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Research Article

**ANALYTICAL METHOD DEVELOPMENT FOR THE
DETERMINATION OF R-PHENETHYLAMINE ASSAY BY
HPLC****Basavarajula Shivacharan*¹, Dr. K. Hariprasad²**Department Of Pharmaceutical Analysis ;Princeton College Of Pharmacy, Pocharam,
Hyderabad, Telangana 500088.**Abstract:**

A novel, specific, accurate, rugged, precise reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative determination of Phenethylamine by using Symmetry Xterra C18 (4.6mm x 150mm, 5 μ m) column with a mobile phase containing a mixture of Acetonitrile and Potassium dihydrogen phosphate buffer adjusted to pH-2.8 with ortho phosphoric acid in the ratio of 35:65. The flow rate was 1.0 ml/min and effluent was monitored at 246 nm and a peak eluted at 4.865 min and column oven temperature was maintained ambient. Calibration curve was plotted with a range from 10- 30 μ g/ml. The LOD and LOQ values of Phenethylamine were found to be 1.3 μ g/ml and 3.9 μ g/ml respectively. The percentage recovery of the Phenethylamine was found to be within the limits. The developed RP-HPLC method was validated according to the current International Conference on Harmonization (ICH) guidelines for specificity, LOD, LOQ, linearity, accuracy, precision, intermediate precision and robustness. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Phenethylamine in bulk drug and in its pharmaceutical dosage form. The proposed method was applied for the analysis of tablet formulations, to improve QC and assure therapeutic efficacy.

Keywords: Phenethylamine, RP-HPLC, Accuracy, Validation, ICH Guidelines.**Corresponding author:****Basavarajula Shivacharan,**Department of Pharmaceutical Analysis, Princeton college of Pharmacy,
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INTRODUCTION:**Analytical Chemistry**^[1]

Chemistry is a science that deals with the composition, structure and properties of substance and with the transformation that they undergo.

Analytical chemistry is a branch of chemistry involved with the analysis of chemical composition of natural and artificial materials. It is the measurement of science consisting of a set of powerful ideas and method that are useful in all fields of science and medicine.

Pharmaceutical analysis is a specialized branch of analytical chemistry which is involved in separating, identifying and determining the relative amounts of components in a sample of matter. Pharmaceutical analysis plays a very important role in quality assurance and quality control of bulk drugs and their formulations. Method of analysis is routinely developed improved validated collaboratively studied and applied. The discipline of analytical chemistry consists of qualitative and quantitative analysis.

Qualitative analysis Refers to analysis in which substances are identified or classified on the basis of their chemical or physical properties, such as chemical reactivity, solubility, molecular weight, melting point, radioactive properties (emission, absorption), mass spectra, nuclear half-life, etc.

Quantitative analysis in which the amount or concentration of an analyte may be determined (estimated) and expressed as a numerical value in appropriate units.

1.2 Instrumental method of chemical analysis

Instrumental method of chemical analysis interacts with all the areas of chemistry and other areas of pure and applied science.

Analytical techniques play an important role in

- Production and evaluation of new drugs in bulk and formulation and also estimation from biological fluids.
- Detection and quantification of impurities and metabolites
- Accelerated stability studies
- In vitro dissolution studies
- Pharmacokinetic studies and drug metabolism studies
- Determination of bioavailability of two or more formulation.

1.2.1 Method of estimation of drugs are divided into

- A. Physical methods.
- B. Chemical methods.
- C. Physicochemical methods.

1. Physical method

Physical method of analysis involves the studying of physical properties of a substance. They include

determination of the solubility transparency or degree of turbidity, density or specific gravity moisture content, melting, freezing and boiling points.

2. Chemical methods

The chemical methods include the gravimetric and volumetric procedures which are based on complex formation acid base precipitation and redox reactions.

1.2.2 Types of chemical analysis

1. **Proximate analysis:** The amount of each element in a sample is determined with no concern as to the actual compounds present.
 2. **Partial analysis** deals with the determination of selected constituent in the sample
 3. **Trace constituent analysis** Concerned with the determination of specified compounds present in minute quantity
 4. **Complete analysis** Proportion of each component of the sample is determined.
- A. **Physicochemical methods** Physicochemical methods include

1.2.3. Spectrophotometric techniques

- UV- visible spectrophotometric techniques
- Fluorescence and Phosphorescence techniques
- Atomic spectrophotometric
- Infrared spectrophotometry
- X- ray spectrophotometry
- Nuclear magnetic resonance spectroscopy
- Mass spectroscopy
- Electron spin resonance spectroscopy

1.2.4. Electrochemical techniques

- Potentiometry
- Voltametry
- Electrogravimetry
- Conductometry
- Amperometry

1.2.5. Chromatographic techniques

- High performance liquid chromatography
- Gas chromatography
- High performance thin layer chromatography
- Thin layer chromatography
- GC-MS
- LC-MS

1.2.6. Miscellaneous techniques

- Thermal analysis
- Kinetic technique
- Electrophoresis.

Table-1: Classification of chromatographic methods

MOBILE PHASE	STATIONARY PHASE
GAS Gas Chromatography (GC)	LIQUID Gas-Liquid Chromatography (GLC)
	SOLID Gas-Solid Chromatography (GSC)
LIQUID Liquid Chromatography (LC)	LIQUID Liquid-Liquid Chromatography (LLC)
	SOLID Liquid-Solid Chromatography (LSC)

1.3. Chromatographic methods ^[2]

Chromatography is the powerful techniques in which differential migration of components take place between two phases, one is stable which is known as stationary phase and another is movable which is known as a mobile phase. Species in the sample undergo repeated interactions (partitions) between the mobile phase and stationary phase. The stationary phase may be solid or a liquid (supported on a solid or a gel), and packed in a column, spread as a layer or film. The mobile phase may be gaseous or liquid. Those solutes, distributed preferentially in the mobile phase, will move rapidly through the system than those distributed preferentially in the stationary phase. This forms the basis of separation of component present in a sample. The distribution of a solute between two phases results from the balance of forces between solute molecules and the molecule of each phase. It reflects the relative attraction or repulsion that molecule or ions of the competing phase shown for the solute and for them. These forces can be polar in nature arising from permanent or induced and dipole moment. In ion exchange chromatography, the forces on the solute molecules are substantially ionic in nature but include polar and non-polar forces as well.

1.3.1 Chromatographic method must having essentially

- Stationary phase
- Mobile phase
- Sample injection system
- Solvent delivery system
- Column (support for stationary phase)
- Detection by detecting agent.

All chromatographic methods involve modifications in these basic components. Chromatographic techniques are predominantly used in the pharmaceutical industry for a large variety of samples.

HPLC is one of the chromatographic techniques is widely used for checking the purity of new drug candidates, monitoring changes or scale ups of synthetic procedures, evaluating new

formulations, and scrutinizing quality control/assurance of final drug products.

1.4. INTRODUCTION TO HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ^[3]

Chromatography may be defined as a non destructive procedure for separating mixture of components into individual components through equilibrium distribution between two phases.

The technique of chromatography is based on the differences in the rate at which the components of a mixture move through a porous medium under the influence of solvent or gas.

1.4.1 Classification of chromatographic process

1. Adsorption chromatography

Gas-solid chromatography

- Liquid-column chromatography
- High performance liquid chromatography
- Thin-layer chromatography

2. Partition Chromatography

- Gas liquid chromatography
- Super critical fluid chromatography
- Liquid liquid chromatography
- Paper chromatography
- High performance liquid chromatography

2. Ion exchange chromatography

3. Permeation chromatography

- Size exclusion chromatography

4. Affinity chromatography

- DNA affinity chromatography

5. Electrophoresis

Capillary electrophoresis

1.4.2 High performance liquid chromatography ^[4]

High performance liquid chromatography is basically a highly improved form of column 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. It also allows using a very much smaller particle size for the column packing material which gives a much

greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture. The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive.

HPLC employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch. The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process if diffusion is minimize

MATERIALS AND METHODS:

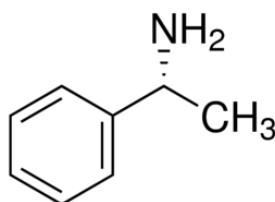
Drug : Phenethylamine
Synonym : Phenethylamine, 2-phenylethylamine, 2-Phenylethanamine, 64-04-0, Benzeneethanamine.

Description : Phenethylamine, or phenylethylamine is an organic compound and a natural monoamine alkaloid, a trace amine, and also the name of a class of chemicals with many members that are well known for their and stimulant effects.

Phenylethylamine functions as a neuromodulator or neurotransmitter in the mammalian central nervous system. It is biosynthesized from the amino acid L-phenylalanine. 2-phenylethylamine is a phenyl ethylamine having the phenyl substituent at the 2-position. It has a role as a human metabolite, an Escherichia coli metabolite and a mouse metabolite. It is a phenyl ethylamine, an aralkylamine and an alkaloid. It is a conjugate base of a 2-phenylethanaminium.

Drug category : Phenethylamine is taken by mouth for improving athletic performance, depression, weight loss, and to improve mood and attention.

Structure :



Chemical name/ Nomenclature / IUPAC Name:

2-phenylethan-1-amine

Molecular Formula : $C_{11}H_{11}N$

Molecular Weight : 121.1796 gm/mole.

Official Pharmacopoeia : USP

PHYSICOCHEMICAL PROPERTIES:

Description (Physical State): Solid

Solubility: It is soluble in water, ethanol, and ether.

Dosage: Capsules

Melting point: -60 °C

pKa : 9.83 (at 25°C)

Log P: 1.41

PHARMACOKINETIC PROPERTIES:

Bioavailability : 1

Half-life : Exogenous: 5–10 minutes;

Absorption : By oral route

Metabolism : PEA is metabolized by phenyl ethanolamine N-methyltransferase (PNMT), monoamine oxidase A (MAO-A), monoamine oxidase B (MAO-B), the semicarbazide-sensitive amine oxidases (SSAOs) AOC2 and AOC3, flavin-containing monooxygenase 3 (FMO3), and aralkylamine N-acetyltransferase (AANAT). N-Methylphenethylamine, an isomer of amphetamine, is produced in humans via the metabolism of phenethylamine by PNMT. β -Phenylacetic acid is the primary urinary metabolite of phenethylamine and is produced via monoamine oxidase metabolism and subsequent aldehyde

dehydrogenase metabolism. Phenyl acetaldehyde is the intermediate product which is produced by monoamine oxidase and then further metabolized into β -phenylacetic acid by aldehyde dehydrogenase.

Excretion : Renal (kidneys)

Adverse effects/Side effects :

Phenethylamine is POSSIBLY UNSAFE for most people when taken by mouth appropriately. Phenethylamine works similar to the drug amphetamine, and may cause similar side effects. Also, it might cause rapid heart rate, anxiety, or agitation.

Indication: Phenethylamine is a chemical that is found naturally in the body. It can also be made in the laboratory. Phenethylamine is taken by mouth for improving athletic performance, depression, weight loss, and to improve mood and attention.

Pharmacodynamics: Phenethylamine, being similar to amphetamine in its action at their common biomolecular targets, releases norepinephrine and dopamine. Phenethylamine also appears to induce acetylcholine release via a glutamate-mediated mechanism.

Phenethylamine has been shown to bind to human trace amine-associated receptor 1 (hTAAR1) as an agonist.

Mechanism of action: Phenethylamine stimulates the body to make certain chemicals that play a role in depression and other psychiatric conditions. People who don't make enough phenethylamine

naturally may be helped by taking phenethylamine as a supplement. However, too much phenethylamine might cause side effects similar to the drug amphetamine. Or That systemic administration of beta-phenyl ethylamine increases acetylcholine release, whereas locally applied beta-phenyl ethylamine decreases striatal acetylcholine release in freely moving rats. Furthermore, the dopaminergic system, through the dopamine D (2) receptor, is involved in the locally applied beta-phenyl ethylamine-induced decr in acetylcholine in the striatum.

INTERACTIONS:

Drug interactions:

7,8-Dichloro-1,2,3,4-tetrahydroisoquinoline: The risk or severity of adverse effects can be increased when Phenethylamine is combined with 7,8-Dichloro-1,2,3,4-tetrahydroisoquinoline.

Acepromazine: The risk or severity of adverse effects can be increased when Acepromazine is combined with Phenethylamine.

Aceprometazine: The risk or severity of adverse effects can be increased when Aceprometazine is combined with Phenethylamine.

Acetazolamide : The risk or severity of adverse effects can be increased when Acetazolamide is combined with Phenethylamine.

Acetophenazine: The risk or severity of adverse effects can be increased when Acetophenazine is combined with Phenethylamine.

Medical Uses:

- **Depression:** Early research shows that taking 10-60 mg of phenethylamine by mouth per day along with 5 mg of the antidepressant selegiline (Anipryl, Eldepryl) twice per day for 4 weeks relieves depression in 60% of people. Of the people who respond to initial treatment with phenethylamine plus selegiline, 86% show continued depression relief when treated for up to 50 weeks.
- **Attention.**
- **Mood.**
- **Weight loss.**
- **Other conditions.**

RESULTS AND DISCUSSION:

HPLC METHOD DEVELOPMENT:

TRAILS

Trail 1:

Column	:	
Inertsil C18 (4.6 x 250mm, 5µm)		
Column temperature	:	45°C
Wavelength	:	
246 nm		
Mobile phase ratio	:	
Acetonitrile: Water (55:45 % v/v)		
Flow rate	:	
1.0mL/min		
Injection volume	:	10 µl
Run time	:	
10minutes		

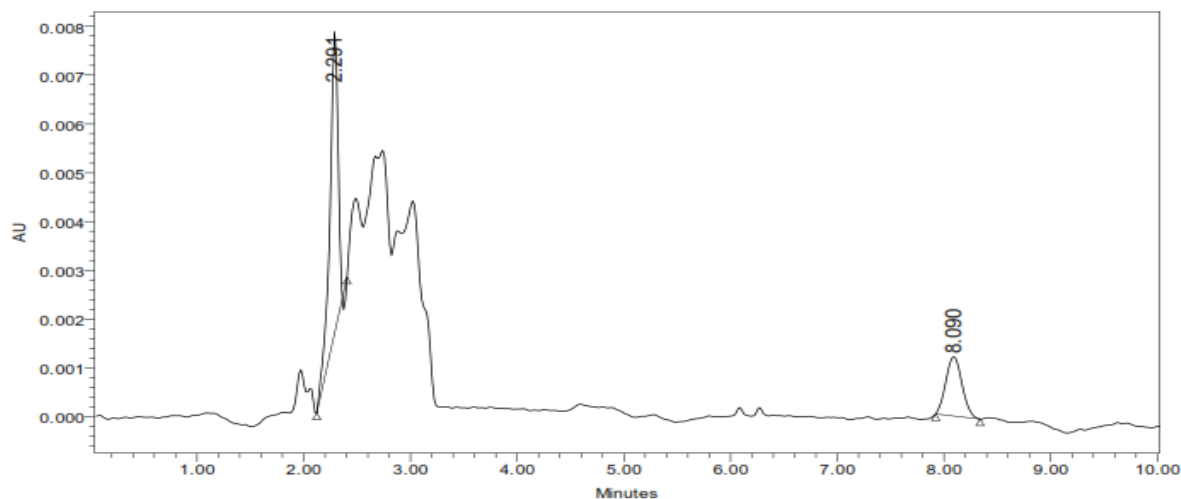


Figure-11: Chromatogram for Trail 1

Table-11: Peak Results for Trail 1

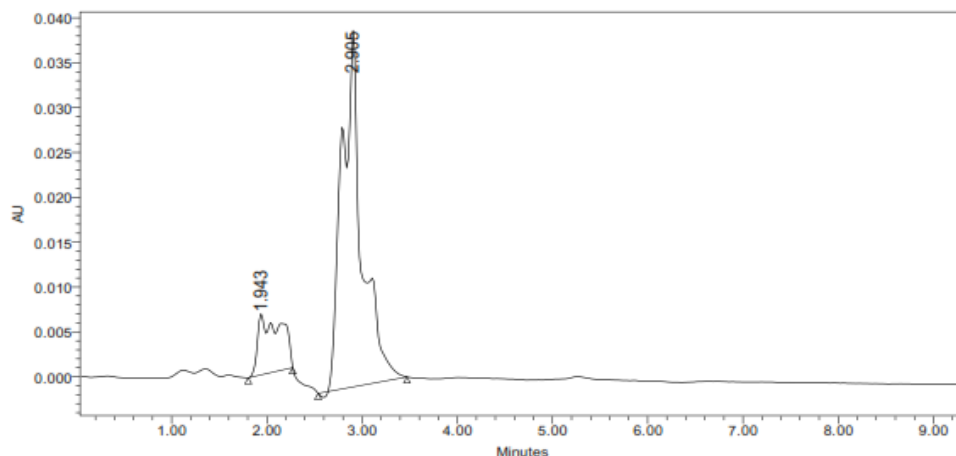
S.No.	Peak Name	R _t	Area	Height	USP Tailing	USP Plate count
1	Phenethylamine	2.291	245523	25416	0.95	1154

Observation:

The trial shows more void peaks and improper baseline in the chromatogram. So more trials were required for obtaining peaks.

Trail 2:

Column : Inertsil C18 (4.6 x 150mm, 5 μ m)
 Column temperature : 30°C
 Wavelength : 246 nm
 Mobile phase ratio : Methanol: Water (70:30)
 Flow rate : 1.0mL/min
 Injection volume : 20 μ l
 Run time : 9 minutes

**Figure-12: Chromatogram for Trail 2****Table-12: Peak Results for Trail 2**

S. No.	Peak name	R _t	Area	Height	USP Tailing	USP plate count
1	Phenethylamine	2.995	365881	54871	2.55	2548

Observation:

This trial show more void peaks and required for obtaining peaks.

improper baseline in the chromatogram, so more trials were

Trail 3:

Column : Zorbax C18 (4.6 x 150mm, 5 μ m)
 Column temperature : 40°C
 Wavelength : 246 nm
 Mobile phase ratio : Methanol: Acetonitrile (70:30)
 Flow rate : 1.0 mL/min
 Injection volume : 20 μ l
 Run time : 8 minutes

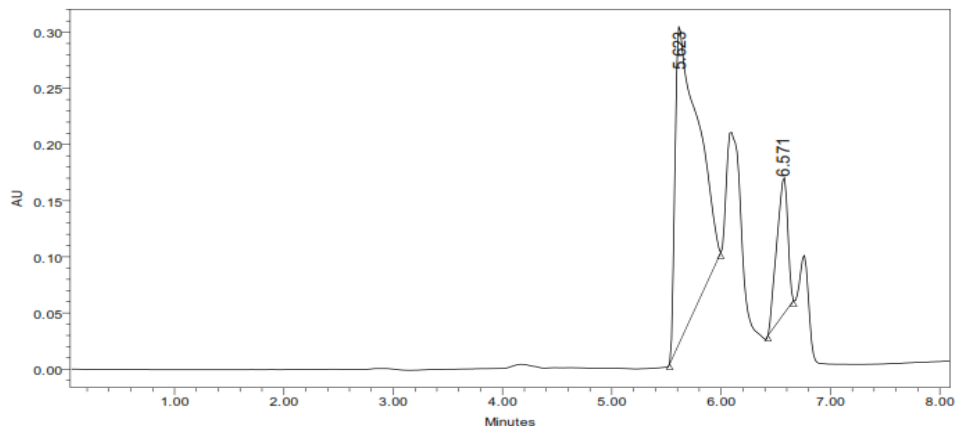
**Figure-13: Chromatogram for Trial 3**

Table-13: Peak Results for Trail 3

S. No.	Peak name	R _t	Area	Height	USP Tailing	USP plate count
1	Phenethylamine	5.623	458745	52641	3.45	1215

Observation:

This trial show more tailing and improper baseline and peak in the chromatogram, so more trials were required for obtaining good peaks.

Trail 4:

Column : Develosil C18 (4.6 x 150mm, 5 μ m)
 Column temperature : 35°C
 Wavelength : 246 nm
 Mobile phase ratio : Methanol: Acetonitrile (40:60)
 Flow rate : 1 mL/min
 Injection volume : 10 μ l
 Run time : 10minutes

Auto-Scaled Chromatogram

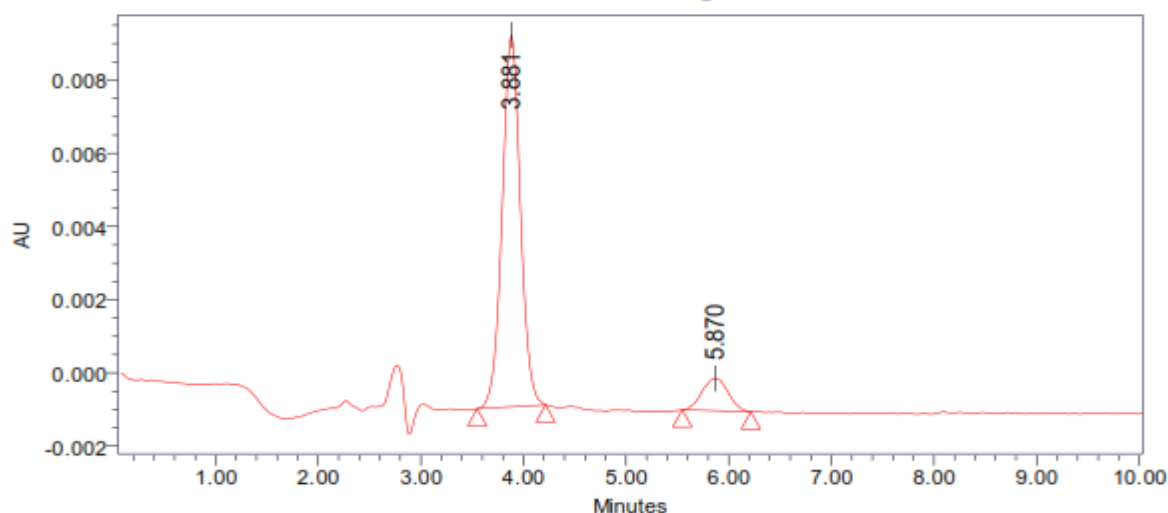


Figure-14: Chromatogram for trial 4

Table-14: Peak results for trail 4

S.No.	Peak name	R _t	Area	Height	USP Tailing	USP plate count
1	Phenethylamine	3.881	465215	32541	0.99	2654

Observation:

This trial does not show Proper base line and more tailing in the chromatogram. So go for further trails to obtain good peaks.

Trail 5:

Column : Phenomenex Gemini C18 (4.6 x 150mm, 5 μ m)
 Column temperature : Ambient
 Wavelength : 246 nm
 Mobile phase ratio : Methanol: Phosphate Buffer (40:60% v/v)
 Flow rate : 1 mL/min
 Auto sampler temperature : Ambient
 Injection volume : 20 μ l
 Run time : 7.5 minutes

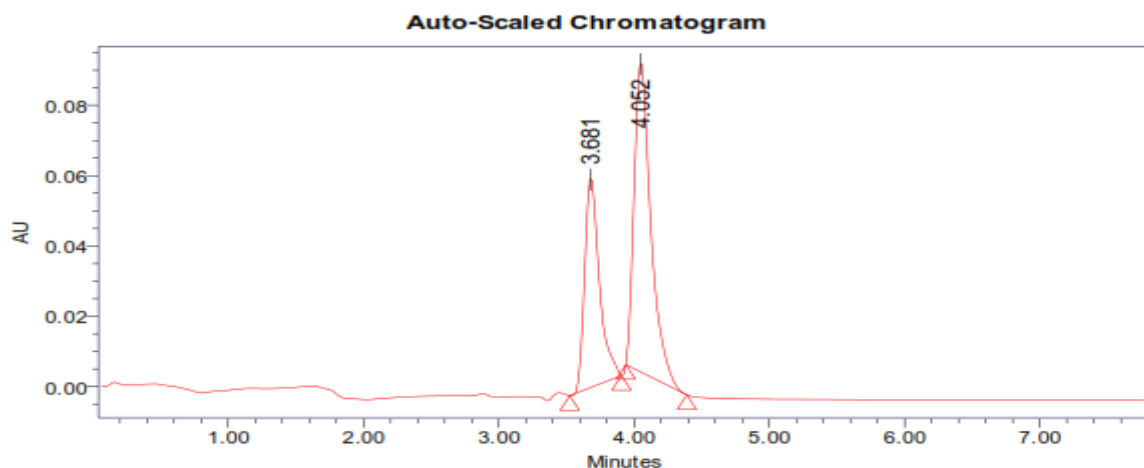


Figure-15: Chromatogram for Trial 5

Table-15: Peak Results for Trail 5

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Phenethylamine	3.681	382541	65874	0.94	2698

Observation:

This trial shows the improper of chromatogram and baseline. So go for further trails.

Trail 6:

Column : Symmetry Xterra C18 (4.6mm x 150mm, 5 μ m)
 Column temperature : Ambient
 Wavelength : 246 nm
 Mobile phase ratio : Acetonitrile: Phosphate Buffer (20:80% v/v)
 Flow rate : 1mL/min
 Auto sampler temperature : Ambient
 Injection volume : 10 μ l
 Run time : 7 minutes

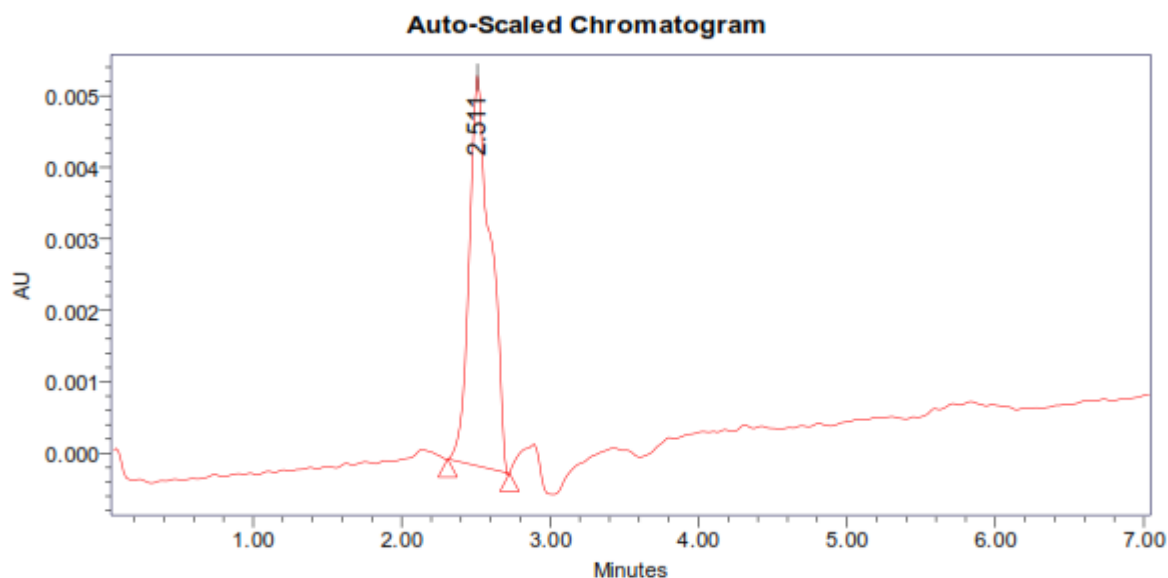


Figure-16: Chromatogram for Trial 6

Table-16: Peak Results for Trail 6

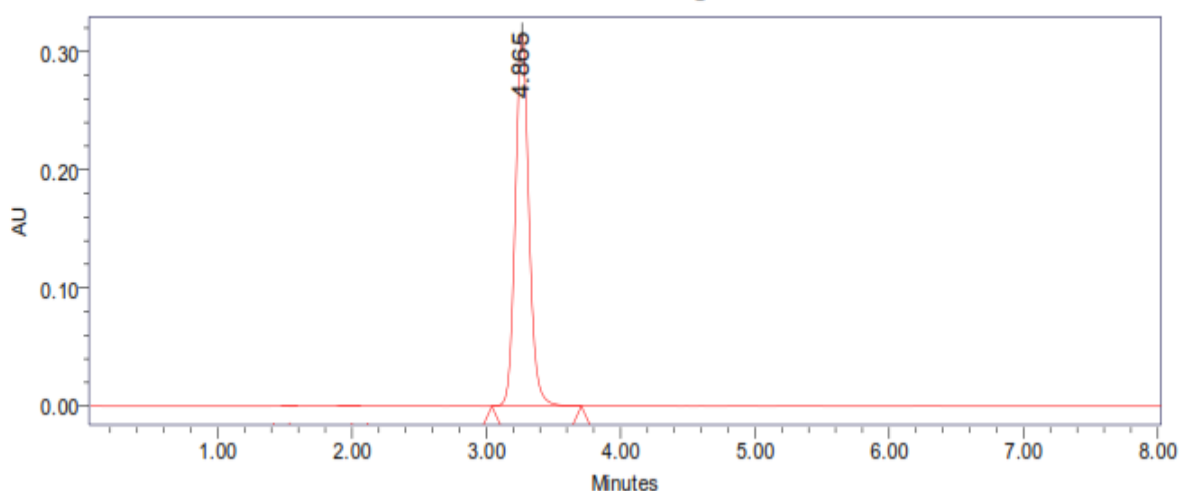
S.No.	Peak name	R _t	Area	Height	USP Tailing	USP plate count
1	Phenethylamine	2.511	126545	32546	1.26	986

Observation:

In this above trail it shows improper peak shape, baseline and less plate count in the chromatogram. More trails required to get good shape peaks.

Optimized Chromatogram (Standard)

Column : Symmetry Xterra C18 (4.6mm x 150mm, 5µm)
 Column temperature : Ambient
 Wavelength : 246 nm
 Mobile phase ratio : Acetonitrile: Phosphate Buffer (pH-2.8) (35:65% v/v)
 Flow rate : 1.0mL/min
 Injection volume : 10 µl
 Run time : 8 minutes

Auto-Scaled Chromatogram**Figure-17: Optimized Chromatogram (Standard)****Table-17: Peak results for Optimized Chromatogram (Standard)**

S.No.	Peak name	R _t	Area	Height	USP Tailing	USP plate count
1	Phenethylamine	4.865	856985	69854	1.25	8547

Observation:

This trial shows proper plate count, peak and baseline in the chromatogram. It's Pass the all system suitability parameters. So it's optimized chromatogram.

Optimized Chromatogram (Sample)

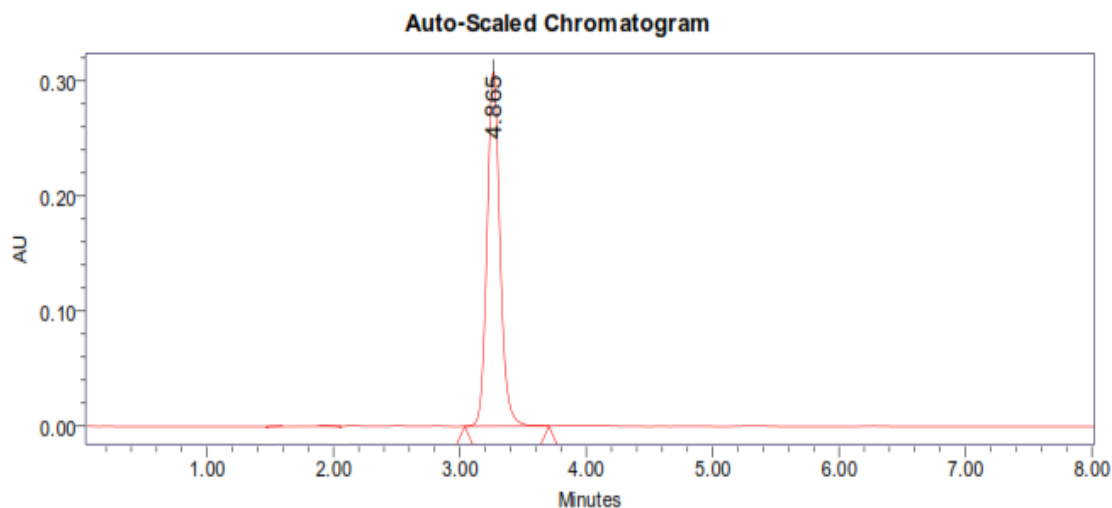


Figure-18: Optimized Chromatogram (Sample)

Table-18: Results of Optimized Chromatogram (Sample)

S.No.	Name	Retention time (min)	Area (μ V sec)	Height (μ V)	USP tailing	USP plate count
1	Phenethylamine	4.865	865845	69857	1.26	8659

Acceptance criteria:

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

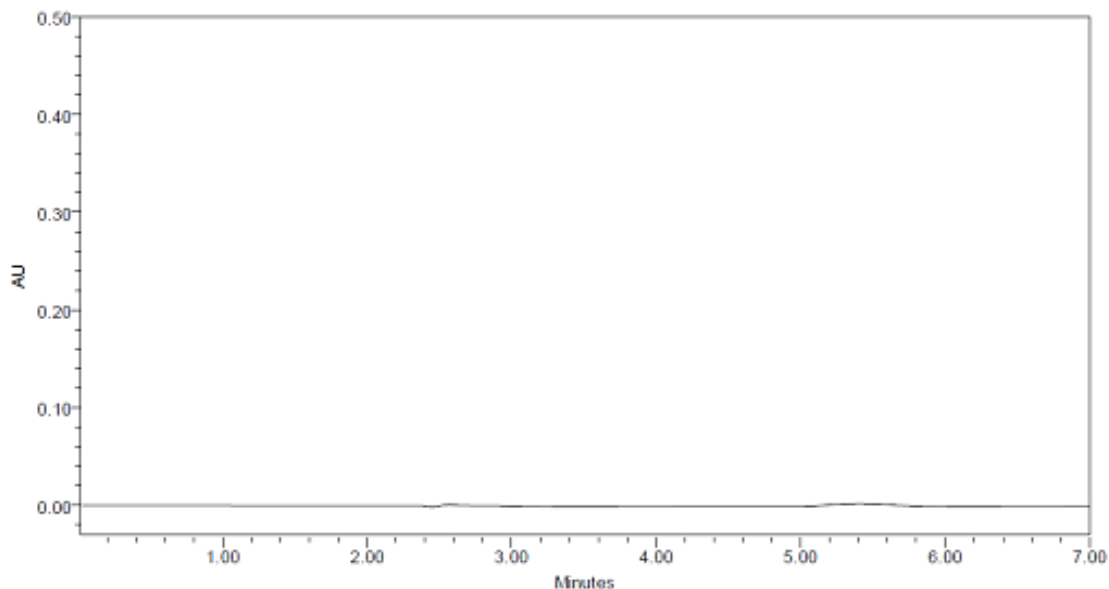
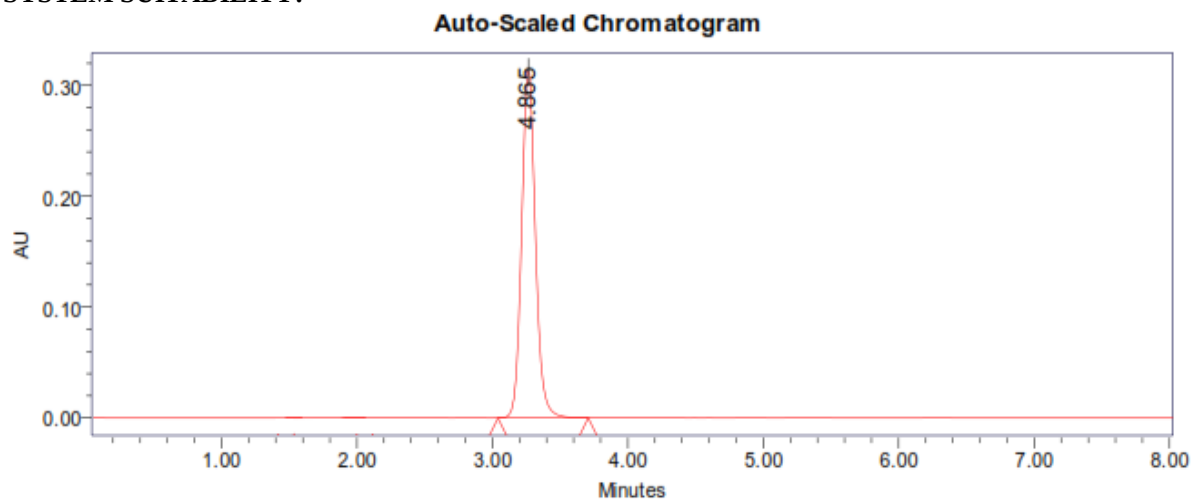
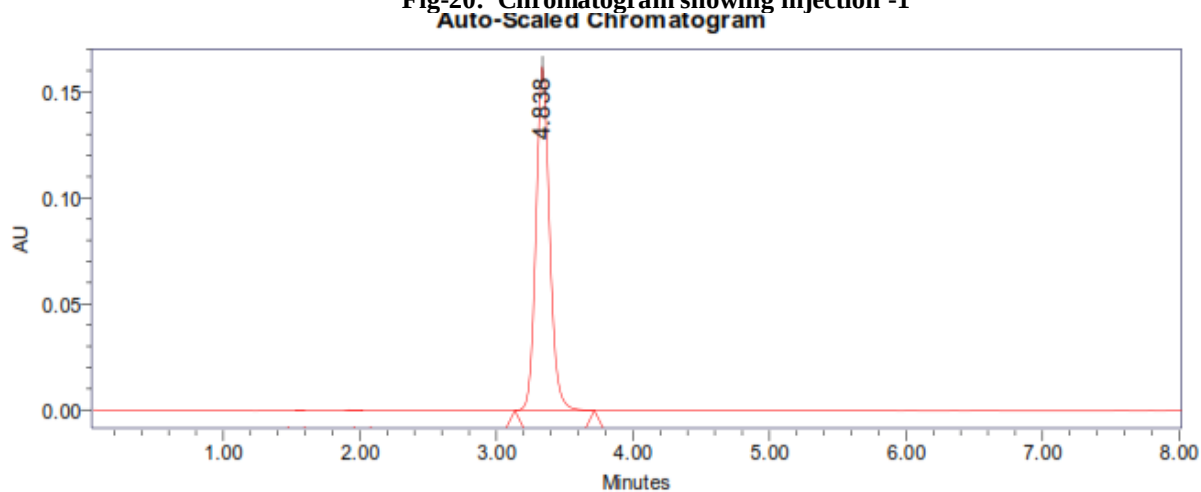
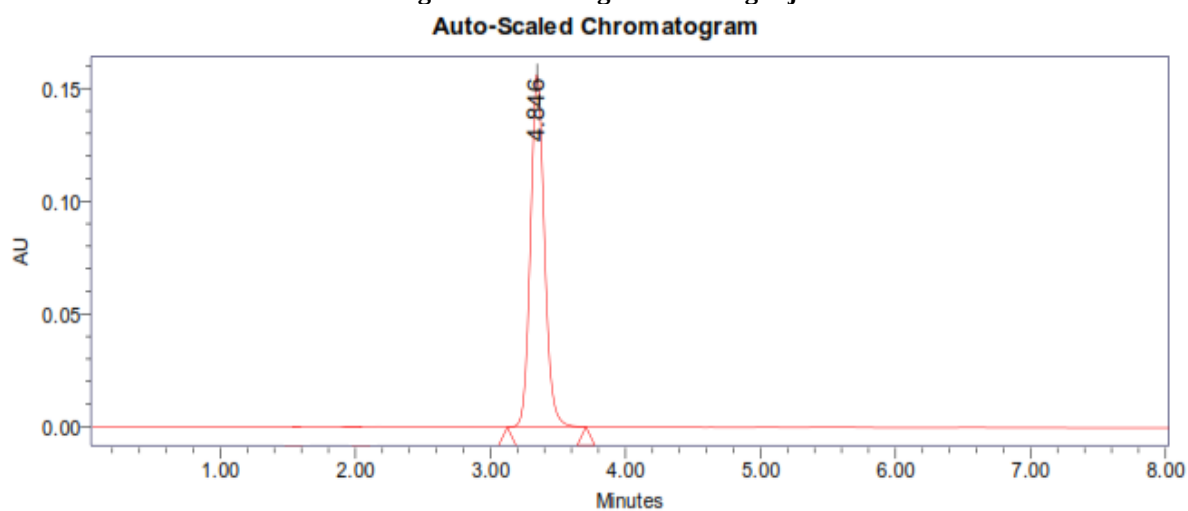
METHOD VALIDATION**Blank:**

Fig-19: Chromatogram showing blank (mobile phase preparation)

SYSTEM SUITABILITY:**Fig-20: Chromatogram showing injection -1****Fig-21: Chromatogram showing injection -2****Fig-22: Chromatogram showing injection -3**

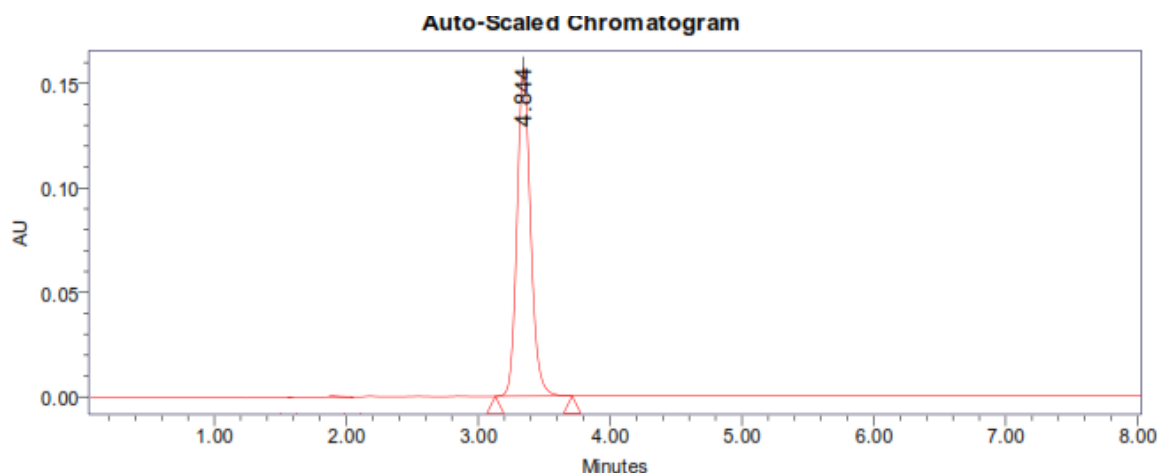


Fig-23: Chromatogram showing injection -4

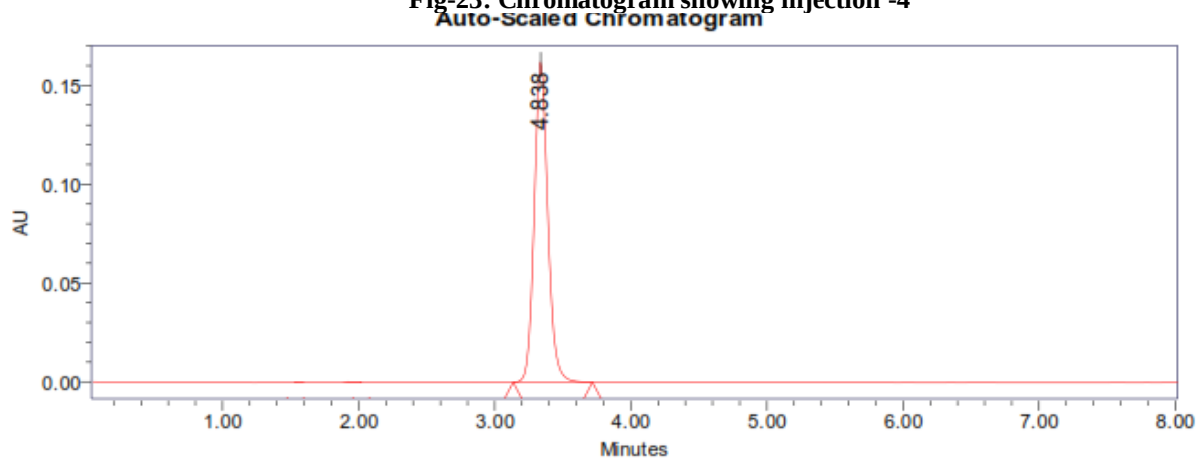


Fig-24: Chromatogram showing injection -5

Table-19: Results of system suitability for Phenethylamine

S.No.	Peak Name	RT	Area (μV*sec)	Height (μV)	USP Plate Count	USP Tailing
1	Phenethylamine	4.865	856985	69854	8569	1.26
2	Phenethylamine	4.838	856857	68954	8547	1.25
3	Phenethylamine	4.846	857894	68975	8596	1.25
4	Phenethylamine	4.844	857468	69854	8541	1.26
5	Phenethylamine	4.838	854785	69856	8616	1.25
Mean			856797.8			
Std. Dev.			1197.992			
% RSD			0.139822			

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 STUDY OF DRUG:

Assay (Standard):

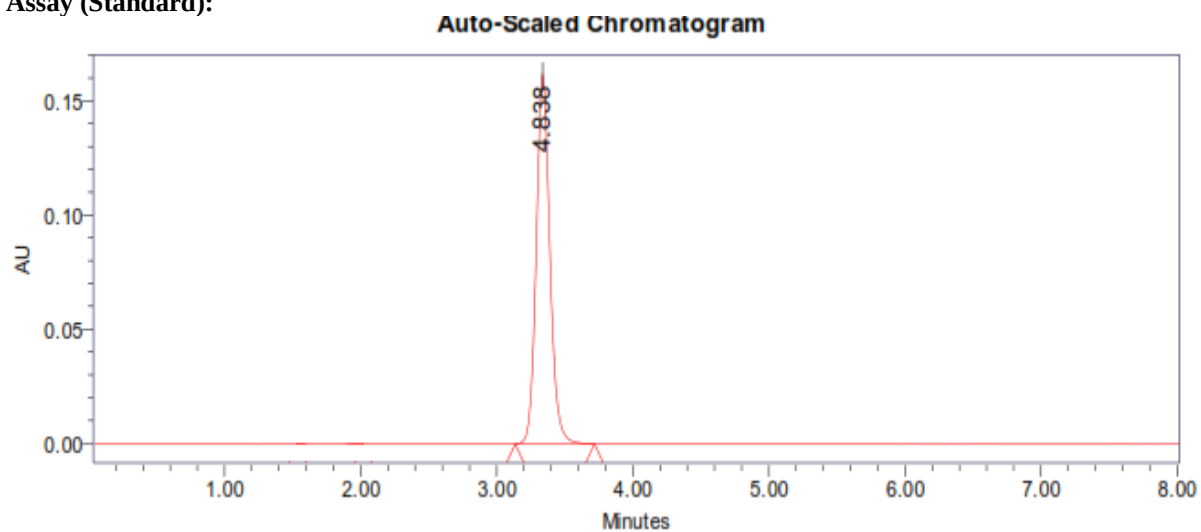


Fig-25: Chromatogram showing injection -1
Auto-Scaled Chromatogram

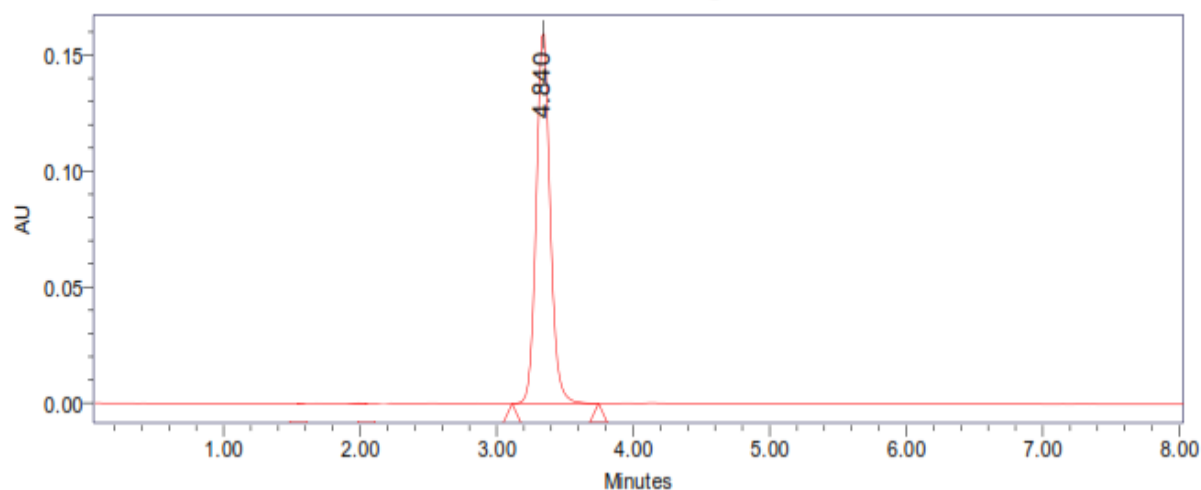
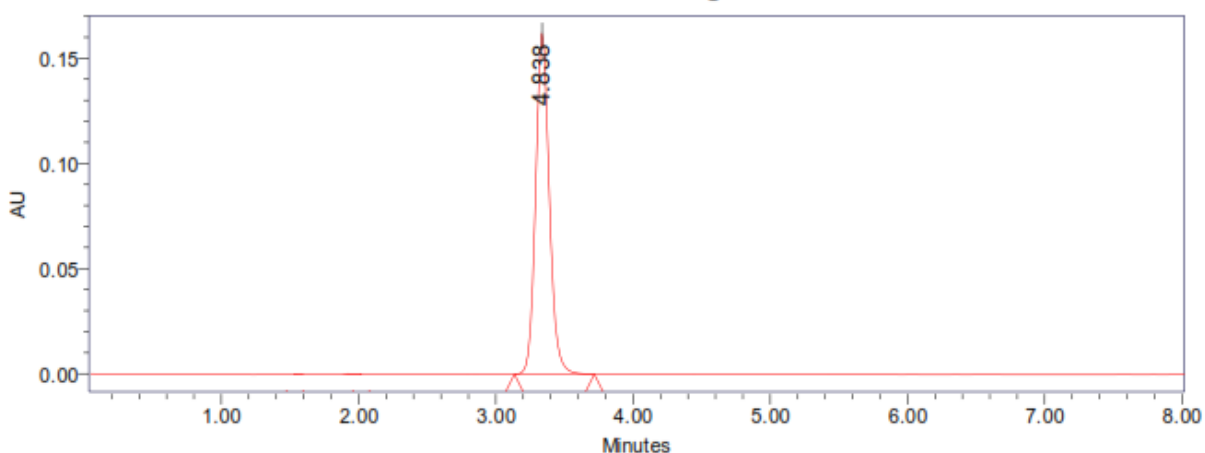


Fig-26: Chromatogram showing injection -2
Auto-Scaled Chromatogram

**CONCLUSION:**

Hence the proposed method was found to be rapid, accurate, precise, specific, robust and economical. The mobile phase is simple to prepare and

economical. This method is also having an advantage that the retention time of the drug is below 5 min and the drug can be assayed with the short time. Thus the method is not time consuming

and can be used in laboratories for the routine analysis of single and combination drugs.

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