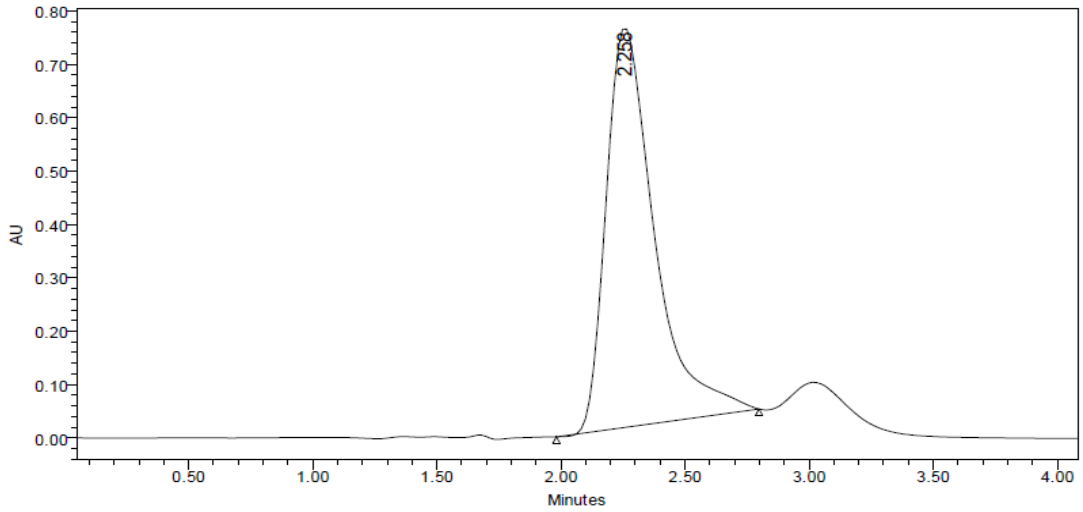

Fig Chromatogram showing trail -1

From the above chromatogram it was observed that the Nadolol peak was splitted

Trial 2

Mobile phase : Phosphate buffer pH 4 : Methanol (40:60%v/v)
Column : Termosil C18 (4.6*150mm) 5µm
Flow rate : 1.0 ml/min
Wavelength : 260 nm
Column temp : Ambient
Sample Temp : Ambient
Injection Volume: 10 µl

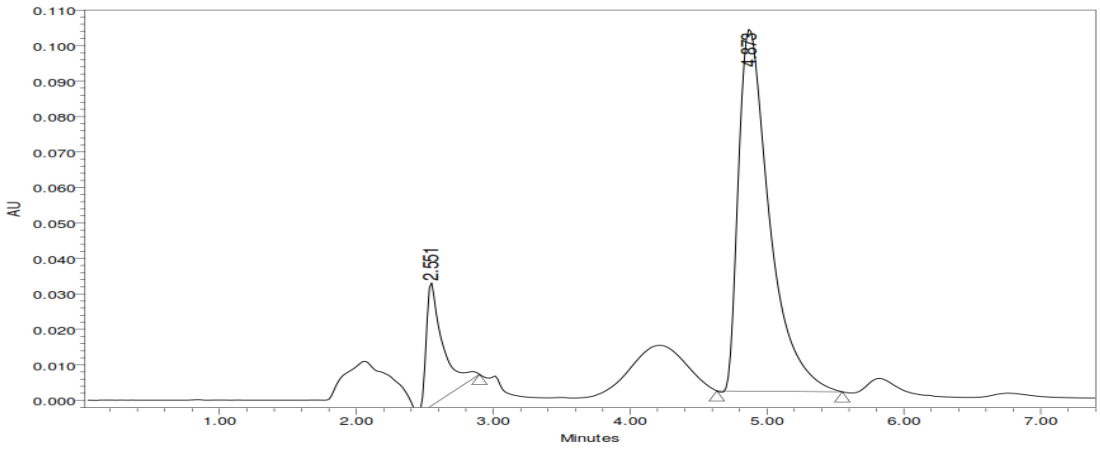

Fig Chromatogram showing trail -2

From the above chromatogram it was observed that the Nadolol and Bendroflumethiazide peaks are splitted

Trial 3

Mobile phase : Phosphate buffer (0.05m) pH 4.0: Methanol (40:60%v/v)
Column : Symmetry C18 5µm (4.6*250mm) Make; waters
Flow rate : 0.8 ml/min
Wavelength : 260 nm
Column temp : Ambient
Sample Temp : Ambient
Injection Volume : 10 µl

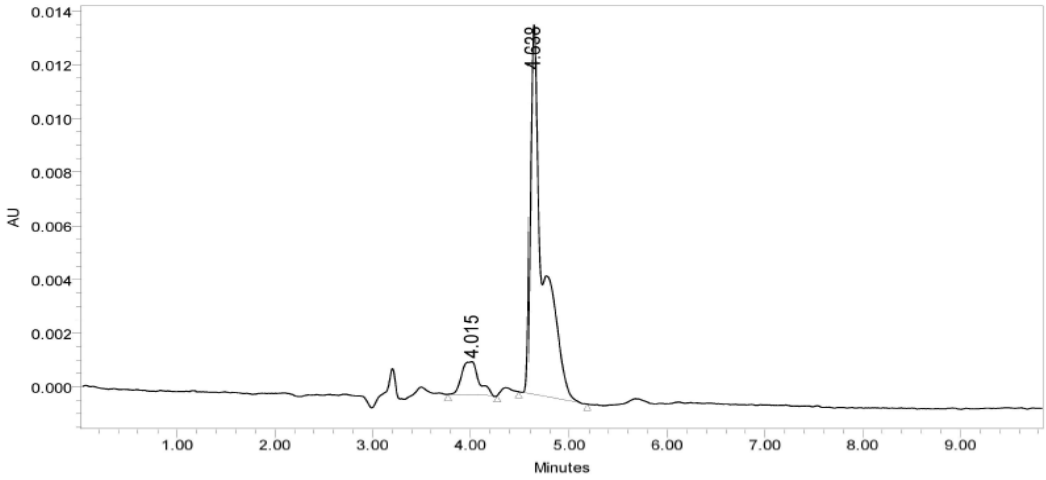

Fig Chromatogram showing trail -3

From the above chromatogram it was observed that the Nadolol and Bendroflumethiazide peaks are splitted

Optimized chromatogram is obtained by following conditions [6]

Column : Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent
Buffer pH : 4.6
Mobile phase : 70% Meoh : 30% phosphate buffer ph-4.6
Flow rate : 1 ml per min
Wavelength : 273 nm
Temperature : ambient.
Run time : 7min.

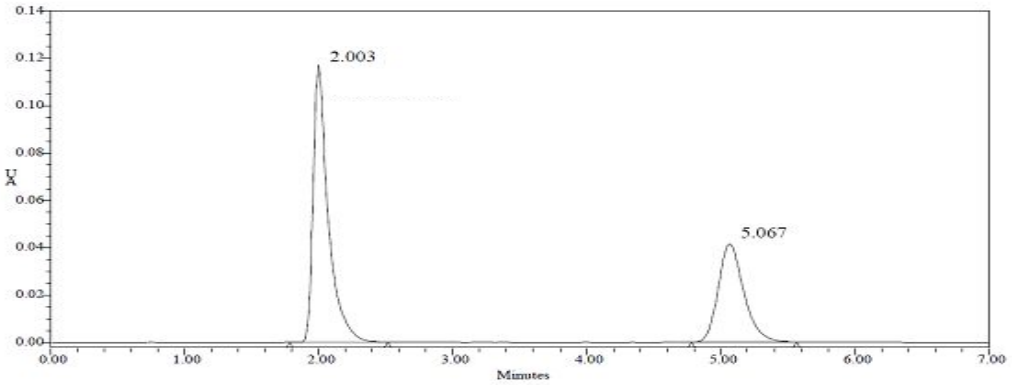

Figure Chromatogram for Nadolol and Bendroflumethiazide

From the above chromatogram it was observed that the Nadolol and Bendroflumethiazide peaks are well separated
Retention time of Nadolol – 2.003 min
Retention time of Bendroflumethiazide - 5.067 min.

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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