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"DEVELOPMENT AND VALIDATION OF A REVERSE PHASE HPLC METHOD FOR ESTIMATION OF ARMODAFINIL IN BULK AND PHARMACEUTICAL FORMULATIONS"

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1. INTRODUCTION

Chromatography

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.[1]

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.[2]

Introduction to chromatography, its types and classification Chromatography

Chromatography is a set of techniques in which separation of chemical substances takes place quantitatively as well as qualitatively.

Terminology used in Chromatography Mobile Phase

In chromatography the substance which is introduced with or along with the sample andcauses elution of the contents of the sample. It may be liquid or gas.

Stationary phase: Stationary phase of the chromatographic system refers to that part which is presentbefore the introduction of sample or solute in the column (as in column chromatography) or on a solidsupport (as in paper or similar chromatography). It may be liquid or solid.

Eluent: The substance which separates the components of the mixture in chromatographic technique.Eluent is that part which brings separation when the solution is passed either from the column or from the solid support.

Eluate: The substance which is separated as a individual component of the mixture is called eluate.

Important types of Chromatographic Techniques

Following are some important types of Chromatographic separation techniques. They are definedthoroughly by explaining their general principle, application, and a brief outline of their instrumentation for a complete understanding.Following are some commonly utilized types of techniques:

i. Gas Chromatography
ii. High Pressure Liquid Chromatography
iii. Supercritical fluid Chromatography
iv. Gel Exclusion Chromatography.

High Pressure Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). The sample is carried by a moving carrier gas stream of helium or nitrogen. HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics, and chemicals.

Sample retention time will vary depending on the

interaction between the stationary phase, the molecules being analyzed, and the solvent, or solvents used. As the sample passes through the column it interacts between the two phases at different rate, primarily due to different polarities in the analytes. Analytes that have the least amount of interaction with the stationary phase or the most amount of interaction with the mobile phase will exit the column faster.

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

HPLC has been used for manufacturing (e.g., during the production process of pharmaceutical and biological products), legal (e.g., detecting performance enhancement drugs in urine), research (e.g., separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (e.g., detecting vitamin D levels in blood serum) purposes.[1]

High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography.

Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster.

All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

Types of HPLC

There are following variants of HPLC, depending upon the phase system (stationary) in the process:

1. Normal Phase HPLC

This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these.

Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

2. Reverse Phase HPLC

The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.

3. Size-exclusion HPLC

The column is filled with material having precisely controlled pore sizes, and the particles are separated according to its their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

4. Ion-Exchange HPLC

The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples.

The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

Instrumentation of HPLC

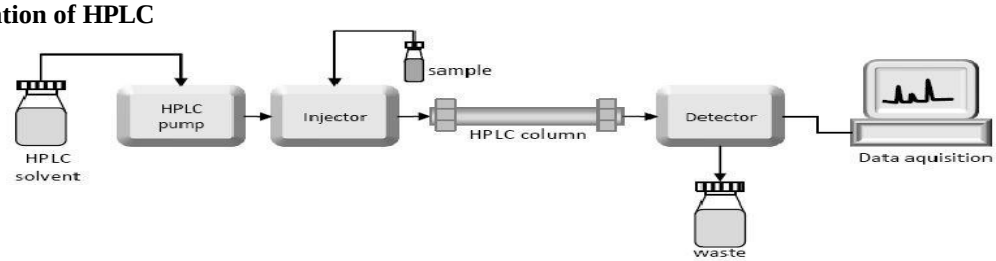


Fig-1: Schematic diagram of HPLC instrumentation.

As shown in the schematic diagram in Figure above, HPLC instrumentation includes a pump, injector, column, detector and integrator or acquisition and display system. The heart of the system is the column where separation occurs.

1. Solvent Reservoir

Mobile phase contents are contained in a glass resorvoir. The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.

Fig. 2: HPLC Solvent Reservoirs.

2. Pump

A pump aspirates the mobile phase from the solvent resorvoir and forces it through the system's column and detecter. Depending on a number of factors including column dimensions, particle size of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated.

Fig. 3: Alliance 2690/5 Model Pump.

3. Sample Injector

The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).

4. Columns

Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 3–10 µm.

Columns with internal diameters of less than 2 mm are often referred to as micro bore columns. Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.

Fig-4: HPLC Columns.

5. Detector

The HPLC detector, located at the end of the column detects the analytes as they elute from the chromatographic column. Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.

6. Data Collection Devices

Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

Applications of HPLC

The information that can be obtained by HPLC includes resolution, identification and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include:

Pharmaceutical Applications

1. To control drug stability.
2. Tablet dissolution study of pharmaceutical dosages form.
3. Pharmaceutical quality control.

Environmental Applications

1. Detection of phenolic compounds in drinking water.
2. Bio-monitoring of pollutants.

Applications in Forensics

1. Quantification of drugs in biological samples.
2. Identification of steroids in blood, urine etc.
3. Forensic analysis of textile dyes.
4. Determination of cocaine and other drugs of abuse in blood, urine etc.

Food and Flavour

1. Measurement of Quality of soft drinks and water.
2. Sugar analysis in fruit juices.
3. Analysis of polycyclic compounds in vegetables.
4. Preservative analysis.

Applications in Clinical Tests

1. Urine analysis, antibiotics analysis in blood.
2. Analysis of bilirubin, biliverdin in hepatic disorders.
3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.

METHOD DEVELOPMENT

Steps for HPLC Method Development

Analytical method development is considered as a critical process in pharmaceuticals. Availability of the different types of columns, operating parameters, mobile phase composition, diluent and pH values make it critical to develop an analytical method. A good analytical method should be simple, used column, mobile phase and buffer should be common. It can be done easily step by step.

Following are the common HPLC method development steps.

1. Selection of HPLC Analytical Method
2. Selection of Chromatographic Conditions
3. Parameter Optimization

1. Selection of HPLC Analytical Method: First of all consult the literature that is available on the product. It will help you to understand the nature of the product that will help to select the different parameters.

A. Sample Preparation: Select method to prepare the sample according to its solubility, filtration requirements, extraction requirements or other special requirements to make a clear solution of HPLC analysis.

B. Chromatography: Reverse phase chromatography is used for most of the samples but when acidic or basic molecules are present in the sample then reverse phase ion suppression (for weak acid or base) or reverse phase ion pairing (for strong acid or base) should be used. The stationary phase should be C18 bonded. Normal phase is used for low or medium polarity analyte especially when it is required to separate the product isomers. Choose cyano bonded phase for normal phase separations. Ion exchange chromatography is best to use for inorganic anion or cation analysis. If analyte has higher molecular weight than size exclusion chromatography is the best to use.

C. Gradient/Isotonic HPLC: Gradient HPLC is helpful in the analysis of complex samples having a number of components. It will help to get higher resolution than isotonic HPLC having constant peak width while in isotonic HPLC peak width increases with the retention time. Gradient HPLC has great sensitivity, especially for the products having longer retention time.

D. Column Size: 100-150 mm columns are used for most of the samples. It reduces the method development and analysis time for the sample. Bigger columns are used for complex samples those take more time in separation. Initially, a flow rate should be kept between 1 and 1.5 ml/min and column particle size should be between 3 and 5 µm.

E. HPLC Detectors: If the analyte has chromophores that enable the compound to be detected by UV than it is better to use UV detector. It is always better to use a UV detector than others. Fluorescence and electrochemical detectors should be used for trace analysis. Samples having high concentration should be analyzed using refractive index detectors.

F. Wavelength: λmax of the sample has the greatest sensitivity to the UV light. It detects the sample components that have chromophores. A wavelength above 200 nm gives greater sensitivity than the lower wavelengths. Wavelengths lower than 200 nm gives more noise, therefore, it should be avoided.

2. Selection of Chromatographic Conditions: After selection of analytical method, different chromatographic conditions are selected. The flow of the analytes through the column depends upon the concentration of the solvent in the mobile phase. The concentration of solvent is generally used to control the retention time. Mobile phase pH and ion pairing reagents also affect the retention time of the sample. Samples having a large number of components are analyzed using the gradient to avoid the large retention time while the samples containing one or two components are analyzed on an isotonic system.

3. Parameter Optimization: After taking the same sample runs some parameters including column dimensions, particle size, run time and flow rate are optimized. It is done to get the best resolution and minimum run time. After proper optimization of the analysis method, it is validated to ensure the consistency of the analytical method. Analytical method validation is now done mandatory by all regulatory authorities.

PARAMETERS FOR HPLC PERFORMANCE CALCULATIONS

Calculating the following values (which can be included in a custom report) used to access overall system performance.

1. Relative retention
2. Theoretical plates
3. Capacity factor
4. Resolution
5. Peak asymmetry
6. Plates per meter

The following information furnishes the parameters used to calculate these system performance values for the separation of two chromatographic components. (Note: where the terms w and t both appear in the same equation they must be expressed the same units).

System suitability parameters:

The theory of chromatography has been used as the basis for system-suitability tests, which are set of quantitative criteria that test the suitability of the chromatographic system to identify and quantify drug related samples by HPLC at any step of the pharmaceutical analysis.

1. Relative retention: The time elapsed between the injection of the sample components in to the column and their detection is known as the retention time (Rt).

$$\alpha = (t2-ta) / (t1-ta)$$

Where,

α =Relative retention.

t_1= Retention time of the peak measured from point of injection.

t_2 = Retention time of the second measured from point of injection.

t_a = Retention time of an inert peak not retained by the column, measured from point of injection.

2. Theoretical plates

$$n = 16 (t_R / w)^2$$

Where,

n =Theoretical plates.

t_R = Retention time of the component.

W = Width of the base of the component peak using tangent method.

3. Capacity factor: The capacity factor describes the thermodynamic basis of the separation and its definition is the ratio of the amounts of the solute at the stationary and mobile phases within the analyte band inside the chromatographic column.

$$K^1 = (t2/t\ a)-1$$

Where,

K^1 = Capacity factor.

t_a = Retention time of an inert peak not retained by the column, measured from point of injection.

4. Resolution: the gap between two peaks

$$R = 2 (t2- t1) / (w2-w1)$$

Where,

R =Resolution between a peak of interest (peak 2) and the peak preceding it (peak 1). W_2 = Width of the base of component peak 2.

W_1 = Width of the base of component peak 1.

5. Peak asymmetry

$$T = W0.05/ 2f$$

Where,

T = Peak asymmetry, or tailing factor.

W0.05 = Distance from the leading edge to the tailing edge of the peak,measured at a point 5 % of the peak height from the baseline.

f= Distance from the peak maximum to the leading edge of the peak.

6. Plate Per Meter

$$N = n/L$$

Where,

N = plates per meter.

L = column length in meters.

Advantages

- HPLC separations can be accomplished in a minutes, in some cases even in seconds.
- High resolution of complex sample mixture into individual components.
- Rapid growth of HPLC is also because of its ability to analyze substances that are unsuitable for gas liquid chromatographic (GLC) analysis due to non-volatility or thermal-instability.

- Quantitative analysis is easily and accurately performed and errors of less than 1 % are common to most HPLC methods.
- Depending on sample type and detector used, it is frequently possible to measure 10^{-9} g or 1 ng of sample. With special detectors, analysis down to 10-12 pg has been reported.
- As HPLC is versatile, it can be applied to wide variety of samples like organic, inorganic, high molecular weight liquids, solids, and ionic-nonionic compounds.

Disadvantages

- HPLC instrumentation is expensive and represents a major investment for many laboratories.
- HPLC cannot handle gas samples.
- HPLC is poor identifier. It provides superior resolution but it does not provide the information that identifies each peak.
- Only one sample can be analyzed at a time.

Finally, at present there is no universal and sensitive detector.

METHOD VALIDATION

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Analytical methods need to be validated or revalidated

- Before their introduction into routine use;
- Whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and
- Whenever the method is changed and the change is outside the original scope of the method.

1. Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s). This definition has the following implications: Identification: to ensure the identity of an analyte. Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc. Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

2. Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

3. 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. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

3.1. Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

3.2. Intermediate precision: Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

3.3. Reproducibility: Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

4. Detection Limit: 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.

5. Quantitation Limit: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

6. Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

7. Range: The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

8. Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method

parameters and provides an indication of its reliability during normal usage.

2. DRUG PROFILE

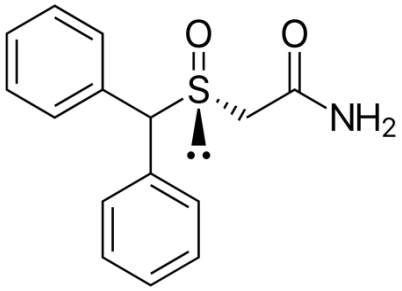
DRUG PROFILE OF ARMODAFINIL

Name of the Drug: Armodafinil

Description: Armodafinil is a 2-[(diphenylmethyl) sulfinyl] acetamide that has R configuration at the sulfur atom. Like its racemate, modafinil, it is used for the treatment of sleeping disorders such as narcolepsy, obstructive sleep apnoea, and shift-work sleep disorder. Peak concentration in the blood later occurs later following administration than with modafinil, so it is thought that Armodafinil may be more effective than modafinil in treating people with excessive daytime sleepiness. It has a role as a central nervous system stimulant and a eugeroic. It is an enantiomer of a (S)-modafinil.

Synonyms: Armodafinil, 112111-43-0, Nuvigil, (R)-Modafinil, (-)-Modafinil, (R)-(-)- Modafinil, CEP-10953, 2-[(R)-(Diphenyl methyl) sulfinyl] acetamide, CRL 40982, Modafinil, (r)-, R-(-)-Modafinil, (-)-(R)-modafinil, CEP 10953, CEP-10952, CRL- 40982, V63XWA605I, CHEMBL1201192, CHEBI:77590, NSC-751850, NSC-758711.

Chemical Structure



IUPAC Name: 2-[(R)-benzhydrylsulfinyl]acetamide

Molecular Formula: $C_{15}H_{15}NO_2S$
Molecular Weight: 273.35g/mol
Pka value(Strongest Acidic): 8.84
Melting Point:164 °C
Log P: 1.53

Bioavailability: Armodafinil is readily absorbed after oral administration.

Physical Appearance: Armodafinil is a white to off-white, crystalline powder

Solubility: Armodafinil was found to bevery slightly soluble in water, sparingly soluble in acetone and soluble in methanol.

Indication: Investigated for use/treatment in sleep disorders, obstructive sleep apnea, schizophrenia and schizoaffective disorders, depression, and bipolar disorders.

Pharmacokinetics:

Absorption: Tmax is 2 hours when fasted and can be delayed approximately 2-4 hours by food, potentially affecting the onset of action.

Volume of distribution: Apparent volume of distribution: 42L.

Protein binding: Specific data unavailable. Similar to modafinil: approximately 60%, primarily to albumin.

Metabolism: In vitro and in vivo data show that Armodafinil undergoes hydrolytic deamidation, S-oxidation, and aromatic ring hydroxylation, with subsequent glucuronide conjugation of the hydroxylated products. Amide hydrolysis is the single most prominent metabolic pathway, with sulfone formation by cytochrome P450 (CYP) 3A4/5 being next in importance. The other oxidative products are formed too slowly in vitro to enable identification of the enzyme(s) responsible. Only two metabolites reach appreciable concentrations in plasma (i.e., R-modafinil acid and modafinil sulfone). Data specific to Armodafinil disposition are not available.

Route of Elimination: Elimination After oral administration of NUVIGIL, Armodafinil exhibits an apparent monoexponential decline from the peak plasma concentration.

Pharmacodynamics: Armodafinil is the enantiopure of the wakefulness-promoting agent modafinil (Provigil), and is indicated to improve wakefulness in adult patients with excessive sleepiness associated with obstructive sleep apnea (OSA), narcolepsy, or shift work disorder (SWD).

Mechanism of action: Nuvigil (Armodafinil) is a single-isomer of modafini. The exact mechanism of action is unknown. Armodafinil belongs to a class of drugs known as eugeroics, which are stimulants that provide long-lasting mental arousal. Pharmacologically, Armodafinil does not bind to or inhibit several receptors and enzymes potentially relevant for sleep/wake regulation. Armodafinil is not a direct- or indirect-acting dopamine receptor agonist. However, in vitro, both Armodafinil and modafinil bind to the dopamine transporter and inhibit dopamine reuptake. [Medilexicon]

Half Life: Terminal half-life is approximately 15 hours.

Drug Interactions

Abemaciclib: The metabolism of Abemaciclib can be increased when combined with Armodafinil.

Abrocitinib: The metabolism of Abrocitinib can be decreased when combined with Armodafinil.

Acalabrutinib: The metabolism of Acalabrutinib can be increased when combined with Armodafinil.

Acenocoumarol: The metabolism of Acenocoumarol can be increased when combined with Armodafinil.

Acetaminophen: The metabolism of Acetaminophen can be increased when combined with Armodafinil.

Acetohexamide: The metabolism of Acetohexamide can be decreased when combined with Armodafinil.

Drug-Food Interactions

- Avoid alcohol.
- Exercise caution with grapefruit products. Armodafinil is partially metabolized by CYP3A4, and grapefruit is a CYP3A4 inhibitor.
- Exercise caution with St. John's Wort. Armodafinil is partially metabolized by CYP3A4, and St. John's Wort is a CYP3A4 inducer.
- Take with or without food. Taking Armodafinil with food can delay the Tmax by 2-4 hours.

Contraindications: Who should not take Armodafinil?

- Manic behaviour.
- Psychotic disorder.
- Suicidal thoughts.
- Depression.
- High blood pressure.
- Mitral valve prolapse syndrome.
- Left ventricular hypertrophy.
- Severe liver disease.

Adverse effects/Side Effects:

- ✓ Blistering, burning, crusting, dryness, or flaking of the skin.
- ✓ Difficult or laboured breathing.
- ✓ Fast, irregular, pounding, or racing heartbeat or pulse.
- ✓ Frequent urination.
- ✓ Headache, severe and throbbing.
- ✓ Increased volume of pale, dilute urine.
- ✓ Itching, scaling, severe redness, soreness, or swelling of the skin.

Medical Uses: Armodafinil is a stimulant used to improve wakefulness in adult patients with excessive sleepiness associated with obstructive sleep apnea, narcolepsy, or shift work disorder.

MARKETED FORMULATION

S. No.	Drug Name	Label Claim	Brand Name	Company
1	Armodafinil	150mg	Waklert-150 Tablet	Sun Pharmaceuticals

3. REVIEW OF LITERATURE

Ramya G, et al. (2021): The present research deals with the development of a stability indicating reverse phase HPLC with PDA detector method for the determination of Armodafinil Agilent XDB-C18, 150×4.6mm, 5µm or Equivalent column. The present research deals with the development of a stability indicating reverse phase HPLC with PDA detector method for the determination of Armodafinil Agilent XDB-C18, 150×4.6mm, 5µm or Equivalent column. The flow rate was kept at 1.0ml/min and the injection volume 10µL and the run time is 8 min and drug Rt is 3.354. The separation was performed at 30°C. Eluents were monitored by PDA detector set at 223nm. The developed method was statistically validated and results for the linearity is 0.999 and for System suitability, theoretical plates are 2500 and its tailing factor is 1.64, Precision is 0.1, LOQ is 1.00µg/ml, LOD is 0.33µg/ml, accuracy is 100.19, Robustness (flow rate, mobile phase) is complied.

P. Vivek Sagar, et al. (2014): Objective: To develop simple reverse phase HPLC method for the estimation of Armodafinil in tablet dosage form. Methods: Chromatography was performed by isocratic elution on a Stainless steel Hibar C18 column with dimensions 4.6 x 250 mm, packed with octadecylsilane bonded to porous silica (C18) with particle size 5 micron. Acetonitrile and water in the ratio of 50:50 v/v is used as mobile phase. The flow rate is 1.0 ml/ min and effluent is monitored at 220 nm. Armodafinil was eluted at a retention time of 3.8 minutes. Results: The standard curve of Armodafinil was linear over a working range of 1–700 µg/ml and gave an average correlation coefficient of 0.999. The limit of quantitation (LOQ) of the drug is 0.1 µg/ ml. Recovery studies were carried out by standard addition method and the recoveries are found satisfactory within the range of 99.3 to 101.5 %. The method is precise with % RSD below Conclusion: The method is validated in terms of robustness and forced degradation studies were carried out and this method can be applied for routine degradation studies and quantification in regular laboratories.

Kambham Venkateswarlu, et al. (2017): The main objective of present study was to develop a RP-HPLC method for estimation of Armodafinil in pharmaceutical dosage forms and characterization of its base hydrolytic product. The method was developed for Armodafinil estimation and base hydrolytic products were characterized. The separation was carried out on C18 column by using mobile phase as mixture of water and methanol (45:55%v/v). Eluents were detected at 220nm at 1ml/min. Stress studies were performed with milder conditions followed by stronger conditions so as to get sufficient degradation around 20%. A total of five degradation products were detected and separated from analyte. The linearity of the proposed method was investigated in the range of 20-120µg/ml for

Armodafinil. The detection limit and quantification limit was found to be 0.01183µg/ml and 0.035µg/ml respectively. The precision % RSD was found to be less than 2% and the recovery was between 98-102%. Armodafinil was found to be more sensitive to the base hydrolysis and yielded its carboxylic acid as degradants. The developed method was stability indicating assay, suitable to quantify Armodafinil in presence of possible degradants. The drug was sensitive to acid, base & photolytic stress and resistant to thermal & oxidation.

Mohammad Habibuddin, et al. (2017): Objective: The objective of the present study is to develop and validate a simple, rapid, sensitive reverse phase HPLC method for the determination of Armodafinil present in bulk and its pharmaceutical formulations. Methods: The chromatographic separation was achieved by using Hypersil ODS C-18 (150 x 4.6 mm, 5µ) in an isocratic mode with mobile phase methanol: phosphate buffer 3.0 (60:40 %v/v) was used. The flow rate was 1 ml/min and effluent was monitored at 225 nm. The method was validated for validation parameters i.e. linearity, accuracy, precision and robustness according to ICH guidelines. Results: The retention time of Armodafinil was 4.2 min and the linearity range of the method was 500-20000ng/ml with regression (r2) coefficient 0.9998. The method was validated for precision, accuracy, robustness and which were found to be within the acceptable limits according to the ICH guidelines. Also, the method was successfully applied for the estimation of Armodafinil in the marketed formulation of Nuvigil and the recovery was found to be>98%. Conclusion: The developed method possess good selectivity, specificity, there is no interference found in the blank at a retention time of ARM and good correlation between the peak area and concentration of the drugs under prescribed conditions. Hence, the method can be applied for routine analysis of Armodafinil.

4. AIM AND OBJECTIVES

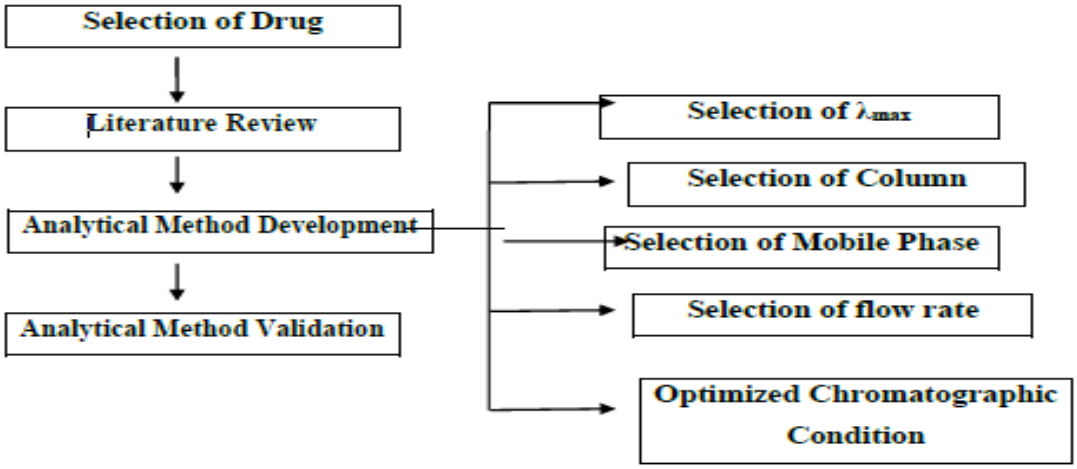
- ✓ Review of literature for Armodafinil gave information regarding its physical and chemical properties, various analytical methods that were conducted alone and in combination with other drugs.
- ✓ Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Armodafinil and single method is available for such estimation by RP-HPLC.
- ✓ Validation is a necessary and important step in both framing and documenting the capabilities of the developed method.
- ✓ The utility of the developed method to determine the content of drug in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient.
- ✓ The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.

The Primary Objective of Proposed Work is:

- ✓ To develop new simple, sensitive, accurate and economical analytical method for the estimation of Armodafinil in bulk and marketed pharmaceutical dosage form.
- ✓ To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Armodafinil in bulk and marketed pharmaceutical dosage form.

5. PLAN OF WORK

- To develop a new analytical method for the estimation of Armodafinil by RP-HPLC in bulk and marketed pharmaceutical dosage form.
- The dissertation work has been carried out in the following steps



6. EXPERIMENTAL METHODS

INSTRUMENTS USED

Table 1: Instruments Used.

S. No	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED

Table 2: Chemicals used.

S. No	Chemical	Brand names
1	Armodafinil	Waklert-150 Tablet (Sun Pharmaceuticals)
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Armodafinil 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 0.1ml of the above Armodafinil 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 and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 35:65% v/v.

Optimization of Column

The method was performed with various C18 columns like, X- bridge column, Xterra, and C18 column. Symmetry ODS C18 (4.6 x 250mm, 5□m) 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: Ambient
Column: Symmetry ODS C18 (4.6×250mm, 5µm)
Mobile phase: Methanol: Phosphate Buffer pH-3.6 (35:65)
Flow rate: 1ml/min
Wavelength: 235nm
Injection Volume: 10µl
Run time: 8 minutes

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.6)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Armodafinil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Armodafinil stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Armodafinil working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Armodafinil stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Weight 10 mg equivalent weight of Armodafinil sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of Armodafinil above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

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

LINEARITY AND RANGE

Accurately weigh and transfer 10 mg of Armodafinil working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (6ppm of Armodafinil)

Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – II (8ppm of Armodafinil)

Take 0.8ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – III (10ppm of Armodafinil)

Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – IV (12ppm of Armodafinil)

Take 0.12ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – V (14ppm of Armodafinil)

Take 0.14ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Procedure

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION REPEATABILITY

Preparation of Armodafinil Product Solution for Precision

Accurately weigh and transfer 10 mg of Armodafinil working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Armodafinil stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

Analyst 1

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

ACCURACY

For preparation of 50% Standard stock solution

Accurately weigh and transfer 10 mg of Armodafinil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.05ml of the above Armodafinil stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution

Accurately weigh and transfer 10 mg of Armodafinil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Armodafinil stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution

Accurately weigh and transfer 10 mg of Armodafinil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the above Armodafinil stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Armodafinil and calculate the individual recovery and mean recovery values.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION (LOD & LOQ)

Preparation of 0.95µg/ml Solution (For LOD)

Accurately weigh and transfer 10 mg of Armodafinil working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.0095ml of the above Armodafinil stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of 2.9µg/ml Solution (For LOQ)

Accurately weigh and transfer 10 mg of Armodafinil working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.029ml of the above Armodafinil stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution

Accurately weigh and transfer 10 mg of Armodafinil working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Armodafinil stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition

The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 40:60, 30:70 instead (35:65), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

7. RESULTS AND DISCUSSION

Trails for the Method Development

Trail 1

Column: Phenomenex C18 (4.6×250mm) 5µm
Column temperature: 40°C
Wavelength: 235nm
Mobile phase ratio: ACN: Water (60:40) V/V
Flow rate: 1ml/min
Injection volume: 10µl
Run time: 9.5min

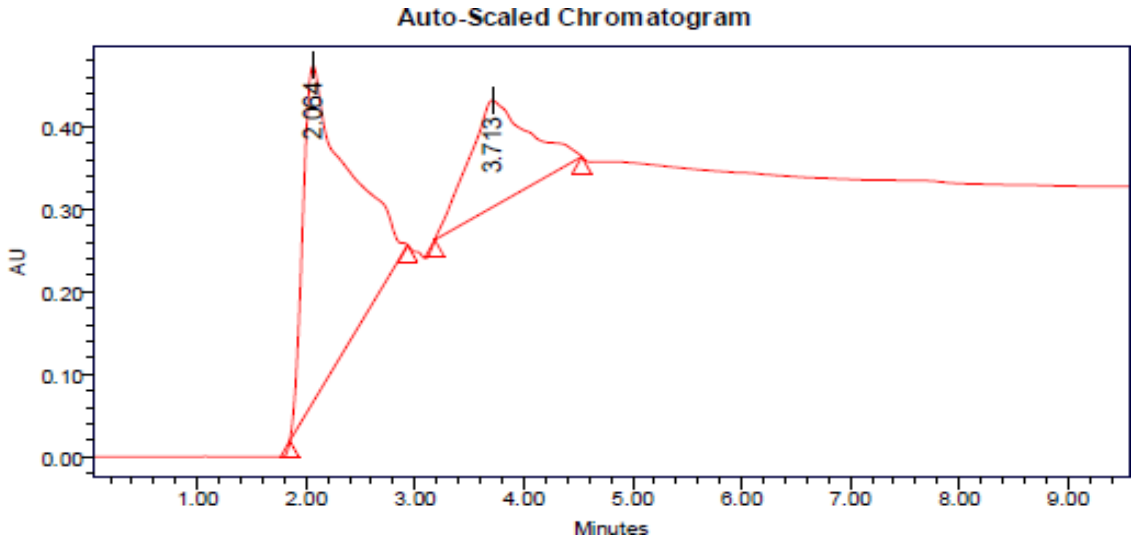


Figure 5: Chromatogram for Trail 1.

Table-3: Results of Chromatogram for Trail 1.

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Armodafinil	2.064	56584	26534	0.95	1965

Observation: In this trial it shows improper separation of peak in the chromatogram. So it requires more trials to obtain good peaks.

Trail 2
Column: Devilosil C18 (4.6×250mm) 5µm
Column temperature: 35°C
Wavelength: 235nm
Mobile phase ratio: Methanol: Water (70:30) V/V
Flow rate: 1.0ml/min
Injection volume: 10µl
Run time: 10min

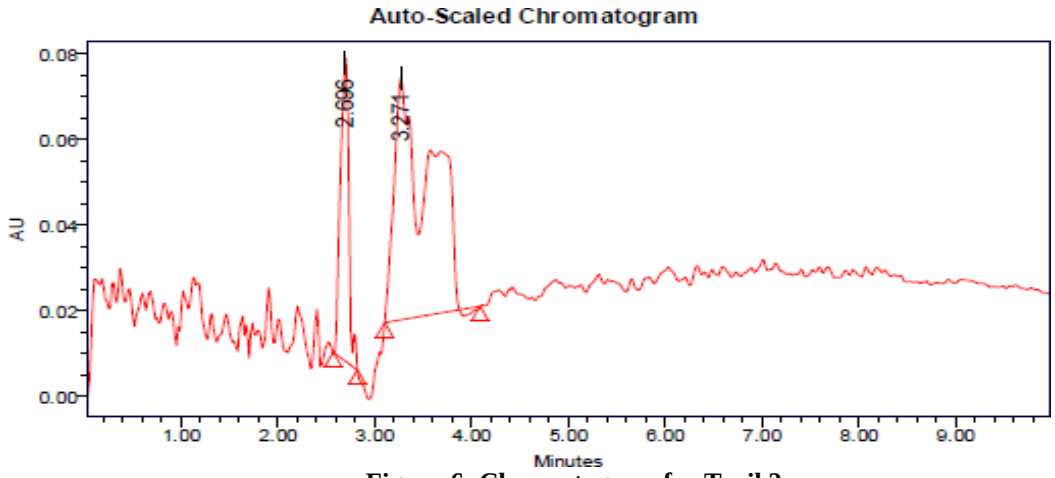


Figure-6: Chromatogram for Trail 2.

Table 4: Results of Chromatogram for Trail 2.

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Armodafinil	2.696	365845	25426	0.91	1896

Observation: In this trial it shows improper separation of peak in the chromatogram and peak splitting was observed. So it requires more trials to obtain good peaks.

Flow rate: 1.0ml/min
Injection volume: 10µl
Run time: 6min

Trail 3:
Column: Apollo C18 (4.6×250mm) 5µm
Column temperature: 35ºC
Wavelength: 235nm
Mobile phase ratio: Methanol: ACN (50:50) V/V

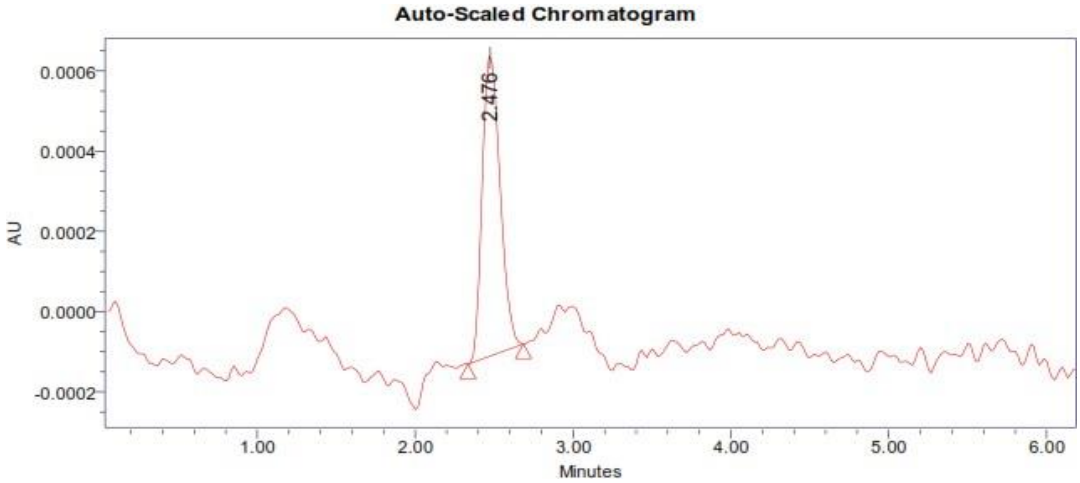


Figure 7: Chromatogram for Trail 3.

Table-5: Results of Chromatogram for Trail 3.

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Armodafinil	2.476	689584	56854	1.06	2698

Observation: In this trial it shows improper separation of peak in the chromatogram and stabilization was good. So it requires more trials to obtain good peaks.

Trail 4:
Column: Symmetry ODS C18 (4.6×250mm, 5µm)
Column temperature: Ambient
Wavelength: 235nm
Mobile phase ratio: Methanol: Phosphate Buffer (30:70) V/V
Flow rate: 1.0ml/min
Injection volume: 10µl
Run time: 10min

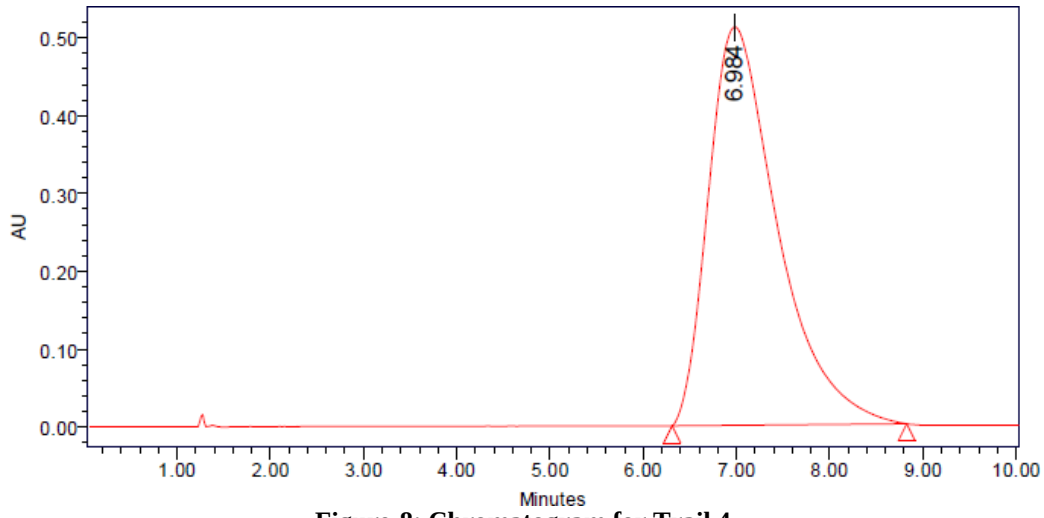


Figure-8: Chromatogram for Trail 4.

Table-6: Results of Chromatogram for Trail 4.

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Armodafinil	6.984	1225687	54857	1.13	3599

Observation: In this trial it shows broad peak and tailing factor is there in the chromatogram. So it requires more trials to obtain good peaks.

Optimized Chromatogram (Standard)
Mobile phase ratio: Methanol: Phosphate Buffer (35:65) V/V
Column: Symmetry ODS C18 (4.6×250mm, 5µm)
Column temperature: Ambient
Wavelength: 235nm
Flow rate: 1ml/min
Injection volume: 10µl
Run time: 8min

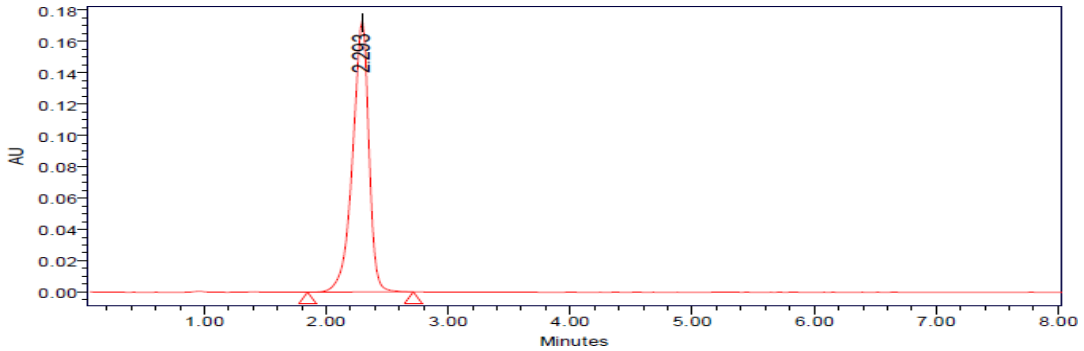


Figure-9: Optimized Chromatogram (Standard).

Table 7: Optimized Chromatogram (Standard).

S. No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Armodafinil	2.293	1658242	185421	1.24	6569

Observation: In this trial it shows proper separation of peak and more plate count in the chromatogram and the tailing factor is within the limit. So it is an optimized chromatogram.

Optimized Chromatogram (Sample)

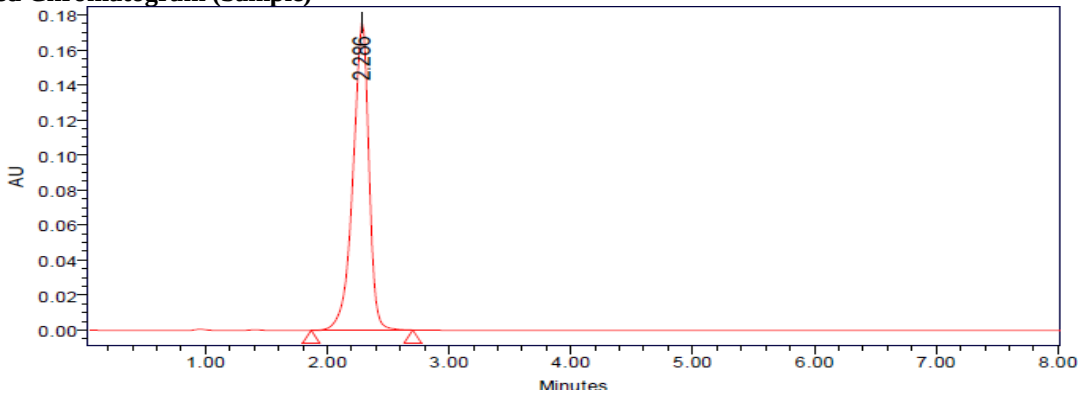


Figure-10: Optimized Chromatogram (Sample).

Table-8: Optimized Chromatogram (Sample)

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Armodafinil	2.286	1689654	185231	1.28	6659

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.

METHOD VALIDATION

Blank:

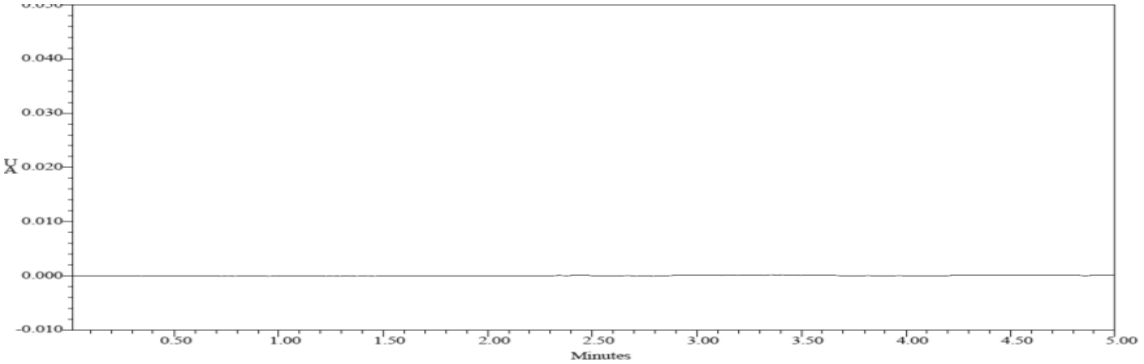


Fig-11: Chromatogram showing blank (mobile phase preparation) System Suitability.

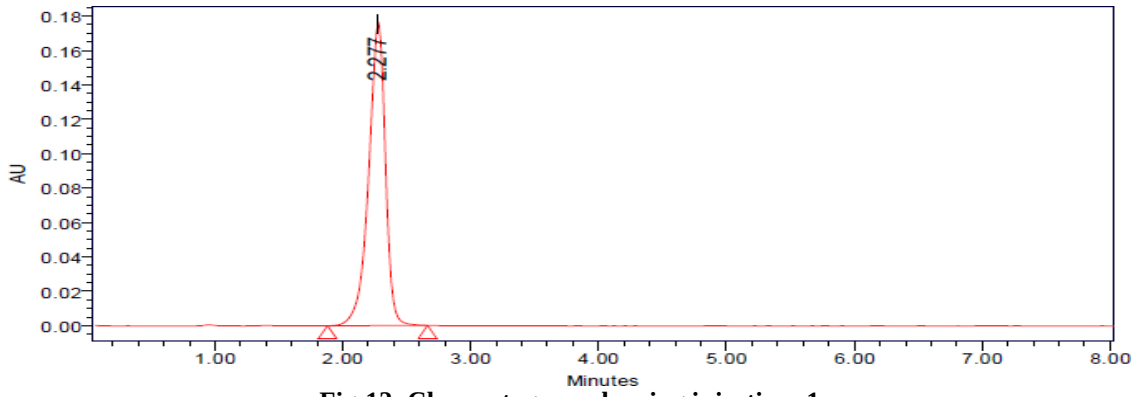


Fig-12: Chromatogram showing injection -1

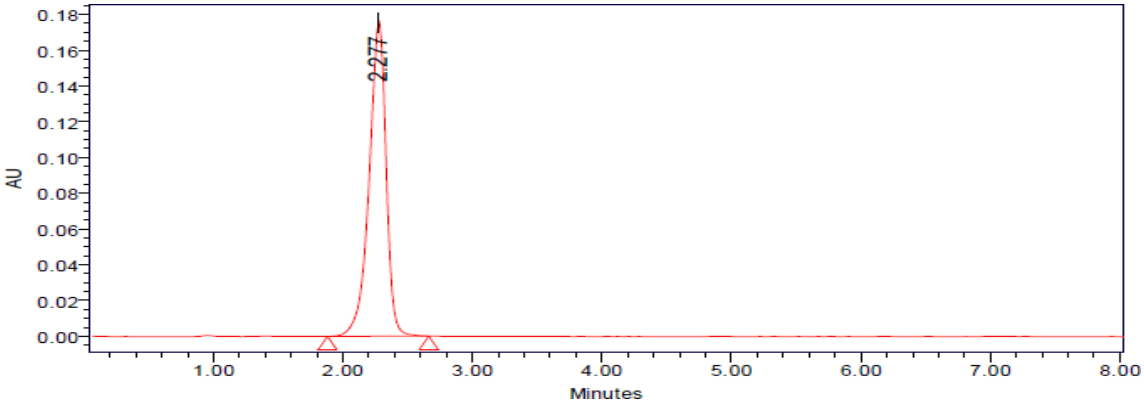


Fig-13: Chromatogram showing injection -2

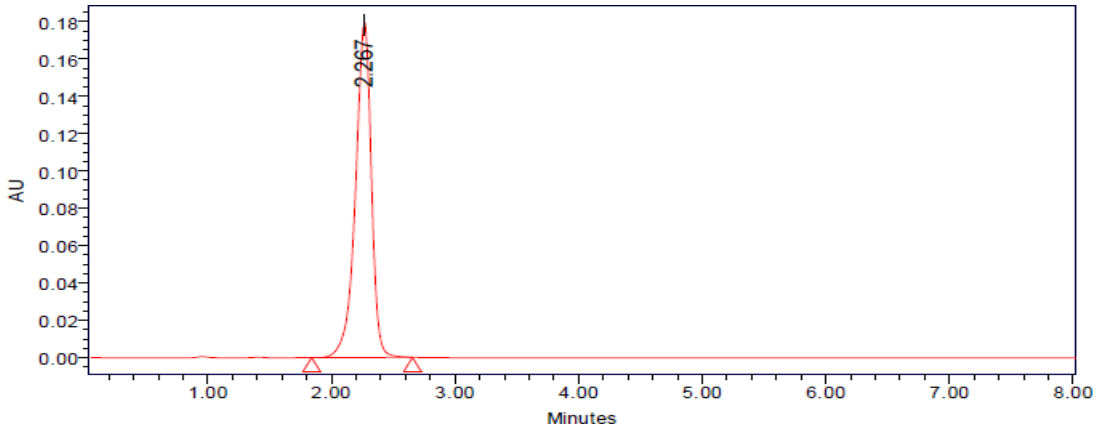


Fig-14: Chromatogram showing injection -3

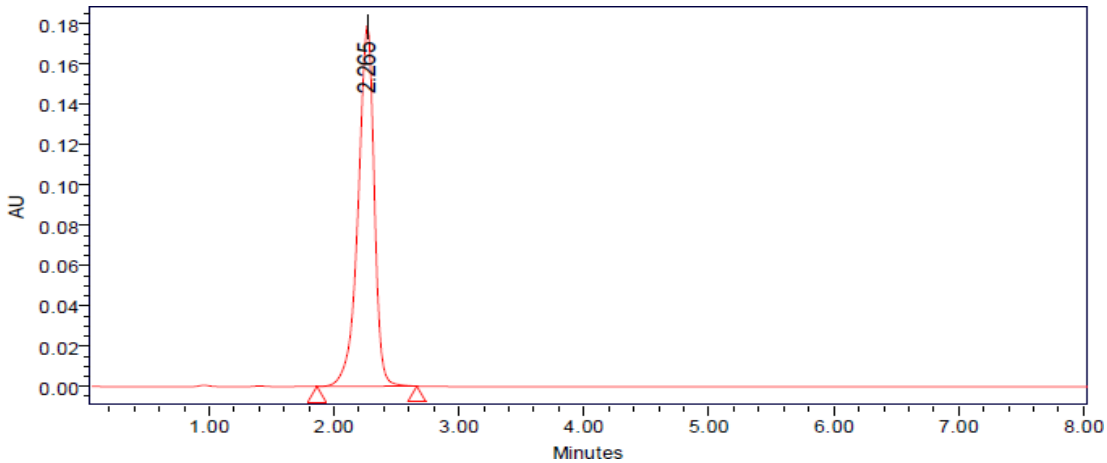


Fig-15: Chromatogram showing injection -4

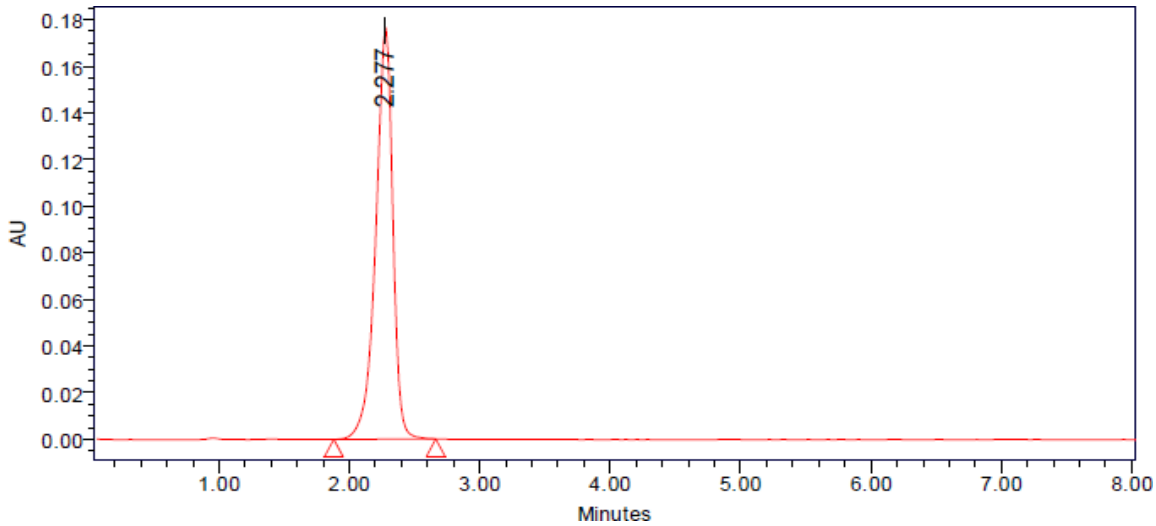


Fig. 16: Chromatogram showing injection -5.

Table-9: Results of system suitability for Armodafinil.

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Armodafinil	2.277	1652847	185647	6589	1.24
2	Armodafinil	2.277	1653658	186254	6587	1.26
3	Armodafinil	2.267	1654521	185475	6584	1.28
4	Armodafinil	2.265	1653564	186594	6582	1.29
5	Armodafinil	2.277	1658745		6895	1.24
Mean			1654667			
Std. Dev.			2355.764			
% RSD			0.142371			

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.

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 quantitate Armodafinil in drug product.

Assay (Standard)

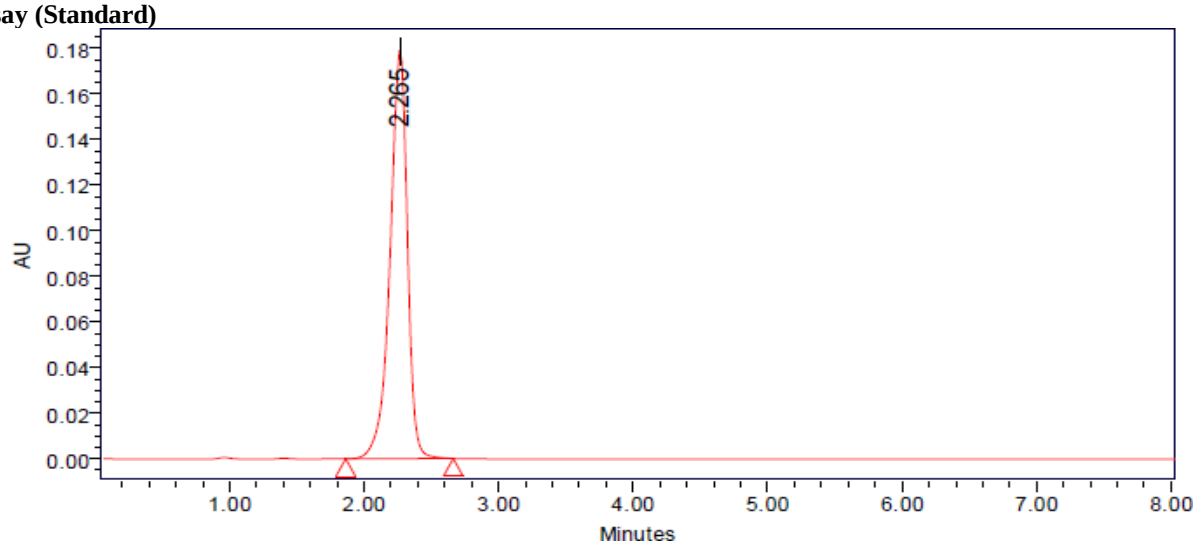


Fig-17: Chromatogram showing assay of standard injection -1.

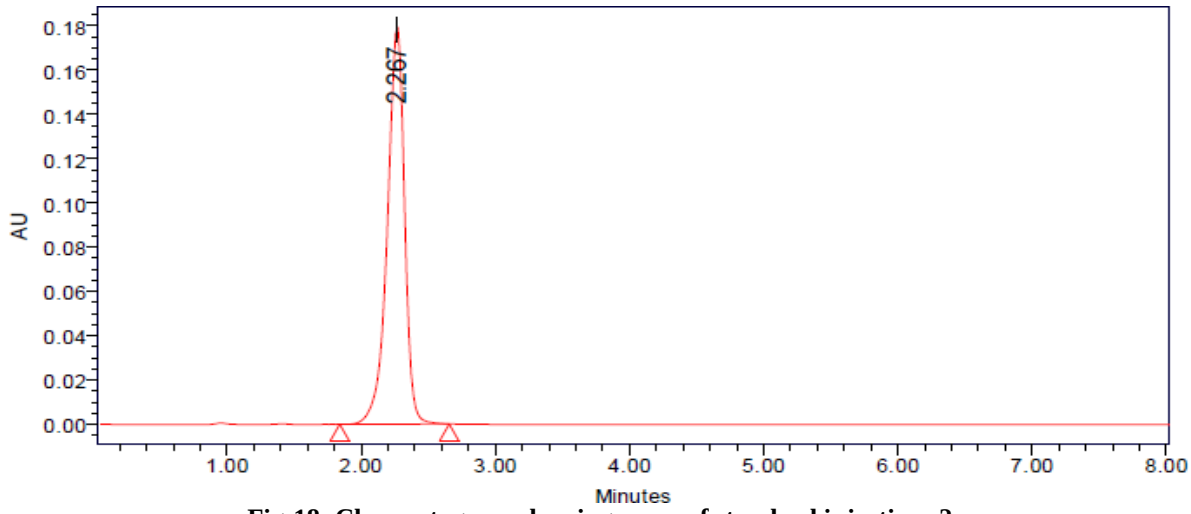


Fig-18: Chromatogram showing assay of standard injection -2

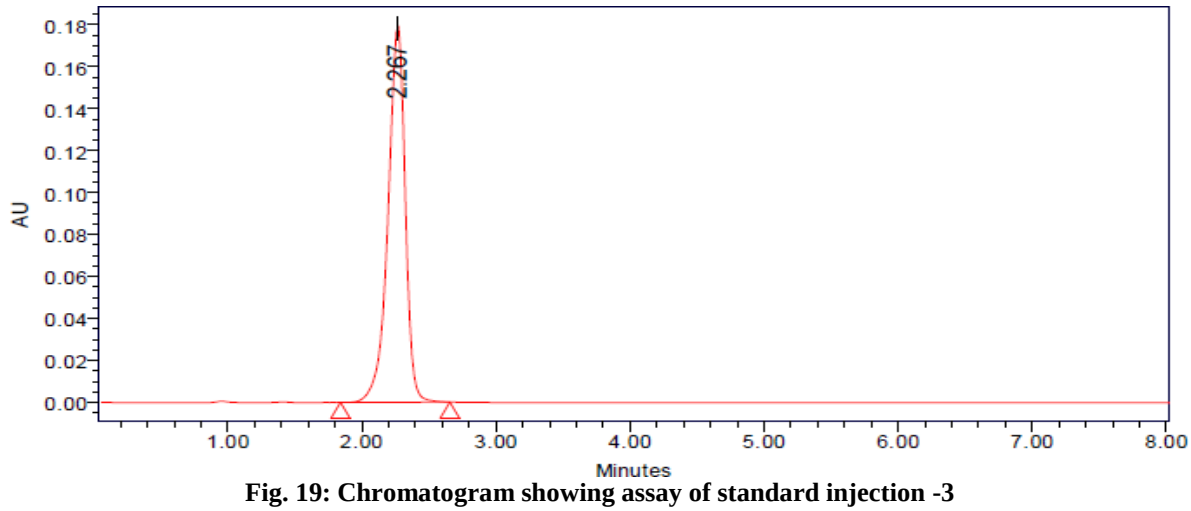


Fig. 19: Chromatogram showing assay of standard injection -3

Table-10: Peak results for assay standard.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Armodafinil	2.265	1658254	185468	1.24	6391	1
2	Armodafinil	2.267	1658475	184524	1.23	6549	2
3	Armodafinil	2.267	1658471	186598	1.25	6682	3

Assay (Sample)

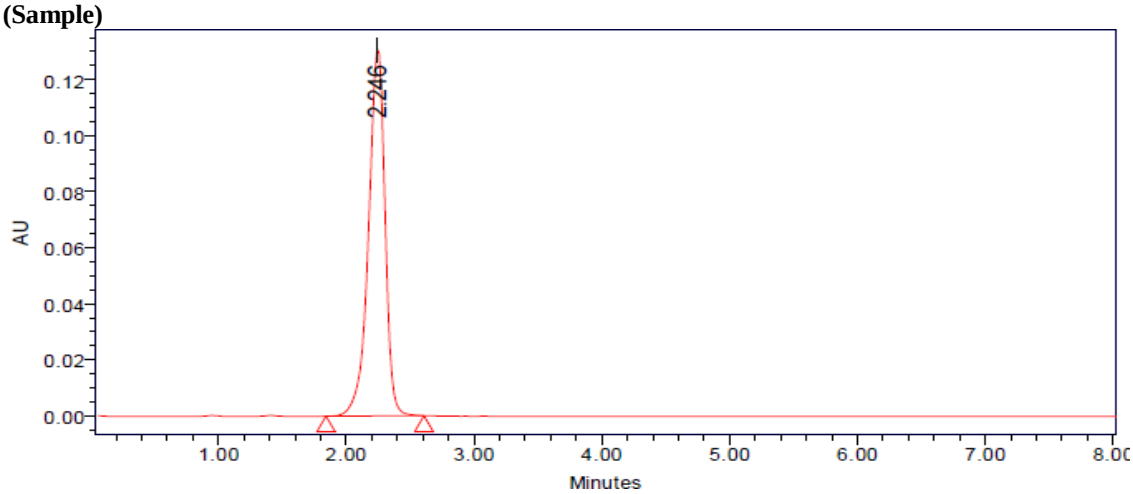


Fig-20: Chromatogram showing assay of sample injection-1.

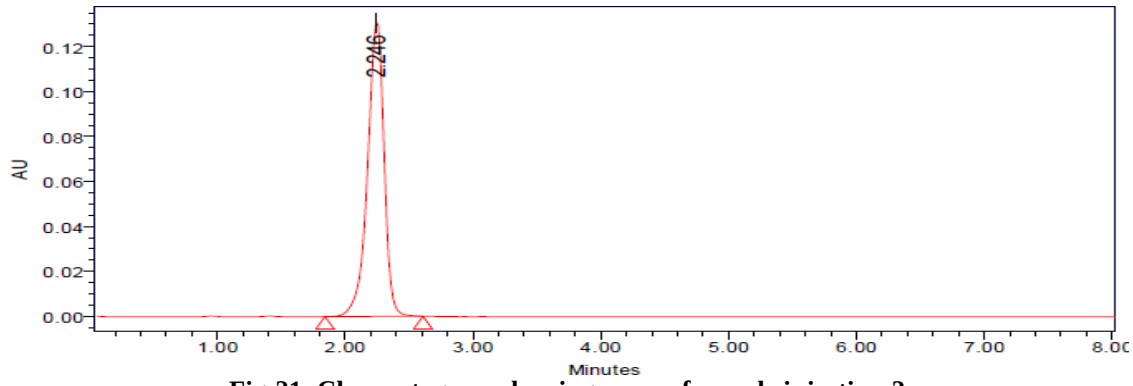


Fig-21: Chromatogram showing assay of sample injection-2.

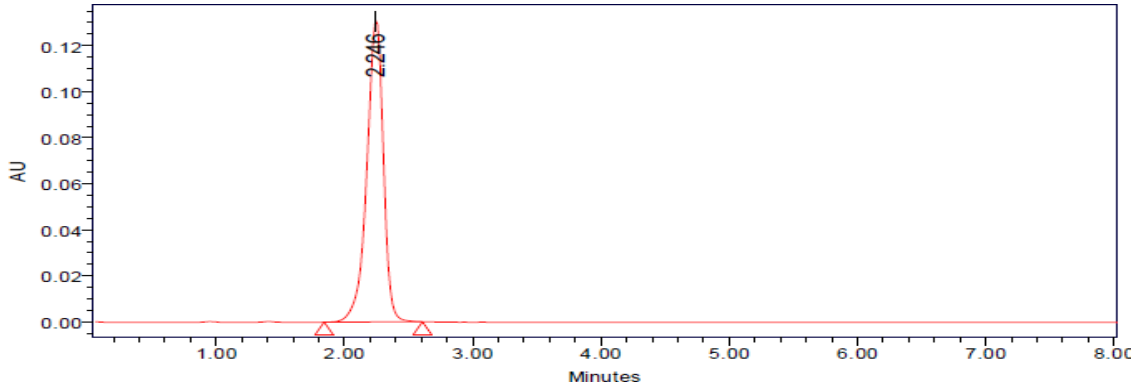


Fig. 22: Chromatogram showing assay of sample injection-3.

Table-11: Peak results for Assay sample

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Armodafinil	2.246	1645879	184574	0.85	6458	1
2	Armodafinil	2.246	1645875	183598	0.86	6584	2
3	Armodafinil	2.246	1658423	185472	0.85	6457	3

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

$$= 99.40\%$$

The % purity of Armodafinil in pharmaceutical dosage form was found to be 99.40%.

LINEARITY

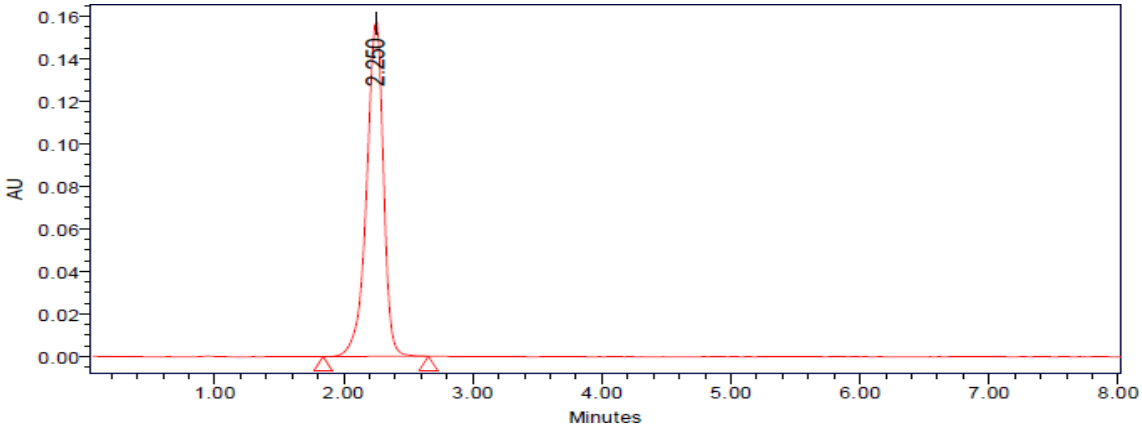


Fig. 23: Chromatogram showing linearity level-1.

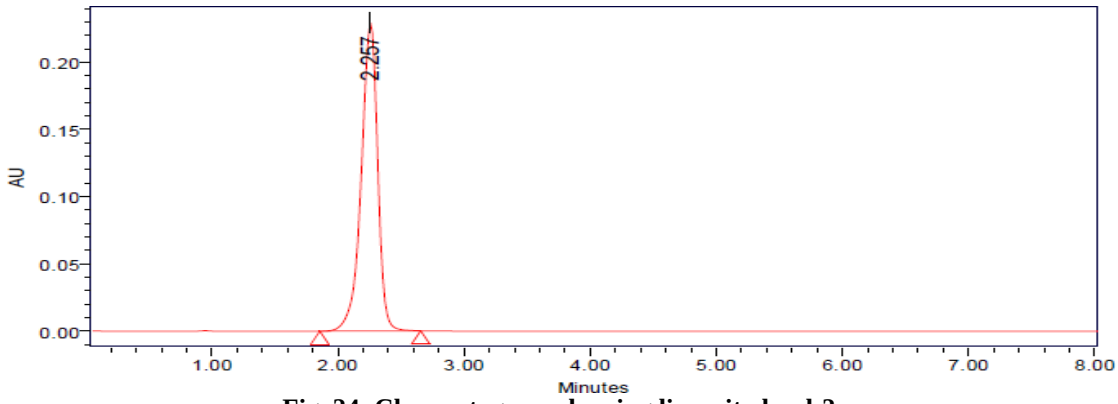


Fig. 24: Chromatogram showing linearity level-2.

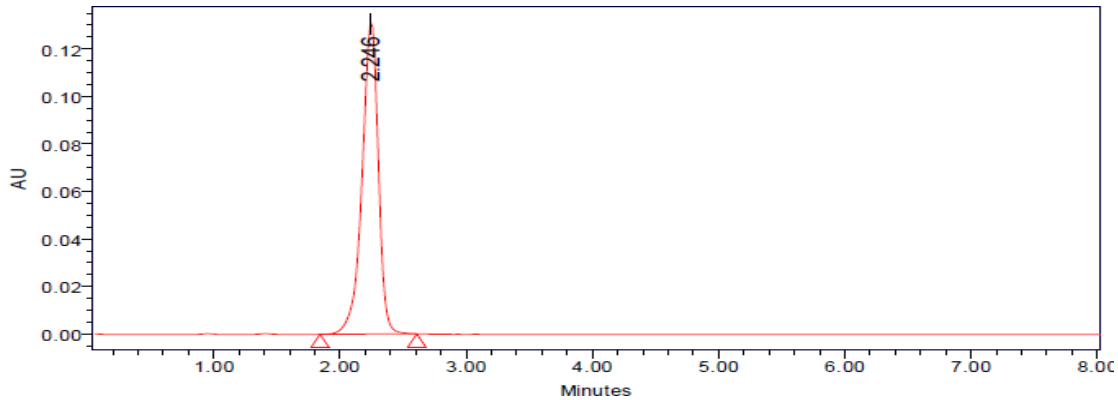


Fig. 25: Chromatogram showing linearity level-3.

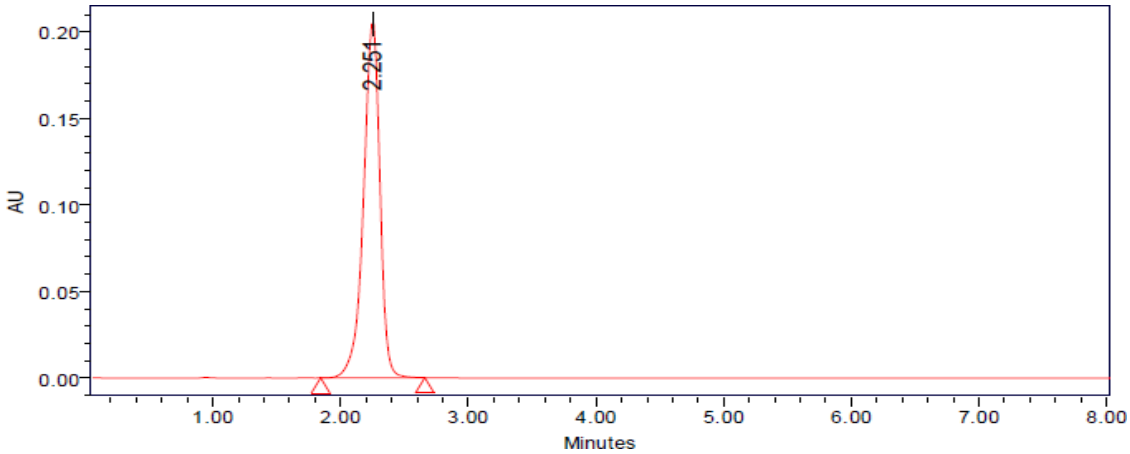


Fig. 26: Chromatogram showing linearity level-4.

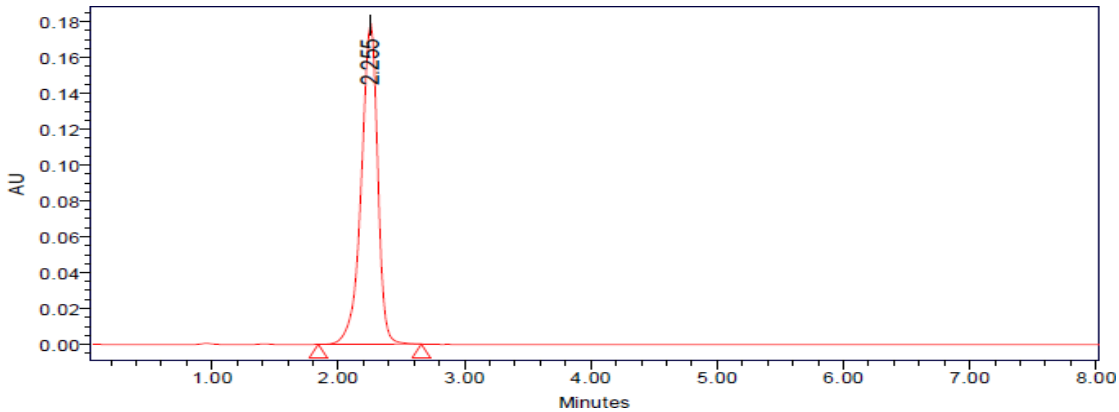


Fig. 27: Chromatogram showing linearity level-5.

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

Table 12: Data for Linearity of Armodafinil.

Concentration ug/ml	Average Peak Area
6	1078475
8	1461129
10	1808358
12	2211573
14	2593778

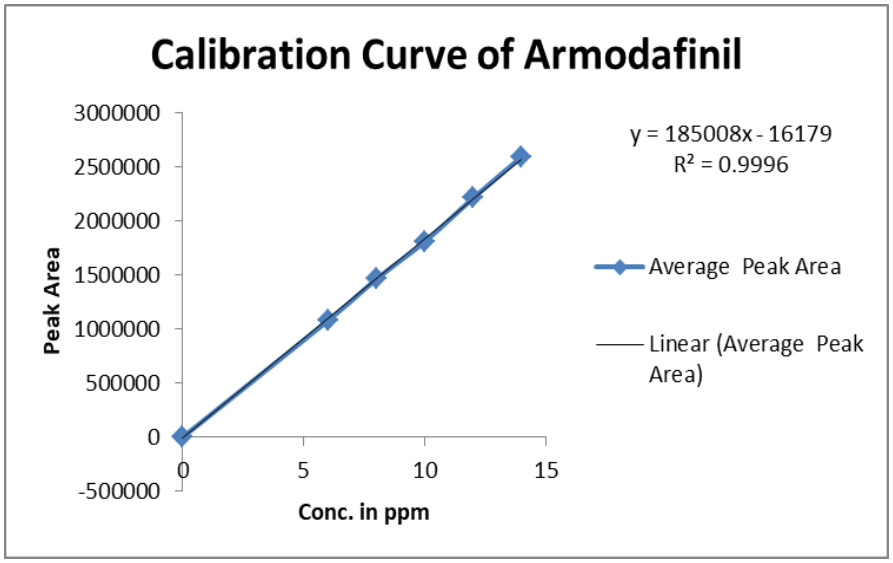


Fig. 28: Linearity Curve of Armodafinil.

LINEARITY PLOT

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

Y = mx + c

Slope (m) = 18500

Intercept (c) = 16179

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 0.16179. 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 Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

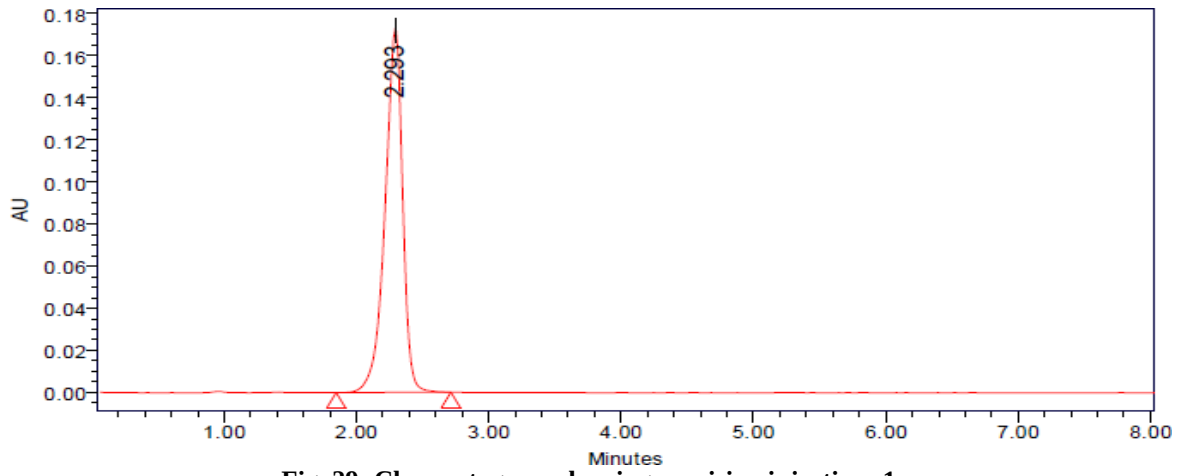


Fig. 29: Chromatogram showing precision injection -1.

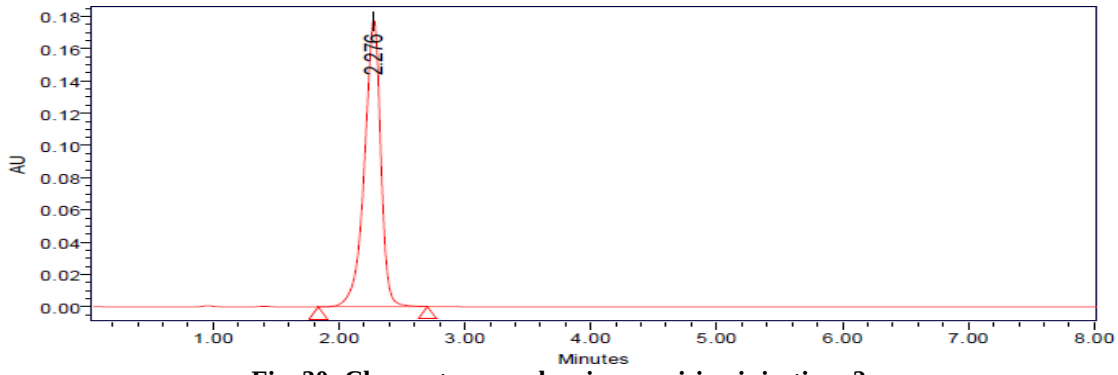


Fig. 30: Chromatogram showing precision injection -2.

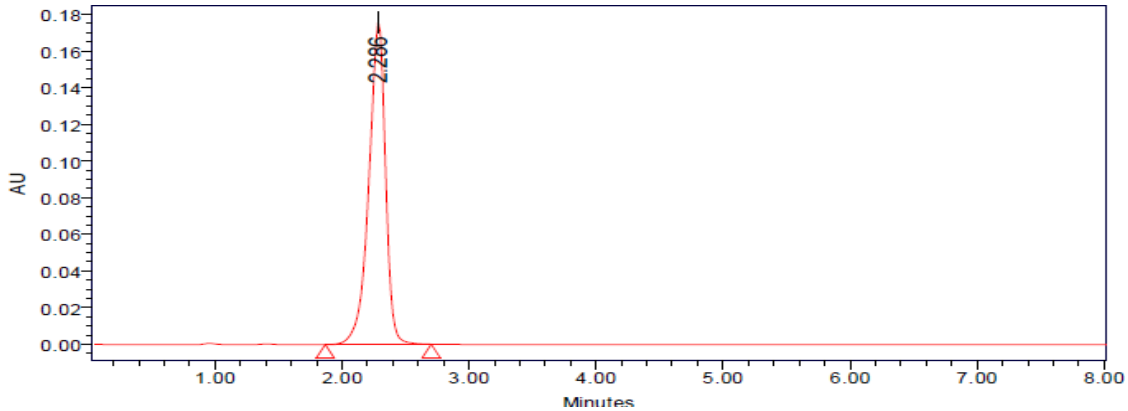


Fig. 31: Chromatogram showing precision injection -3.

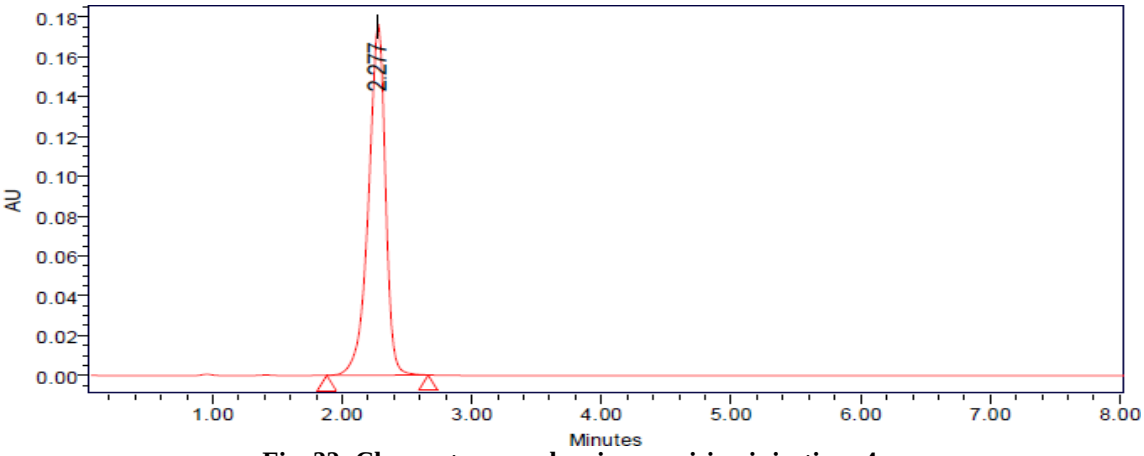


Fig. 32: Chromatogram showing precision injection -4.

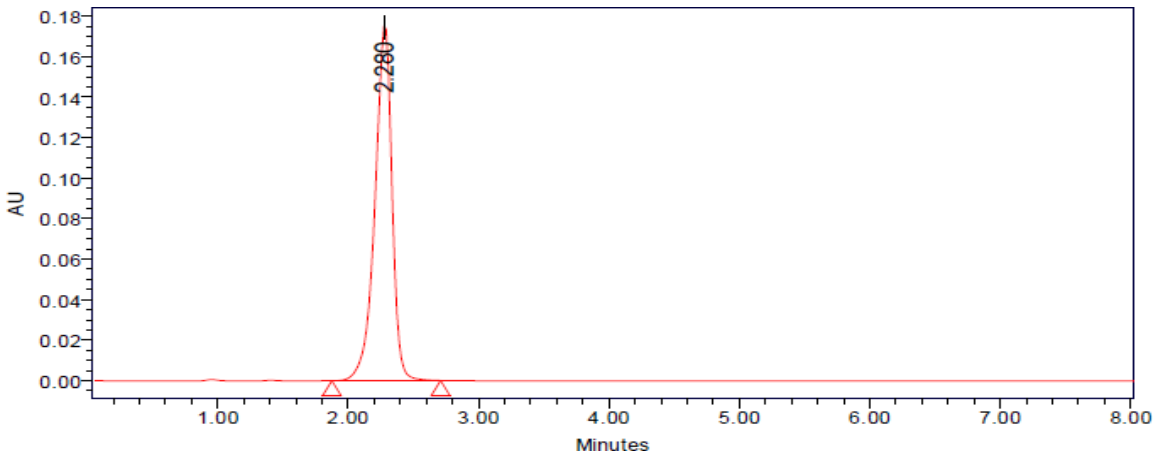


Fig. 33: Chromatogram showing precision injection -5.

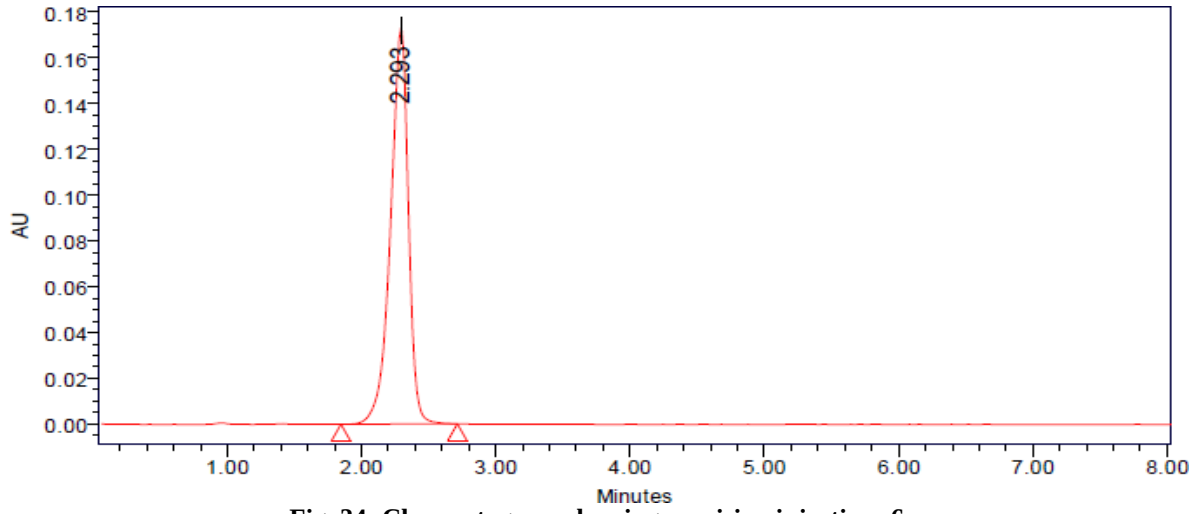


Fig. 34: Chromatogram showing precision injection -6.

Table-13: Results of repeatability for Armodafinil.

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Armodafinil	2.293	1658954	186958	1.26	6785
2	Armodafinil	2.276	1658745	187548	1.27	6854
3	Armodafinil	2.286	1659865	189854	1.26	6852
4	Armodafinil	2.277	1653254	186985	1.25	6784
5	Armodafinil	2.280	1654781	189542	1.24	6895
6	Armodafinil	2.293	1661324	187586	1.28	6965
Mean			1657821			
Std.dev			3120.433			
%RSD			0.188225			

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

Analyst 1

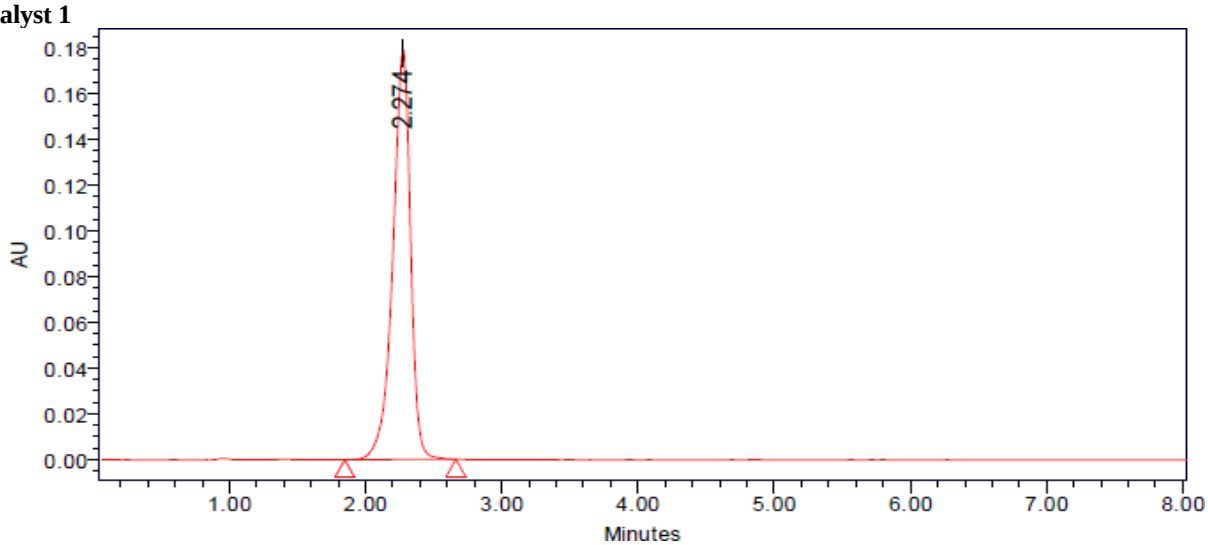


Fig. 35: Chromatogram showing Analyst 1 injection -1.

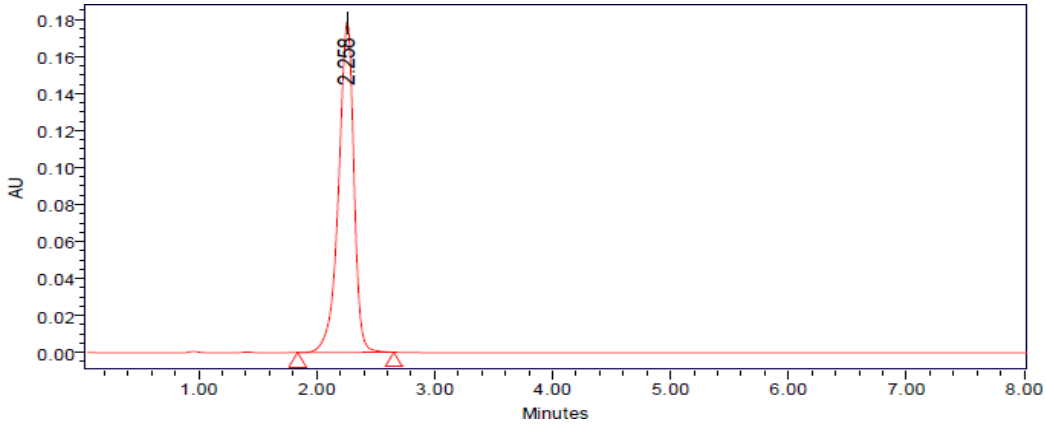


Fig. 36: Chromatogram showing Analyst 1 injection -2.

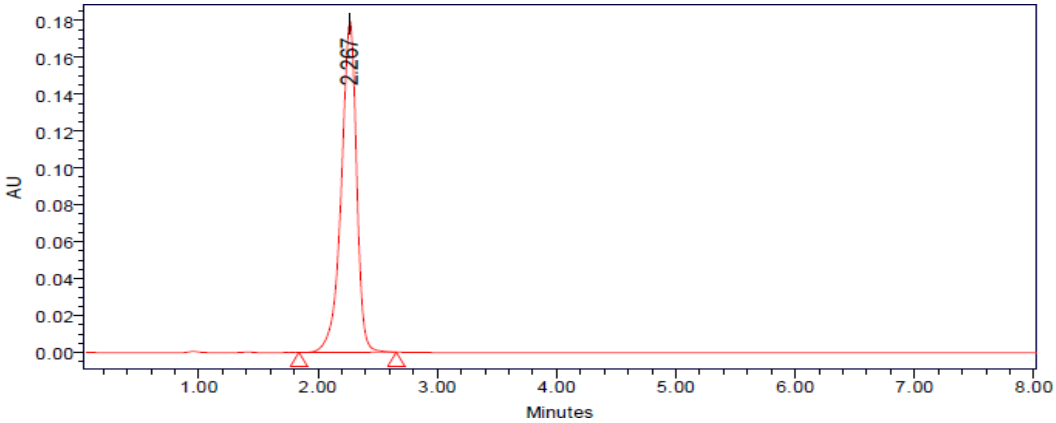


Fig. 37: Chromatogram showing Analyst 1 injection -3.

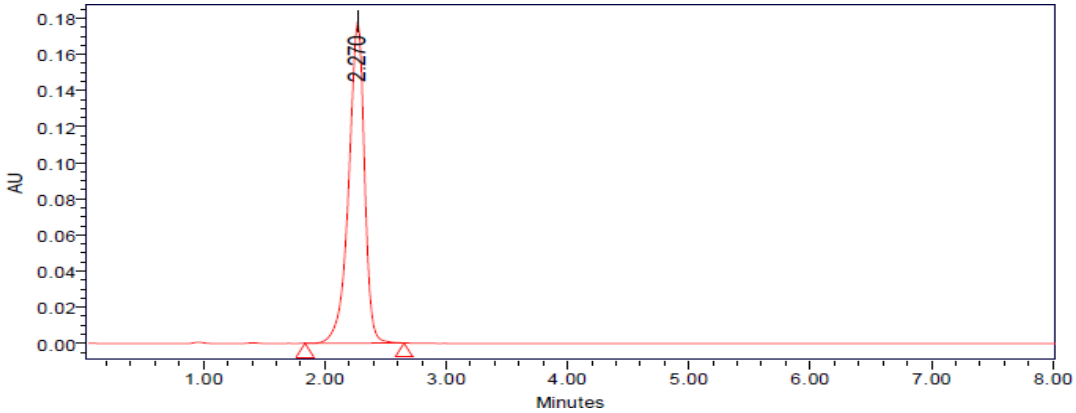


Fig. 38: Chromatogram showing Analyst 1 injection -4.

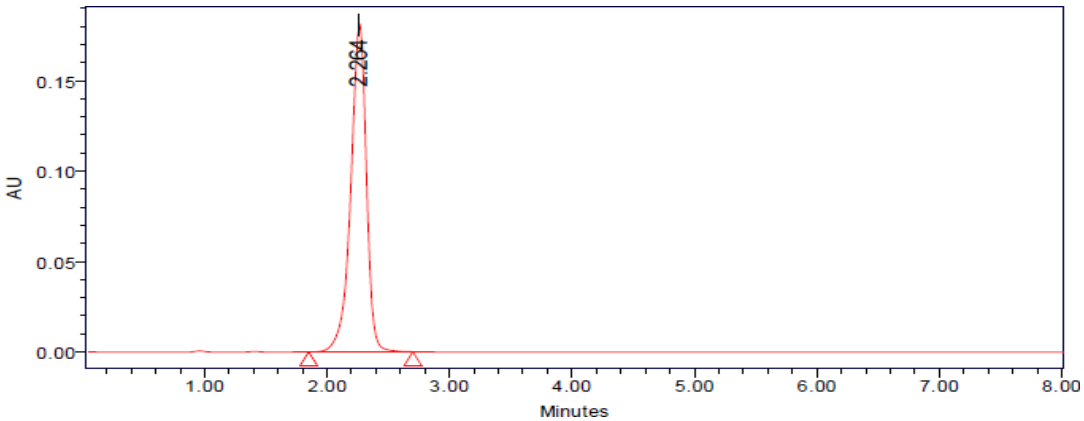


Fig. 39: Chromatogram showing Analyst 1 injection -5.

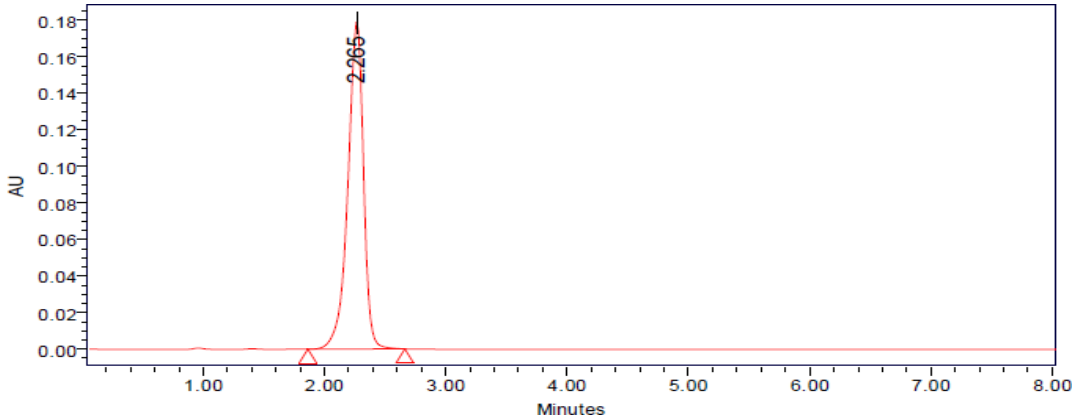


Fig. 40: Chromatogram showing Analyst 1 injection -6.

Table-14: Results of Intermediate precision for Armodafinil

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Armodafinil	2.274	1678541	186589	6587	1.26
2	Armodafinil	2.258	1685985	186598	6321	1.26
3	Armodafinil	2.267	1685745	186985	6385	1.25
4	Armodafinil	2.270	1685987	187854	6580	1.26
5	Armodafinil	2.264	1698526	187549	6721	1.27
6	Armodafinil	2.265	1685943	186598	6637	1.26
Mean			1686788			
Std. Dev.			6463.466			
% RSD			0.383182			

Acceptance Criteria

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

Analyst 2

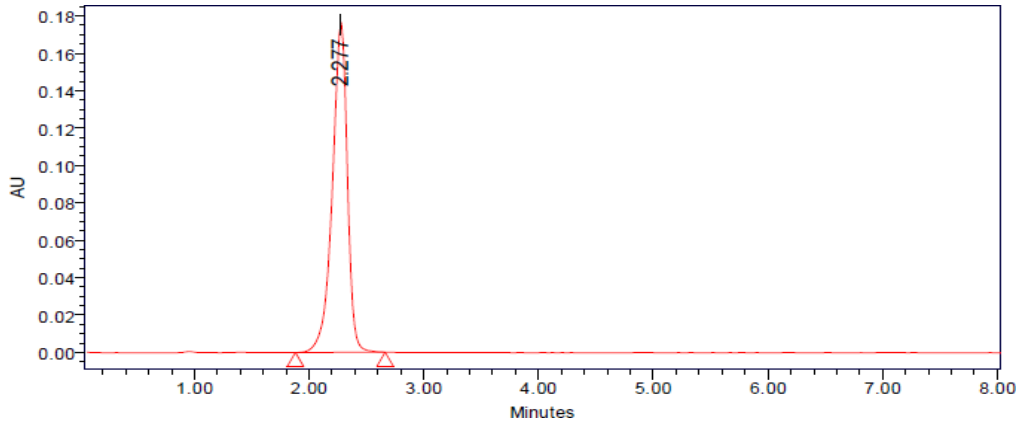


Fig. 41: Chromatogram showing Analyst 2 injection -1.

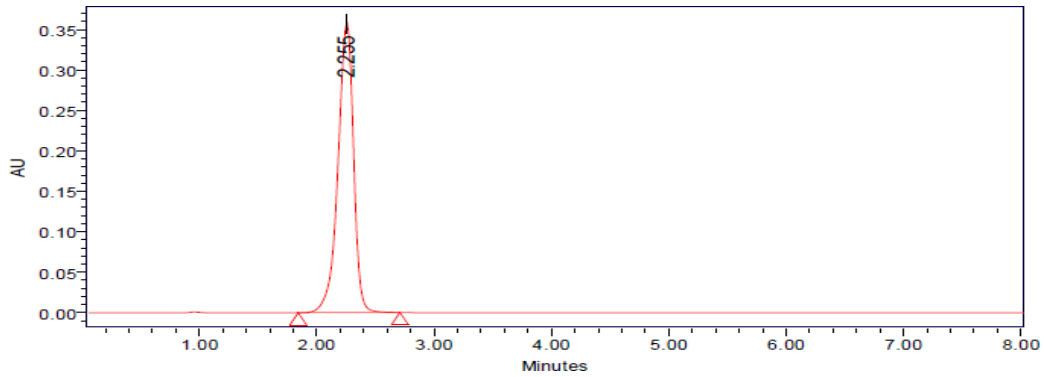


Fig. 42: Chromatogram showing Analyst 2 injection -2.

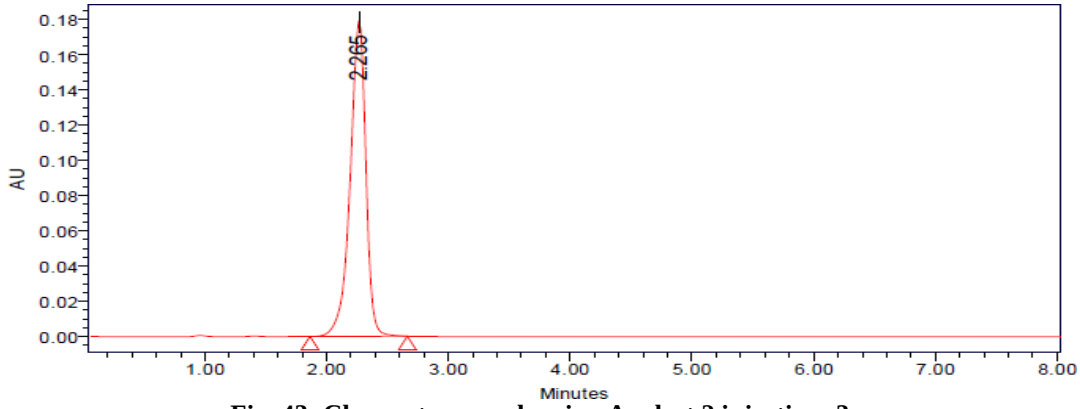


Fig. 43: Chromatogram showing Analyst 2 injection -3.

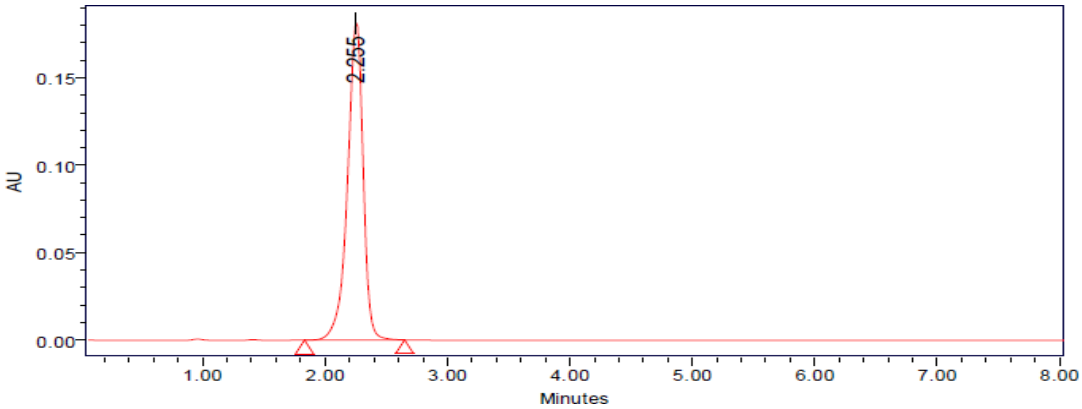


Fig. 44: Chromatogram showing Analyst 2 injection -4.

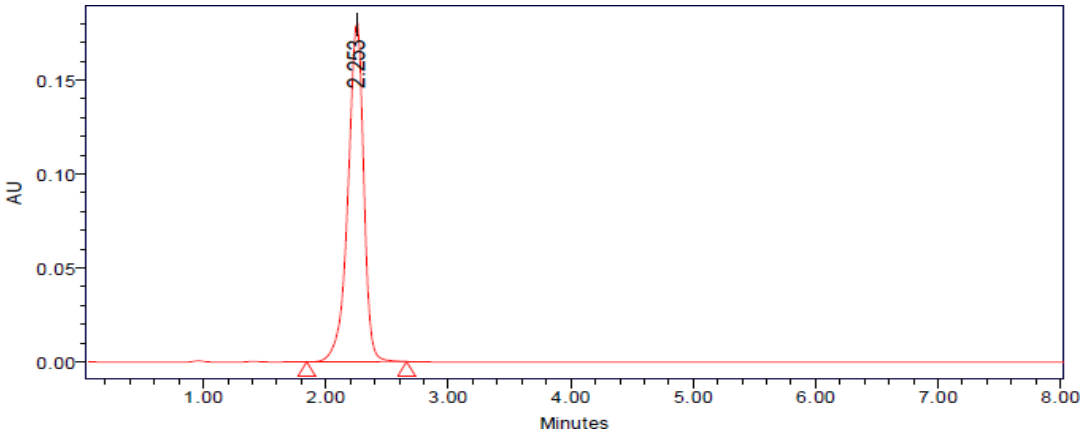


Fig. 45: Chromatogram showing Analyst 2 injection -5.

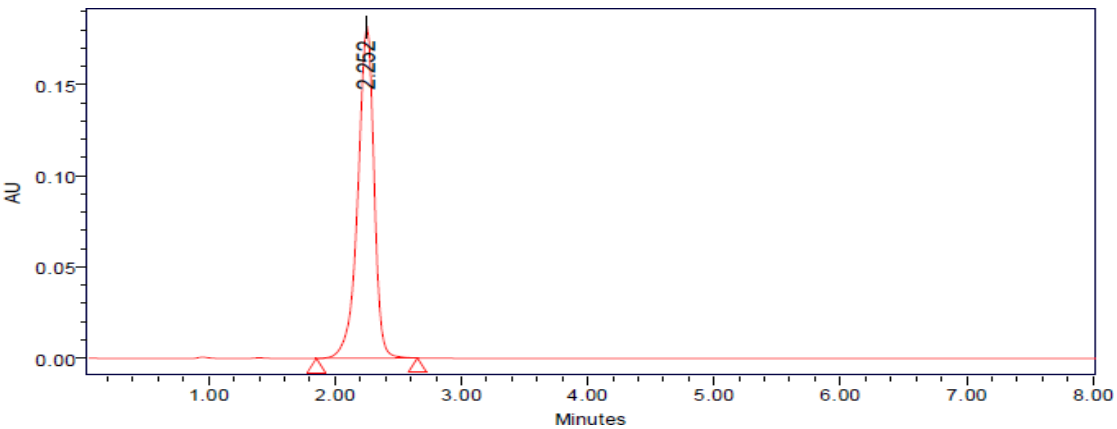


Fig. 46: Chromatogram showing Analyst 2 injection -6.

Table 15: Results of Intermediate precision Analyst 2 for Armodafinil.

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USPTailing
1	Armodafinil	2.277	1665847	167481	6854	1.25
2	Armodafinil	2.255	1658989	167854	6785	1.26
3	Armodafinil	2.265	1659845	167895	6854	1.24
4	Armodafinil	2.255	1665964	167854	6895	1.26
5	Armodafinil	2.253	1659863	168585	6459	1.25
6	Armodafinil	2.252	1665986	167859	6456	1.26
Mean			1662749			
Std. Dev.			3501.766			
% RSD			0.210601			

Acceptance Criteria

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

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

Accuracy 50%

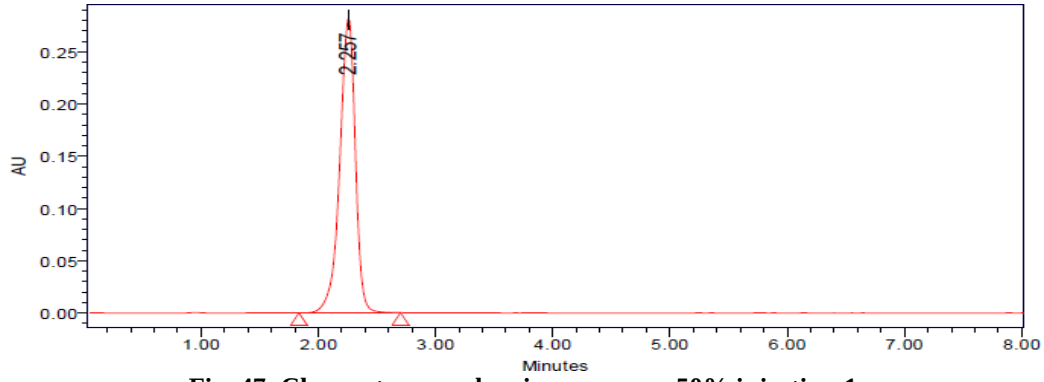


Fig. 47: Chromatogram showing accuracy-50% injection-1.

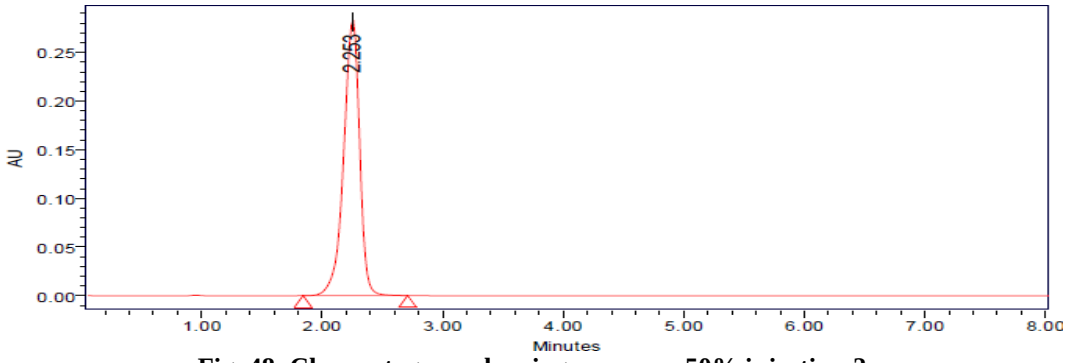


Fig. 48: Chromatogram showing accuracy-50% injection-2.

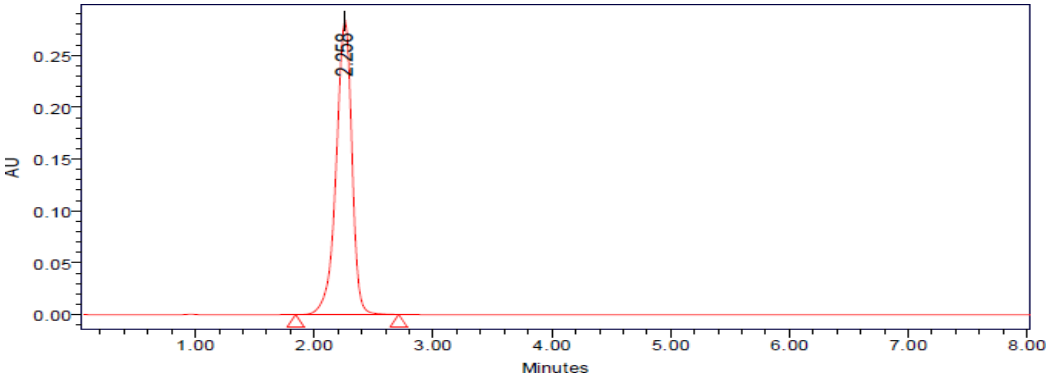


Fig-49: Chromatogram showing accuracy-50% injection-3.

Table 16: Results of Accuracy for concentration-50%.

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Armodafinil	2.257	108982	92715	0.99	4695	1
2	Armodafinil	2.253	108659	92548	1.02	4658	2
3	Armodafinil	2.258	109564	92685	1.00	4785	3

Accuracy 100%

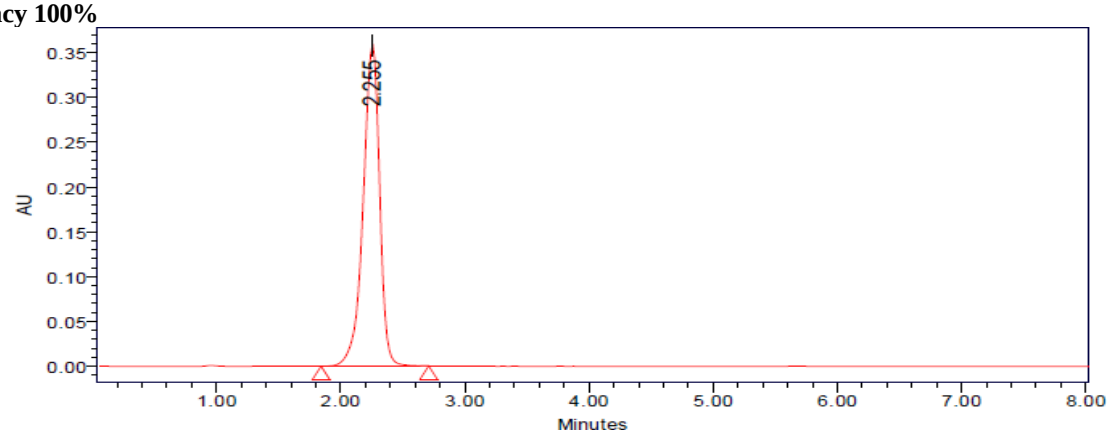


Fig-50: Chromatogram showing accuracy-100% injection-1.

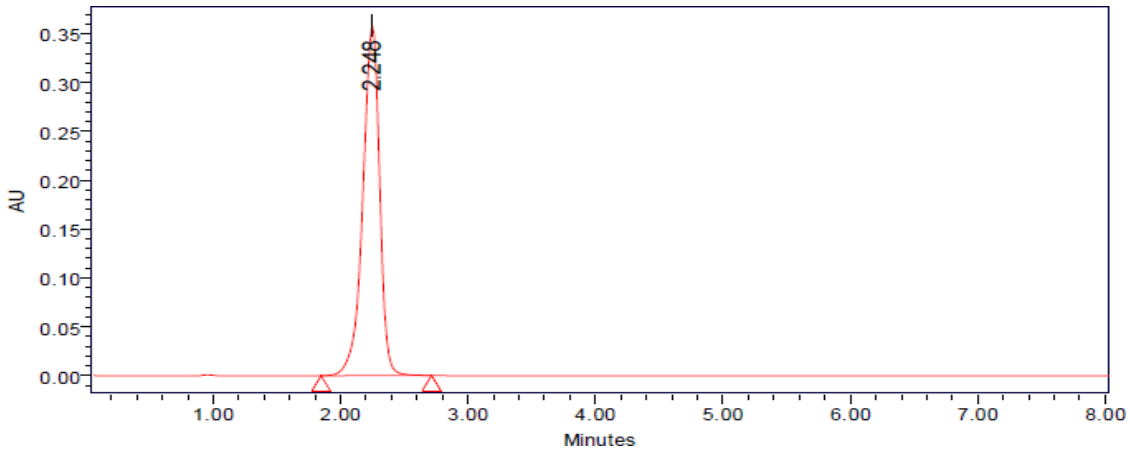


Fig. 51: Chromatogram showing accuracy-100% injection-2.

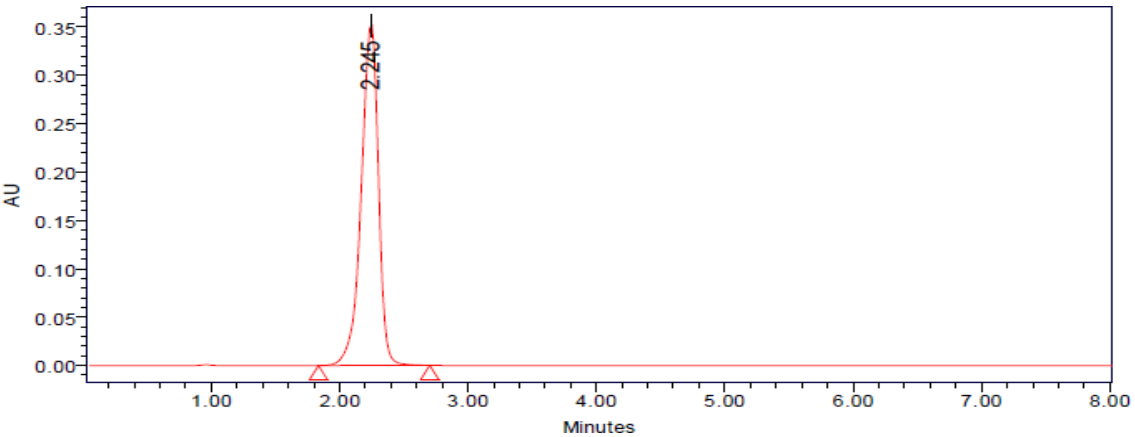


Fig. 52: Chromatogram showing accuracy-100% injection-3.

Table-17: Results of Accuracy for concentration-100%.

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Armodafinil	2.255	201689	186598	1.26	6852	1
2	Armodafinil	2.248	201874	185476	1.25	6585	2
3	Armodafinil	2.245	202998	186579	1.26	6658	3

Accuracy 150%

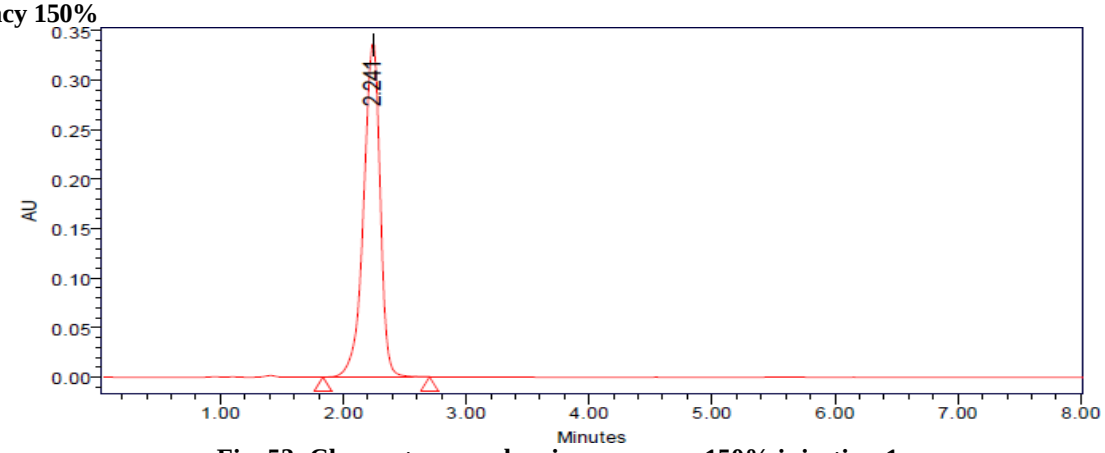


Fig. 53: Chromatogram showing accuracy-150% injection-1.

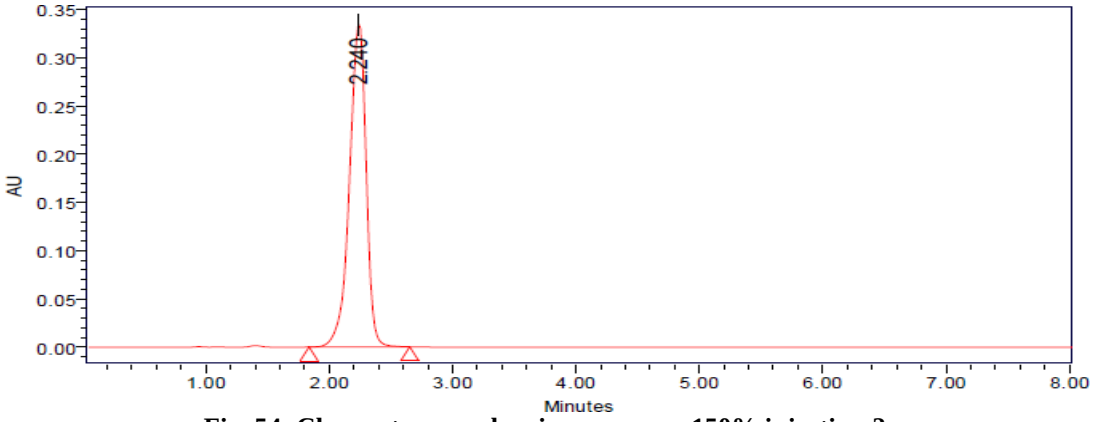


Fig. 54: Chromatogram showing accuracy-150% injection-2.

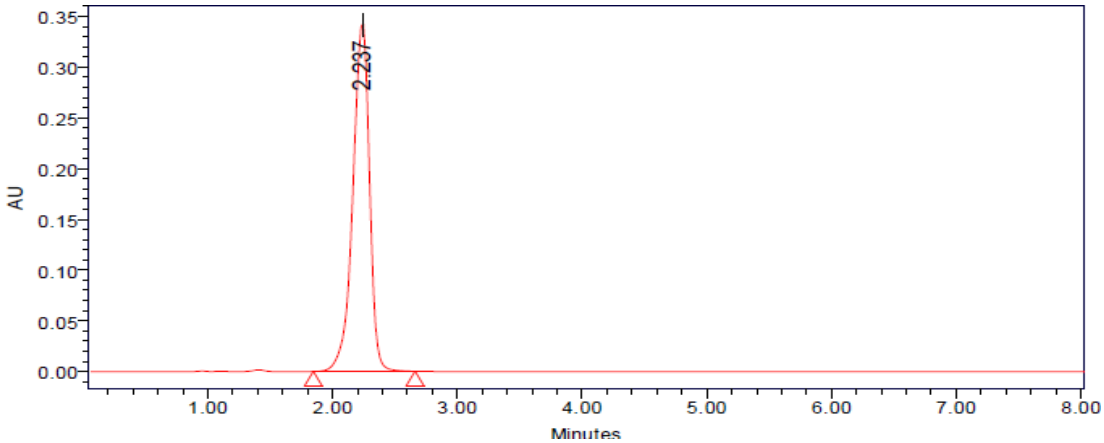


Fig. 55: Chromatogram showing accuracy-150% injection-3.

Table-18: Results of Accuracy for concentration-150%.

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Armodafinil	2.241	296765	236543	1.32	7042	1
2	Armodafinil	2.240	297546	236895	1.31	6926	2
3	Armodafinil	2.237	296786	238546	1.32	7059	3

Table 19: The Accuracy Results for Armodafinil.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109068.3	5	5.021	100.420%	100.72%
100%	202187	10	10.054	100.540%	
150%	297032.3	15	15.181	101.206%	

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

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 × σ / s

Where

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

Result

= 0.95µg/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×σ/S

Where

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

Result

= 2.9µg/ml

LOD	0.95925344
LOQ	2.906828605

SE of Intercept = Excel Function (Data Analysis → Regression) SD of Intercept = SE of Intercept * √N
LOD = 3.3 * (SD of Intercept/Slope) LOQ = 10 * (SD of Intercept/Slope)

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 Armodafinil. The method is robust only in less flow condition. The standard of Armodafinil was 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

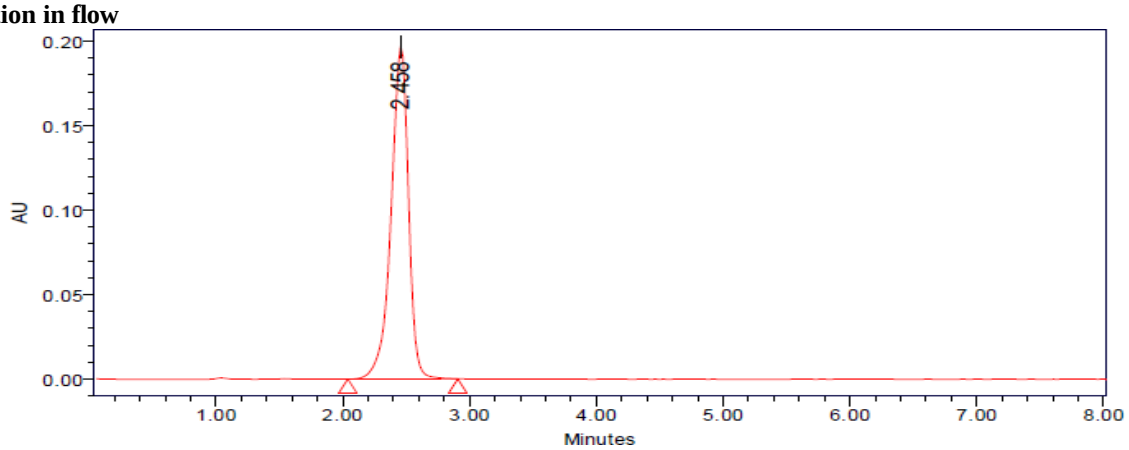


Figure-56: Chromatogram showing less flow of 0.9ml/min.

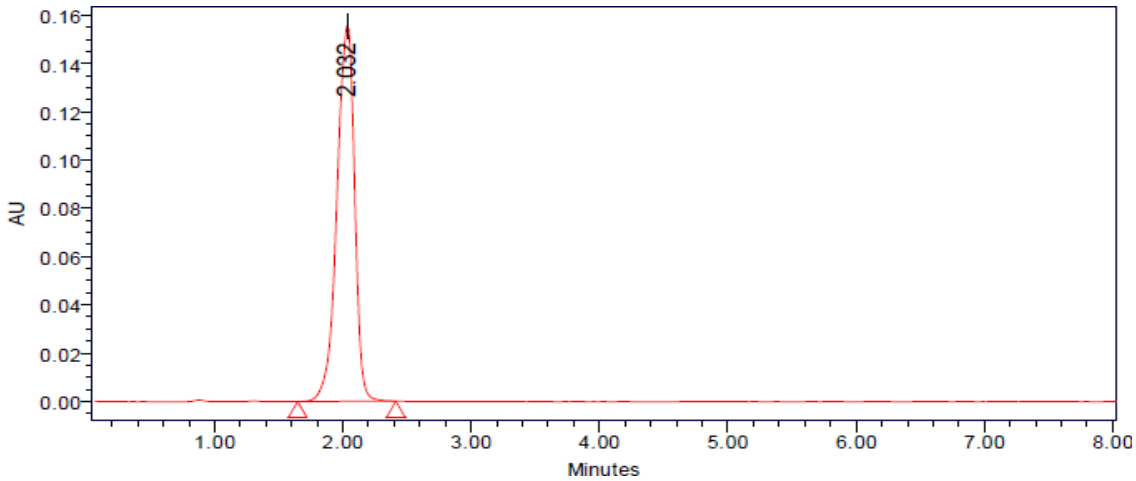


Figure 57: Chromatogram showing more flow of 1.1 ml/min.

Table 20: Results for Robustness.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1658242	2.312	6569	1.24
Less Flow rate of 0.9 mL/min	1854215	2.458	6865	1.35
More Flow rate of 1.1 mL/min	1758468	2.032	6254	1.32

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

8. SUMMARY

The analytical method was developed by studying different parameters.

First of all, maximum absorbance was found to be at 235nm and the peak purity was excellent.

Injection volume was selected to be 10µl which gave a good peak area.

The column used for study was Symmetry ODS C18 (4.6×250mm, 5µm)because it was giving good peak.

Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.

Mobile phase is Methanol: Phosphate Buffer pH-3.6 in the ratio of 35:65 v/v was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery.

Run time was selected to be 8min because analyze gave peak around 2.276and also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range of 6-14ppm of theArmodafinil target concentration.

The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

9. CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Armodafinil in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.

Armodafinil was found to be very slightly soluble in water, soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF).

Methanol: Phosphate Buffer (35:65) v/v was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Armodafinilin bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

On the occasion of presenting this thesis, it is my privilege to express my sincere thanks to my esteemed research guide **Dr. Syed Hafeez Ahmed Sir,** Department of Pharmaceutical Analysis, Shadan College of Pharmacy, Hyderabad, for his guidance and constant encouragement that framed the foundation of this project.

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I am thankful to all teaching staff for their constant help and encouragement during my studies. I sincerely thank **Dr. Shaik Mohd Khasim**, Director, Shadan College of Pharmacy, for supporting us throughout the project.

My sincere thanks to **office staff, Librarian**, and especially the **non-teaching staff** who have always extended their full-hearted support during my project work.

Above all, I thank **my parents** and all my family members for their blessings and encouragement, without which it would not have been possible for me to complete the study.

Above all “Thank You” to **the Almighty**, who has given me this opportunity to extend my gratitude to all those

people who have helped me and guided me throughout my work and life, I bow my head in complete submission before Him for the blessings poured on me.

Despite all this co-operation rendered generously by one and all, I am solely responsible for any and all the errors and short comings of this dissertation.

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LIST OF ABBREVIATIONS

HPLC	-	High Performance liquid chromatography
UV	-	Ultra violet spectroscopy
TLC	-	Thin layer chromatography
LOD	-	Limit of Detection
LOQ	-	Limit of Quantitation
S.D	-	Standard Deviation
%RSD	-	Percentage Relative Standard Deviation
RS	-	Peak Resolution
M.P	-	Mobile Phase
Mg	-	milligrams
µg	-	Micrograms
ml	-	Milliliters
%	-	Percentage
w/w	-	Weight/weight
v/v	-	volume/volume
µg/ml	-	micrograms per milliliter
nm	-	Nanometer
Rt	-	Retention Time
Min	-	Minutes
ICH	-	International conference on Harmonization