Research Article

NOVEL RP - HPLC METHOD AND DEVELOPMENT AND VALIDATION FOR ESTIMATION OF PHENTERMINE USING BULK AND PHARMACEUTICAL DOSAGE FORM

Sowmya G
Osmania university of technology, Hyderabad, India.
Author email: gandlasowmya810@gmail.com

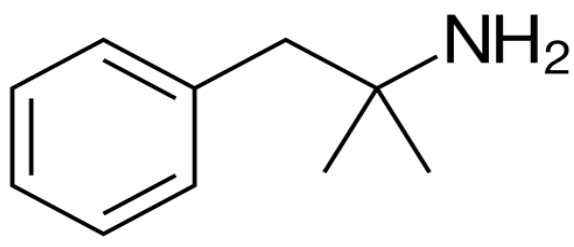
ABSTRACT

A simple and selective HPLC method is described for the determination of Phentermine. Chromatographic separation was achieved on a c 18 column using mobile phase consisting of a mixture of 40 volumes of Methanol, 40 volumes of Acetonitrile and 20 volumes of Water with detection of 263 nm. Linearity was observed in the range 50-150 µg /ml for Phentermine (r 2 =0.990) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Phentermine was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

Key words: Phentermine, linearity , retention time, drugs estimation, HPLC , high resolution.

INTRODUCTION

Phentermine also known as **α,α-dimethylphenethylamine**, is a psychostimulant drug of the substituted amphetamine chemical class, with pharmacology similar to amphetamine. It is used medically as an appetite suppressant for short term use, as an adjunct to exercise and reducing calorie intake. IUPAC name for phentermine is 2-methyl-1-Phenylpropan-2-amine.It comes under category of central nervous System agents, central nervous system stimulants, centrally acting Anti obesity products[12] .Phentermine is indicated in the management of exogenous obesity as a short term (a few weeks) adjunct in a regimen of weight reduction based on caloric restriction. Phentermine hydrochloride is a sympathomimetic amine with pharmacologic activity similar to the prototype drugs of this class used in obesity, the amphetamines. Actions include central nervous system stimulation and elevation of blood pressure[12]. Protein biniding is approximately 96.3%[13]. Indication is for the treatment and management of obesity. Reverse phase HPLC is preffered. In this type, the stationary phase is non-polar and the mobile phase is polar, non-polar compounds are retained for longer periods as they have more affinity towards the stationary phase[1,2]. Hence, polar compounds travel faster and are eluted first. Aim is to develop and validate new HPLC method[7] for Phentermine in pharmaceutical dosage form. Solubility determination of Phentermine in various solvents and buffers, Determine the absorption maxima of Phentermine in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development4,[8], Optimize the mobile phase and flow rates for proper resolution and retention times[5], Validate the developed method as per ICH guidelines[9,10,11,] this all process involved in plan of work. A simple and selective HPLC method is described for the determination of Phentermine[1,3]. Chromatographic separation[2] was achieved on a c_{18} column using mobile phase consisting of a mixture of 40 volumes of Methanol, 40 volumes of Acetonitrile and 20 volumes of Water with detection of 263 nm. Linearity was observed in the range 50-150 µg /ml for Phentermine (r^2 =0.990) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated[6,10,11]. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.



Chemical structure of phentermine[13]

MATERIAL AND METHODS

Table 1: Instruments used

UV-Visible Spectrophotometer	Nicolet evolution 100
UV-Visible Spectrophotometer software	Vision Pro
HPLC software	Open lab EZ chrome
HPLC	Agilent Technologies
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Mettler Toledo
Syringe	Hamilton
HPLC Column	Inertsil ODS 3V(150x4.6mm) 4µm

Table 2: Reagents used

Water	HPLC Grade
Methanol	HPLC Grade

Potassium Dihydrogen Phosphate	AR Grade
Acetonitrile	HPLC Grade
Dipotassium hydrogen phosphate	AR Grade
Orthophosphoric acid	HPLC Grade

Table 3: Drugs used

Phentermine (API)	Gift Samples obtained from Chandra labs, Hyd.
Phentermine Phentermine **(Adipex - P 37.5 mg)** **Tablet dosage form**	Obtained from local pharmacy

Mobile Phase:

A mixture of, 40 volumes of Methanol, 40 volumes of methanol and 20 volumes of Water. The mobile phase was sonicated for 10 min to remove gases.

METHOD DEVELOPMENT AND VALIDATION

In this present work , an analytical method based of HPLC using detection was developed and validated for assay determination of phentermine in pharmaceutical dosage form. Solubility studies and deermination of working wavelength was conducted. Here 5 trails were conducted from which trail 5 was optimized and with optimized chormatographic conditions. Preparation of mixed standard solution was performed and observation was noted down. Assay of standard and sample preparations was performed for 5 preparations in both. The amount of phentermine in taken dosage forms for assay result was found to be 100.9% in both respectively. HPLC method validaton was condcucted for system suitability and system precision, method precision, linearity and range, specificity, accuracy,robustness and intermedite precision (Ruggedness) was performed and validated.

RESULTS AND DISCUSSION

Solubility Studies

These studies are carried out at 25 ^{0}C

Phentermine:

Slightly soluble in water, Freely soluble in Ethanol, and Methanol, Acetonitrile and Phosphate buffer.

Determination of Working Wavelength (λmax)

Preparation of standard stock solution of Phentermine

10 mg of Phentermine was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 μg /ml of solution by diluting 1ml to 10ml with methanol.

Results

The wavelength of maximum absorption (λ_{max}) of the drug, 10 μg/ml solution of the drug in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the 8.3 and The absorption curve shows characteristic absorption maxima at 263nm for Phentermine, selected as detector wavelength for the HPLC chromatographic method.

METHOD DEVELOPMENT OF PHENTERMINE

Trial - 1

Chromatographic conditions

Mobile phase : Phosphate buffer : ACN
Ph : 4.0
Ratio : 37:63
Column : Inertsil ODS 3V (250×4.6× 5μ)
wavelength : 230 nm
Flow rate : 1ml/min

Preparation of mixed standard solution

weigh accurately 10 mg of Phentermine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 μg/ml of Phentermine is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Observation:

- Although the Efficiency was not satisfactory for Phentermine .
- Theoretical plates for the Phentermine less than 2000.
- Hence it was not taken for optimization.

Trial- 2

Chromatographic conditions

Mobile phase : KH_2PO_4:Methonol
pH : 6.0

Ratio : 55:45
Column : Inertsil ODS 3V (250×4.6 ×5µ)
wavelength : 230nm
Flow rate : 1ml/min

Preparation of mixed standard solution

Weigh accurately 10 mg of Phentermine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Phentermine is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Observation:

- Efficiency was not good.
- The run time is very more.
- The peaks of Phentermine showed tailing.
- Hence it was not taken for optimization.

Trial- 3:

Chromatographic conditions

Mobile phase : Phosphate buffer:ACN:Methanol
Ph : 4.0
Ratio : 60:10:30
Column : Inertsil ODS 3V, (250×4.6× 5µ)
Wavelength : 230nm
Flow rate : 1ml/min

Preparation of mixed standard solution

Weigh accurately 10 mg of Phentermine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Phentermine is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Observation:

- Asymmetry factor for Phentermine does not meet the system suitability requirements.
- Efficiency was very less.
- Hence it was not taken for optimization.

Trial- 4:

Chromatographic conditions

Mobile phase : phosphate buffer:ACN
pH : -
Ratio : 20:80
Column : Inertsil ODS , (250×4.6× 5µ)
Wavelength : 230 nm
Flow rate : 1ml/min

Preparation of mixed standard solution

Weigh accurately 10 mg of Phentermine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Phentermine is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Observation:

- Peak Asymmetry factor for Phentermine does not meet the system suitability requirements.
- Tailing factor is very more.
- hence it was not taken for optimization.

Trial-5 : (Optimized):

Chromatographic conditions

Mobile pha : Methanol: ACN : WATER
pH : -
Ratio : 40:40:20
Column : Inertsil ODS 3V column,C18(250x4.6 ID) 5µm
Wavelength : 230nm
Flow rate : 1.0ml/min

Preparation of mixed standard solution

weigh accurately 10 mg of Phentermine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Phentermine is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Observation:

- All the system suitability requirements were met.
- The peak Asymmetry factor was less than 2 for both Phentermine
- The efficiency was more than 2000 Phentermine.
- Resolution between two peaks >1.5.
- hence this method was for optimized.

Table 4: Optimized chromatographic conditions

Mobile phase	METHANOL:ACN : WATER(40:40:20)
Ph	-
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm

Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	230
Injection volume	20 µl
Run time	6 min
Retention time	About 2.520 min for Phentermine

Assay

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10 mg of Phentermine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Phentermine is prepared by diluting 0.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Sample preparation : weigh accurately 10 Tablets **(Adipex - P 37.5 mg)** weigh accurately 10 mg of Phentermine in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Phentermine is prepared by diluting 0.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Calculation

The amount of Phentermine present in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{\text{LC}} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of Phentermine in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

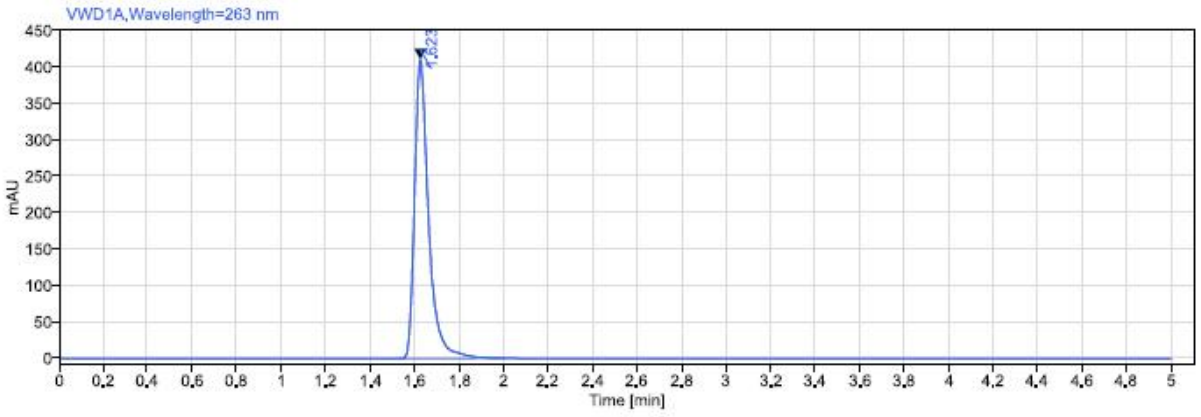


Fig.1.1: Chromatogram of Assay standard preparation-1

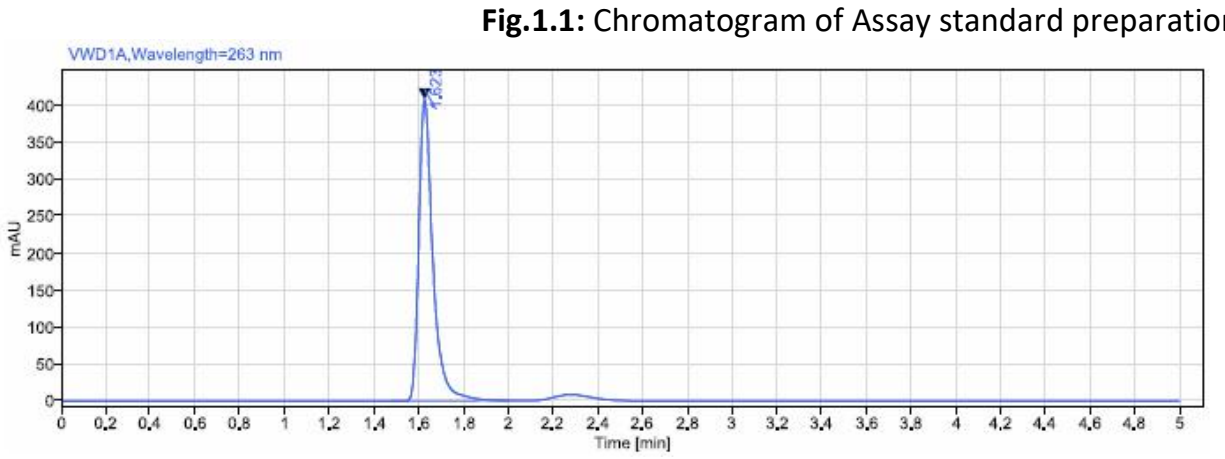


Fig.1.2 : Chromatogram of Assay standard preparation-2

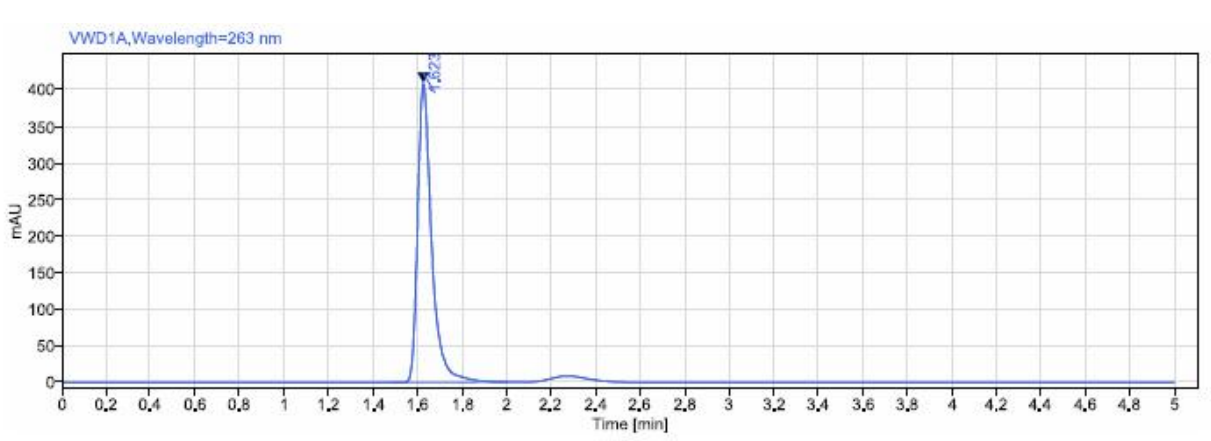


Fig.1.3 : Chromatogram of Assay standard preparation-3

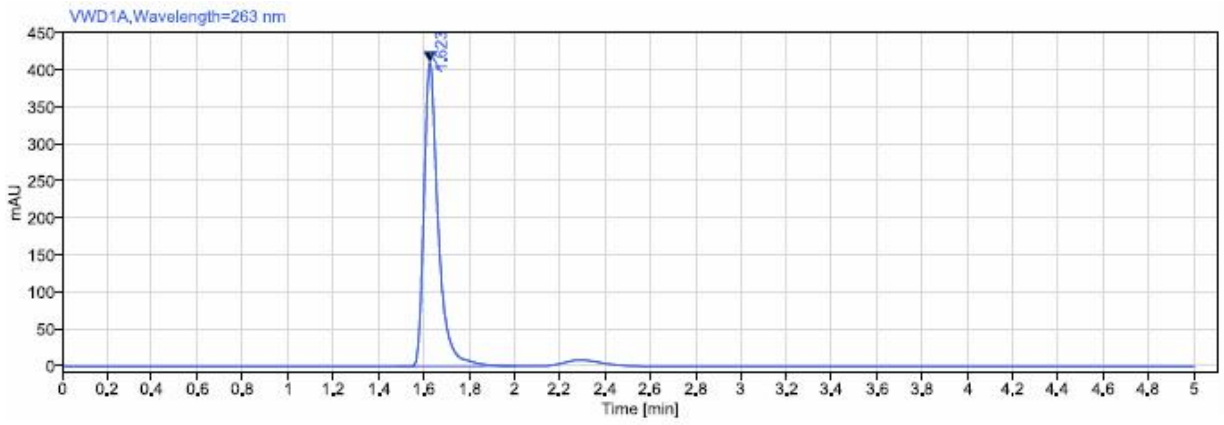


Fig. 1.4: Chromatogram of Assay standard preparation-4

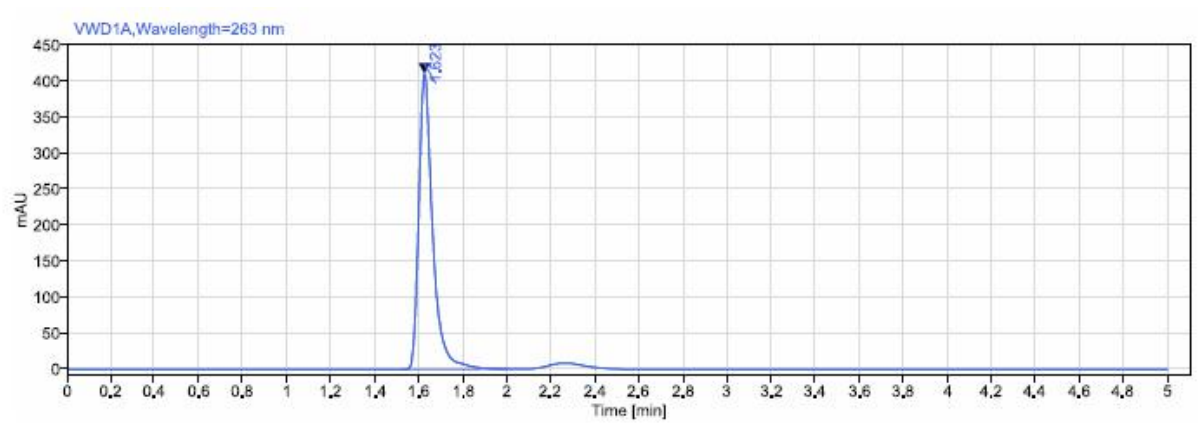


Fig1.5 : Chromatogram of Assay standard preparation-5

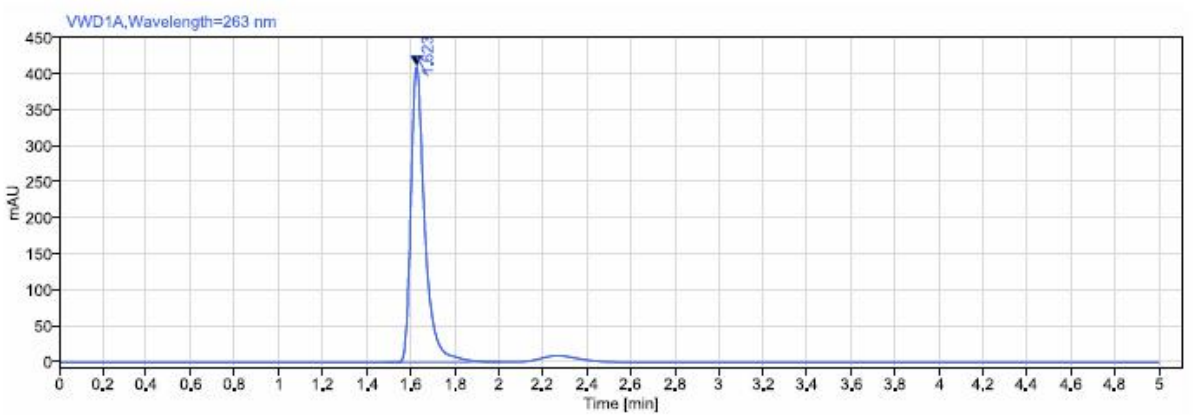


Fig2.1: Chromatogram of Assay sample preparation-1

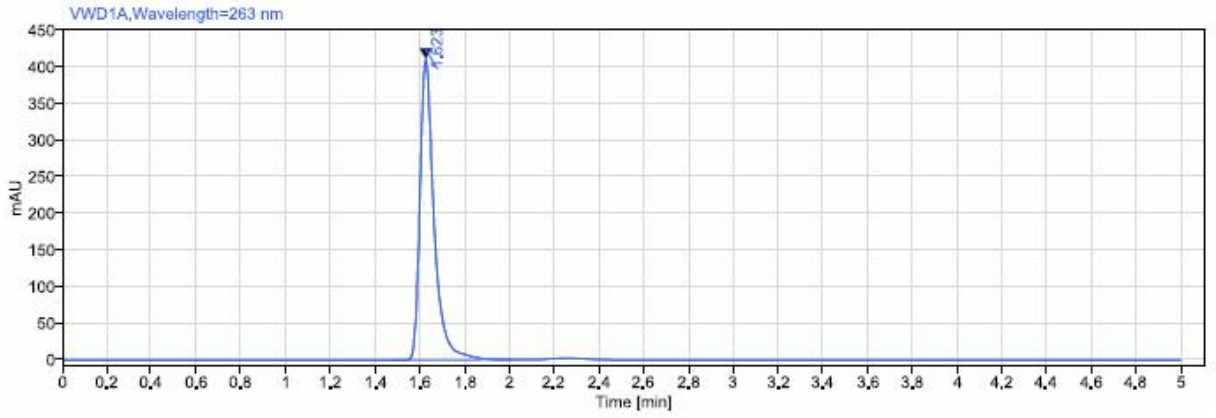


Fig2.2 : Chromatogram of Assay sample preparation-2

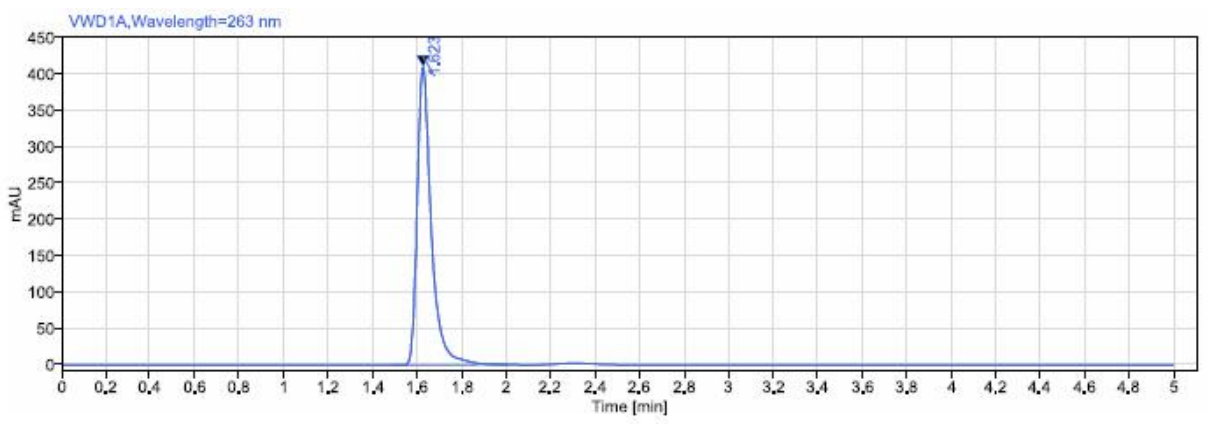


Fig2.3 : Chromatogram of Assay sample preparation-3

Fig2.4 : Chromatogram of Assay sample preparation-4

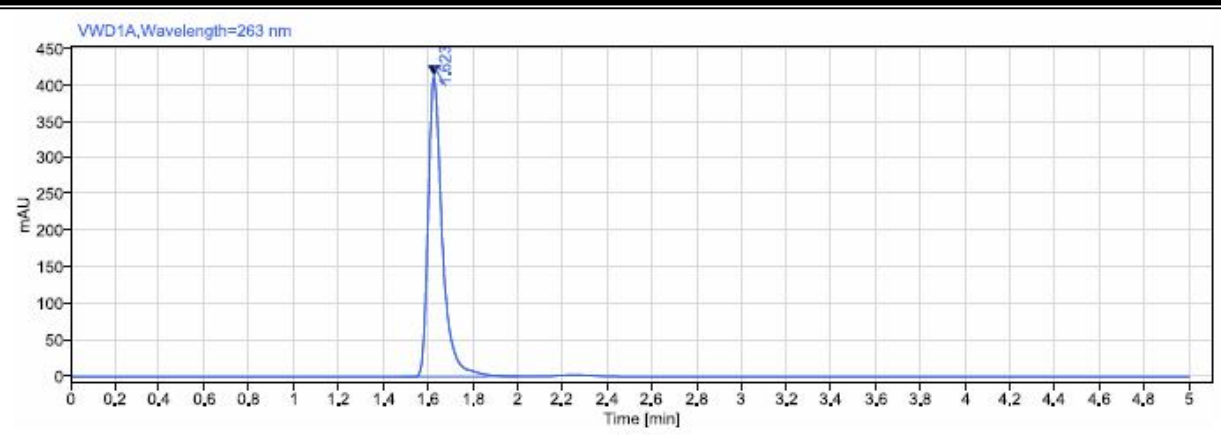


Fig2.5: Chromatogram of Assay sample preparation-5

Table No 5: Assay Results

Phentermine		
	Standard Area	**Sample Area**
Injection-1	1795.43	1810.55
Injection-2	1793.89	1810.74
Injection-3	1794.86	1810.81
Injection-4	1794.23	1810.81
Injection-5	1794.88	1811.4
Average Area	1794.66	1810.86
Standard deviation	0.60	
%RSD	0.03	
Assay(%purity)	100.90	

Observation

The amount of Phentermine present in the taken dosage form was found to be 100.90% respectively.

VALIDATION

HPLC METHOD VALIDATION

System Suitability& System precision

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 20µg/mL of PHENTERMINE was injected six times and the chromatograms were recorded for the same.

Table 6 **:** Results for system suitability of PHENTERMINE.

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.622	1806.55	3700	1.53
2	1.623	1807.57	3686	1.48
3	1.623	1808.12	3682	1.48
4	1.623	1808.46	3682	1.48
5	1.623	1809.14	3682	1.48
6	1.623	1809.18	3685	1.47
Mean	1.623	1808.170	-	-
SD	0.00041	1.00	-	-
%RSD	0.025	0.06	-	-

Acceptance criteria

1. The % RSD for the retention time of PHENTERMINE Peaks from 6 replicate injections of each Standard solution should be not more than 2.0
2. The % RSD for the peak area responses of PHENTERMINE peak from 6 replicate injections of each standard solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the PHENTERMINE peaks is not less than 2000.

4. The Tailing factor (TP) for the PHENTERMINE peak is not more than 2.0.

Result

The plate count and tailing factor results were found to be within the limits and The % RSD was found to be 1.2 so system is suitable and giving precise results

Method precision

Method precision was determined by injecting sample solutions of concentration PHENTERMINE (20µg/mL) for six timesare prepared separately.

The chromatograms were recorded and the results were summarized in Table 7

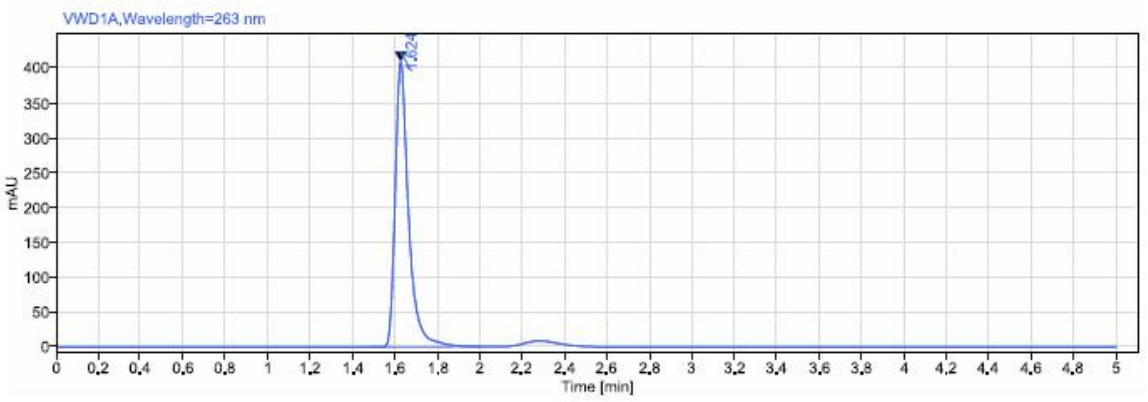


Fig.3.1 **:** Chromatogram of Method Precision-01

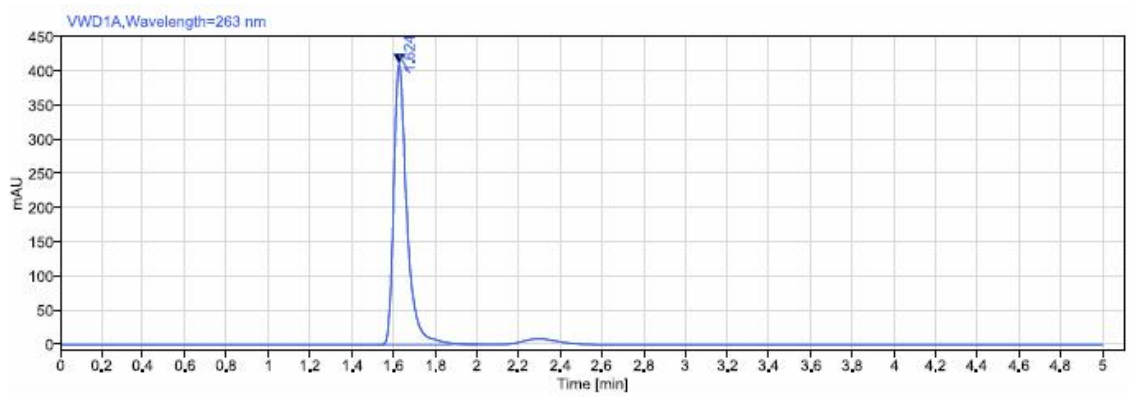


Fig.3.2**:** Chromatogram of Method Precision-02

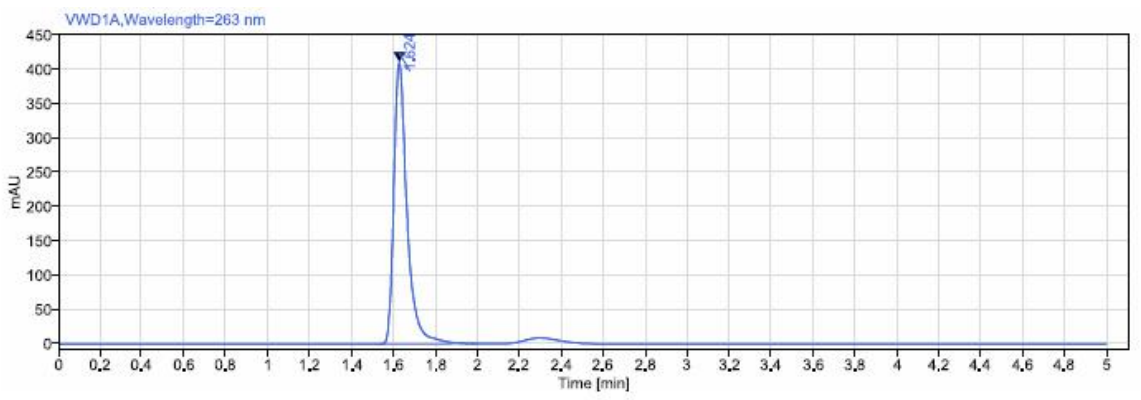


Fig.3.3**:** Chromatogram of Method Precision-03

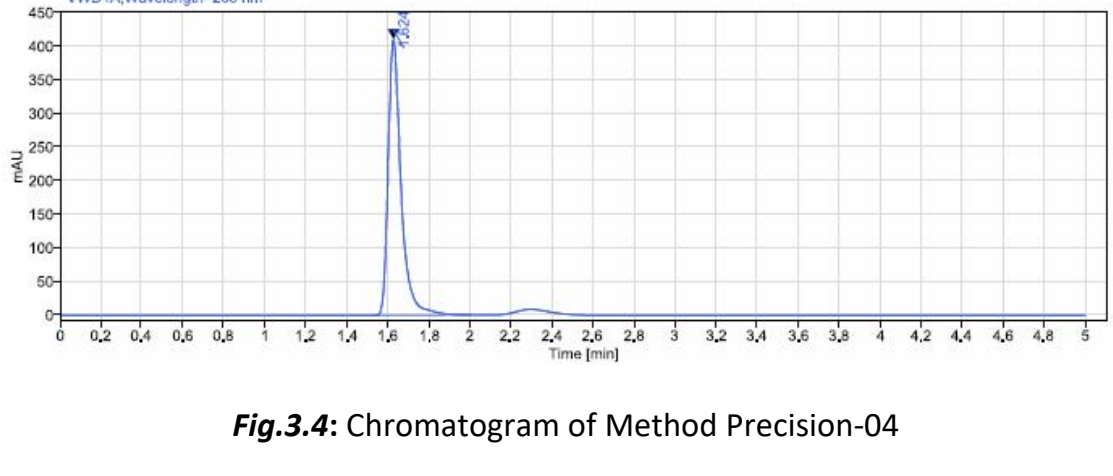


Fig.3.4**:** Chromatogram of Method Precision-04

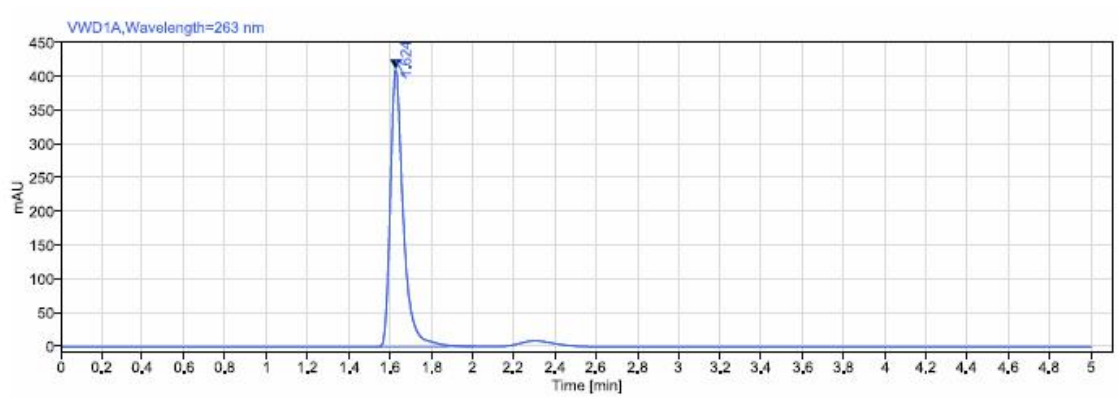


Fig.3.5**:** Chromatogram of Method Precision-05

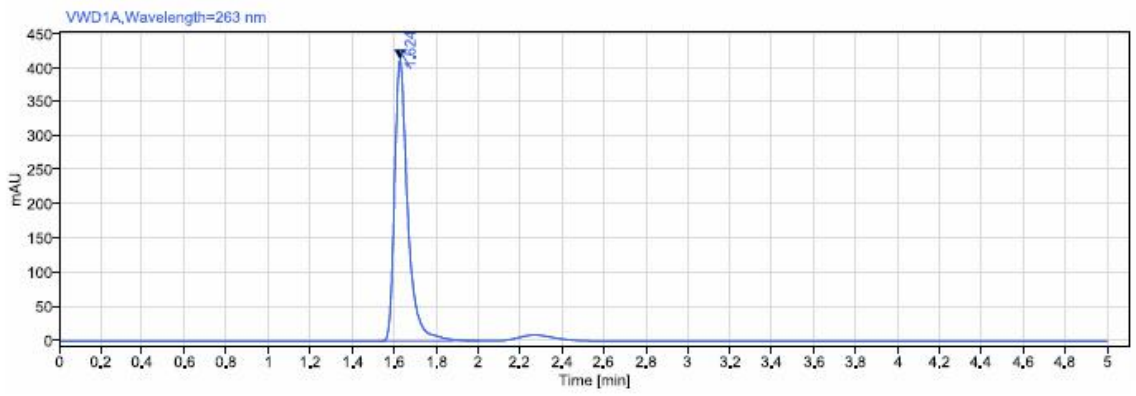


***Fig.3.6*:** Chromatogram of Method Precision-06

***Table 7*:** Method precision results for PHENTERMINE

Phentermine		
S.No.	RT	AREA
1	1.623	1792.8
2	1.624	1793.47
3	1.624	1795.84
4	1.624	1797.36
5	1.624	1798.51
6	1.624	1800.08
AVG	1.6238	1796.3433
SD	0.0004	2.86
%RSD	0.025	0.16

Result

The %RSD of Assay for 6 Samples determinations of PHENTERMINE found to be within the acceptance criteria (less than 2.0%). hence method is precise.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of PHENTERMINE were prepared by dissolving 100 mg of PHENTERMINE in 100 mL of Diluent. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min further dilutions were given in the Table8

***Table 8* :** Linearity Preparations.

Preparations	**Volume from standard stock transferred in mL**	**Volume made up in mL (with mobile phase)**	**Conc. obtained (µg/mL)**
			PHENTERMINE
Preparation 1	0.5	10	50
Preparation2	0.8	10	80
Preparation 3	1	10	100
Preparation 4	1.2	10	120
Preparation 5	1.5	10	150

The above prepared PHENTERMINE were in injected into the system and the chromatograms were recorded as given in Fig.

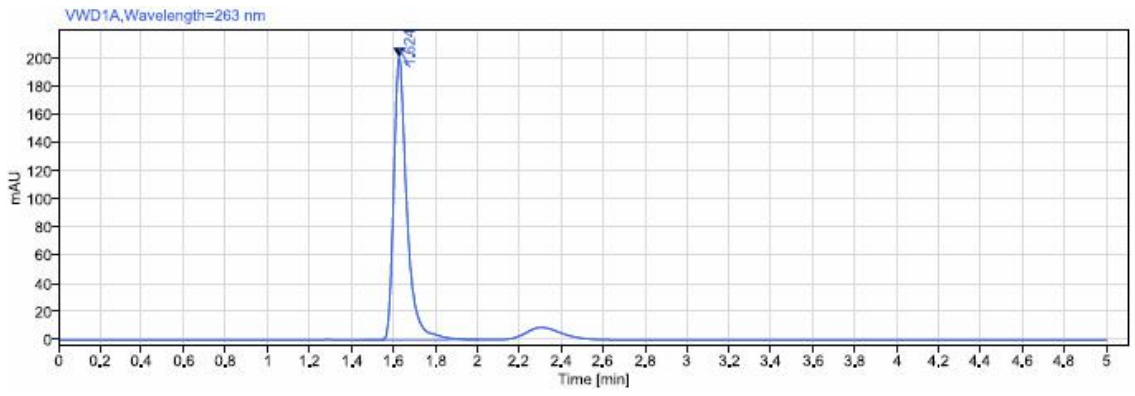


***Fig 4.1*:** Chromatogram of linearity for preparation 1.

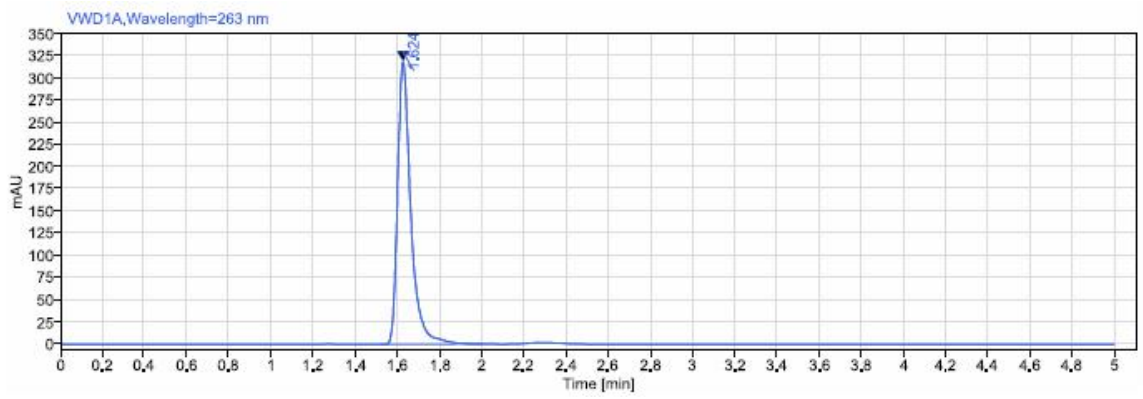


Fig 4.2*:* Chromatogram of linearity for preparation 2.

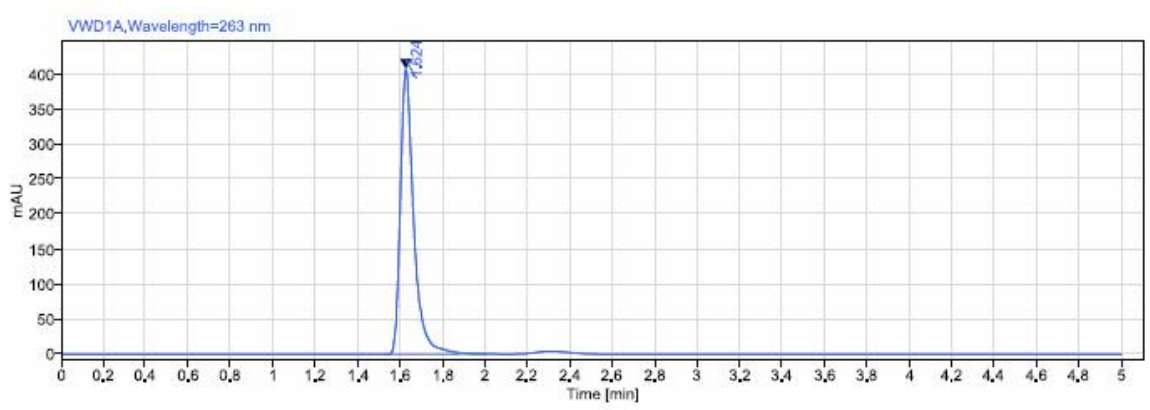


Fig 4.3**:** Chromatogram of linearity for preparation 3.

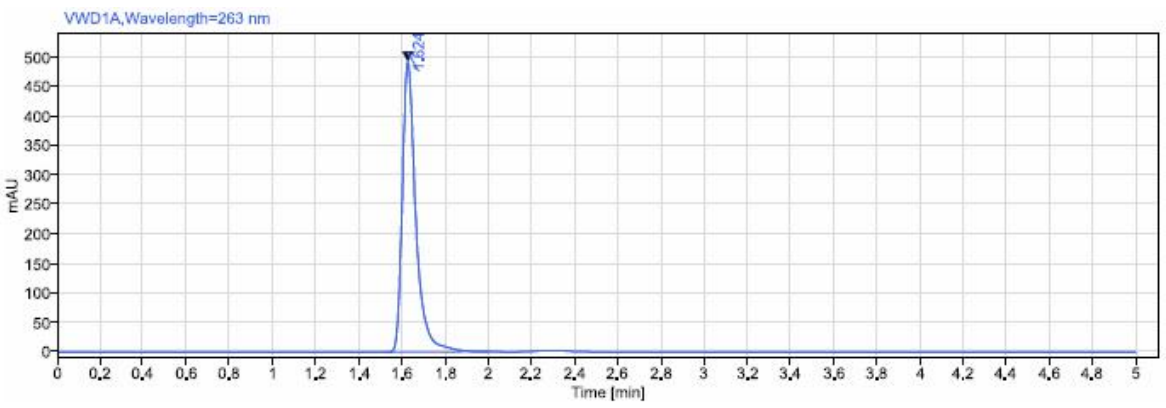


Fig 4.4**:** Chromatogram of linearity for preparation 4.

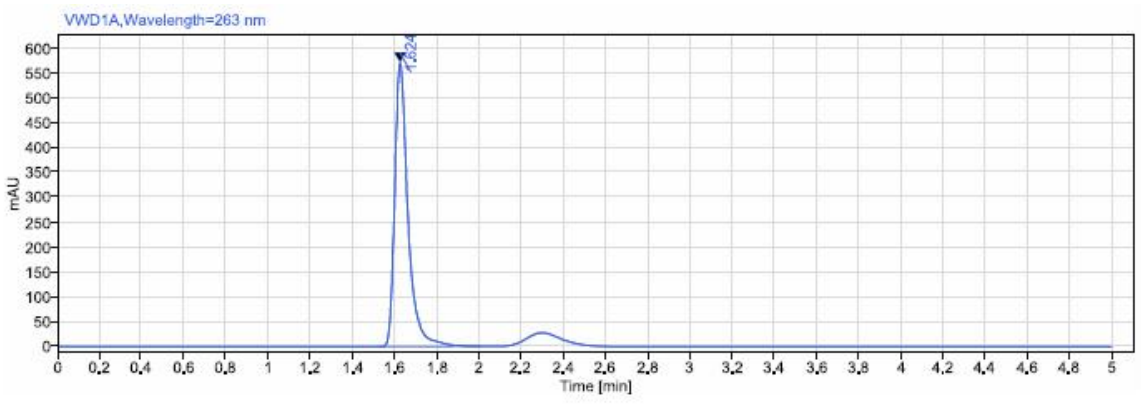


Fig 4.5**:** Chromatogram of linearity for preparation 5.

A graph was plotted for PHENTERMINE against the concentrations of the solutions and the peak areas . The correlation coefficient R^2 was determined and was found to be 1.00 for PHENTERMINE

Table 10 **:** Linearity data of PHENTERMINE.

S.No	Concentration (µg/mL)	Area
1	50	874.8
2	80	1394.81
3	100	1784.51
4	120	2157.39
5	150	2502.4

***Fig 5*:** Graph for Linearity data of PHENTERMINE.

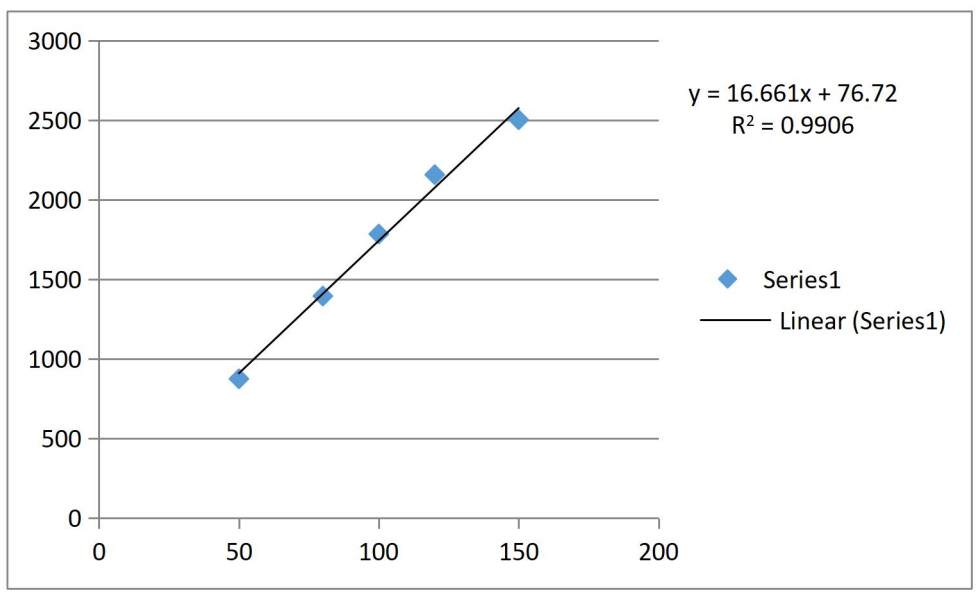


***Table 11*:** Linearity results of PHENTERMINE.

S.No	**Parameter**	PHENTERMINE
1	Correlation coefficient	0.990
2	Slope	16.66
3	Intercept	76.72

Acceptance criteria

The relationship between the concentration (in %) of PHENTERMINE and area of PHENTERMINE should be linear in the specified range and the correlation should not be less than 0.99.

Result

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparation 0.990

Specificity

A study to establish & determine the interference of blank and placebo as conducted. Analysis was performed on placebo intriplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of blank and placebo solutions had shown no peaks at the retention times of PHENTERMINE.

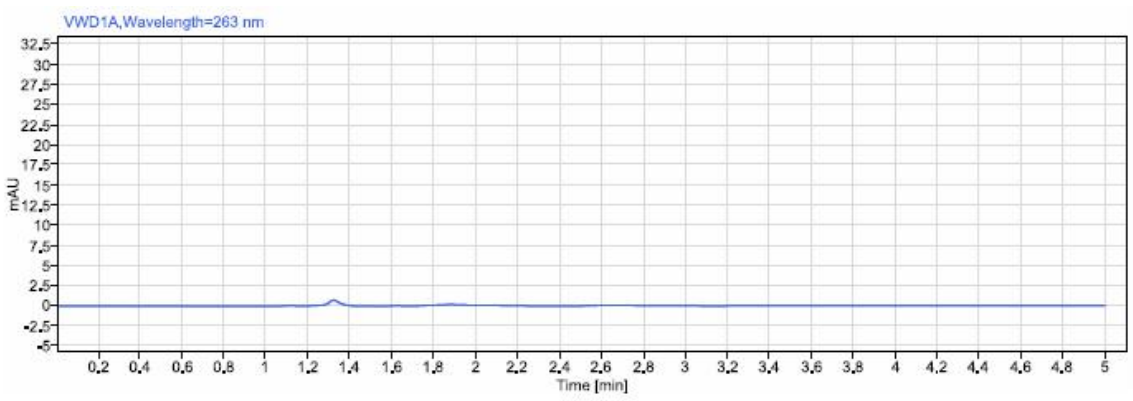


***Fig 6.1*:** Chromatogram of Placebo

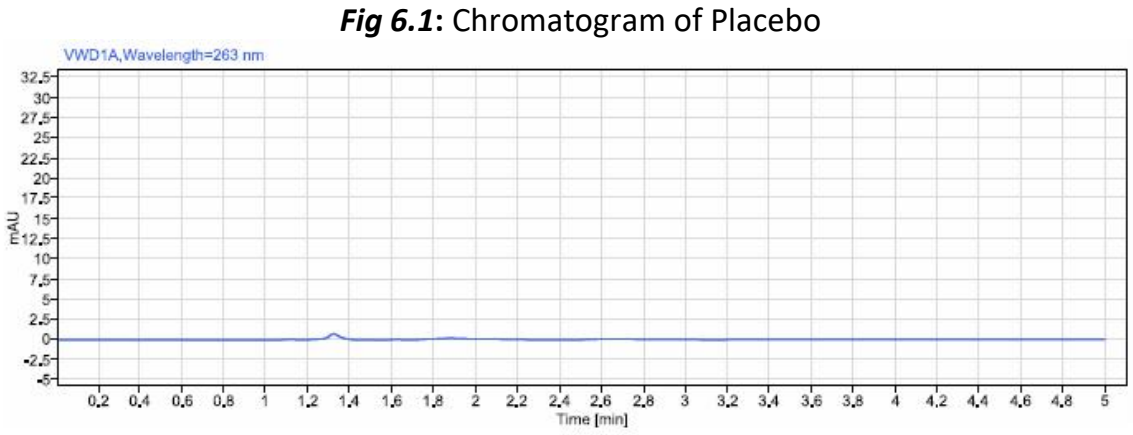


***Fig 6.2*:** Chromatogram of Blank

Result

It was observed that diluent or excipient peaks do not interfere with the PHENTERMINE Peak.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in Table 12.

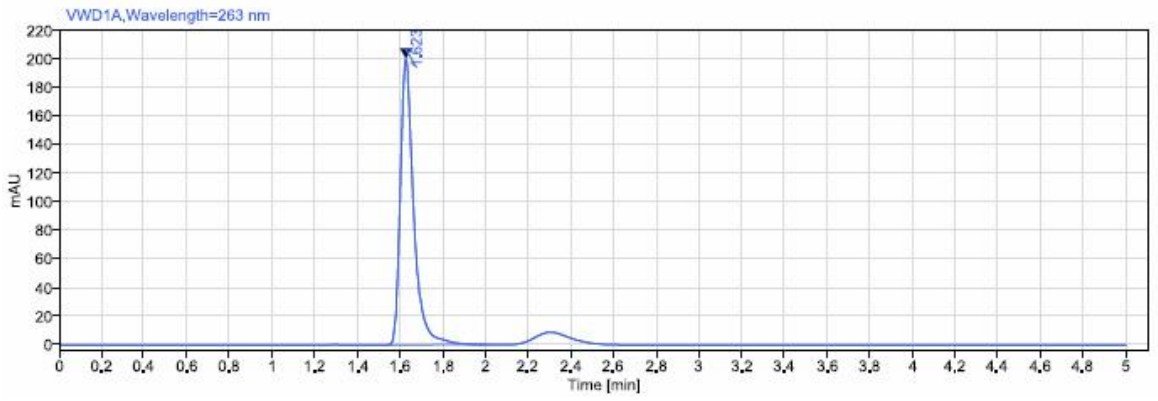


Fig 7.1: Chromatogram of 50% recovery-1

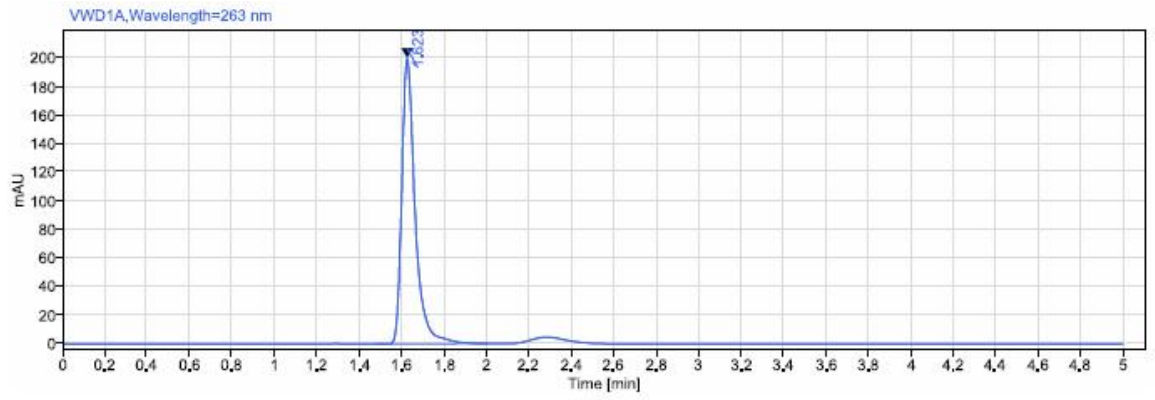


Fig 7.2: Chromatogram of 50% recovery-2

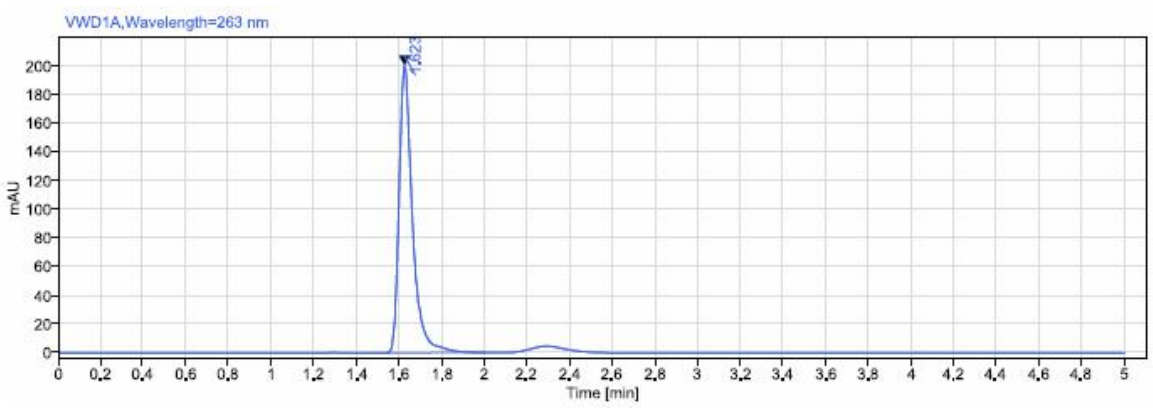


Fig7.3 : Chromatogram of 50% recovery-3

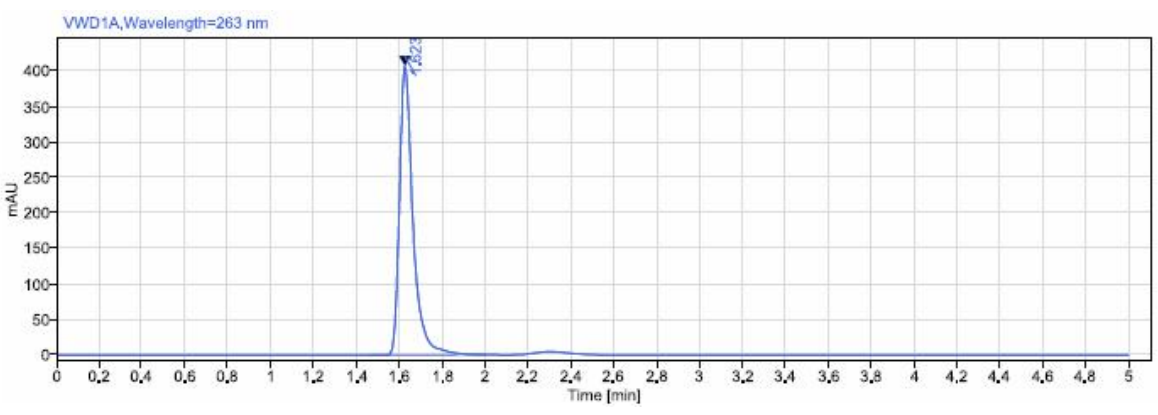


***Fig 8.1*:** Chromatogram of 100% recovery-1

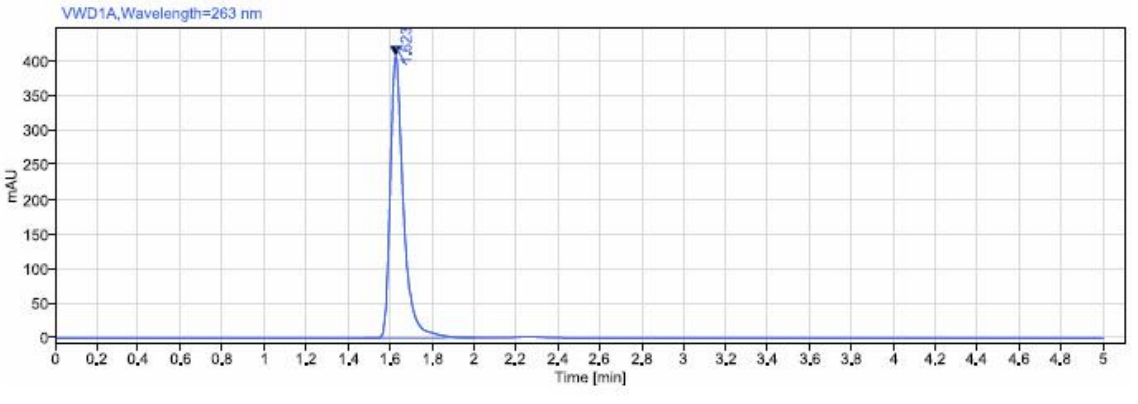


***Fig 8.2*:** Chromatogram of 100% recovery-2

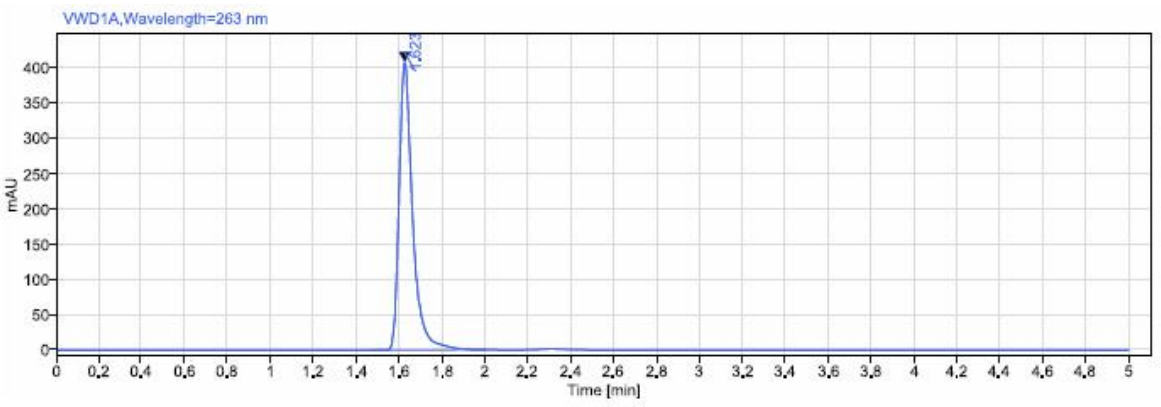


***Fig 8.3*:** Chromatogram of 100% recovery-3

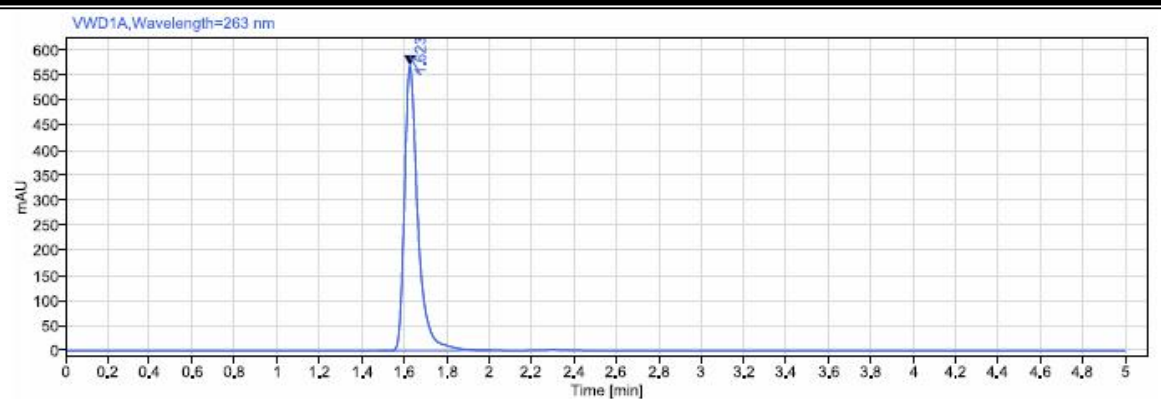


Fig 9.1 *:* Chromatogram of 150% Recovery-1.

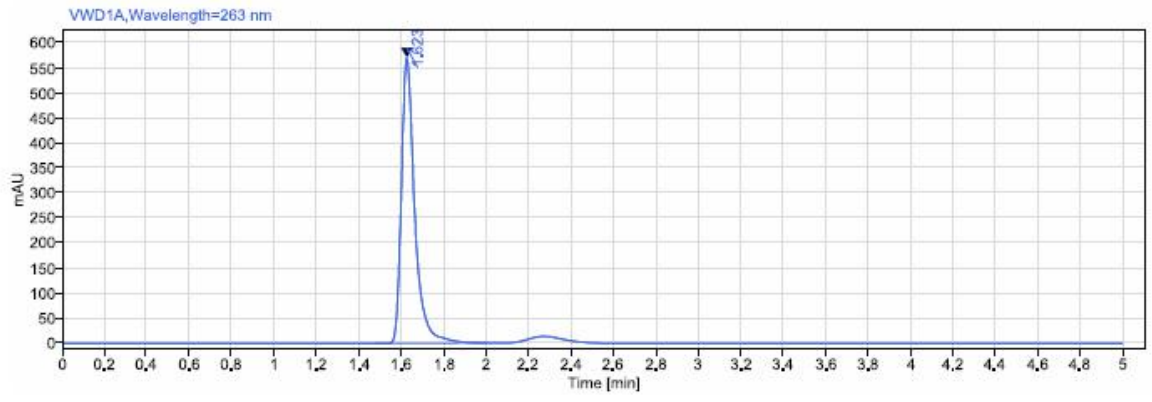


Fig 9.2*:* Chromatogram of 150% Recovery-2.

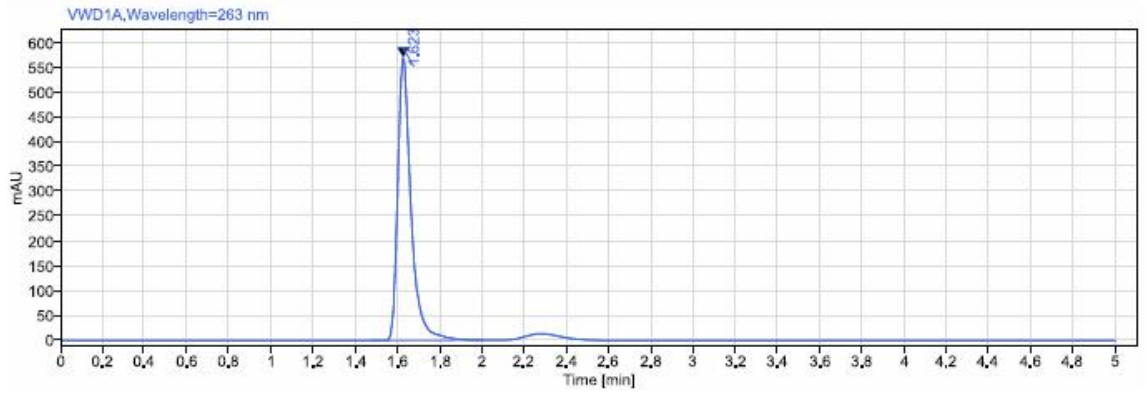


Fig9.3 *:* Chromatogram of 150% Recovery-3.

Amoxycillin						
Name of the Sample	Standard Weight in mg	Area	Conc Added (µg/ml)	Conc Recovered (µg/ml)	%Recovery	Average
50% Recovery_01	50	14507698	50	49.99	100.0	99.4
50% Recovery_02	50	14368377	50	49.51	99.0	
50% Recovery_03	50	14348431	50	49.44	98.9	
100% Recovery_01	100	28777447	100	99.16	99.2	
100% Recovery_02	100	28784075	100	99.19	99.2	
100% Recovery_03	100	28669699	100	98.79	98.8	
150% Recovery_01	150	43496072	150	149.88	99.9	
150% Recovery_02	150	43483295	150	149.84	99.9	
150% Recovery_03	150	43236035	150	148.98	99.3	

Table 12: Results for Recovery of PHENTERMINE.

Acceptance criteria

The Average % recovery of PHENTERMINE between 98% and 102%.

Result

The percentage mean recovery of PHENTERMINE %

LIMIT OF DETECTION

$$\mathrm{LOD} = \frac{3.3\sigma}{S}$$

= 3.3 * (0.6)/76.72

= 0.025µg/ml PHENTERMINE

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Observation:

The LOD for this method was found to be 0.025μg/ml PHENTERMINE

LIMIT OF QUANTIFICATION (LOQ)

$$\mathrm{LOQ} = \frac{10\sigma}{S}$$

= 10* (0.6)/76.72

= 0.078μg/ml PHENTERMINE

Where

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

OBSERVATION :

The LOQ for this method was found to be 0.078μg/ml PHENTERMINE

Robustness

The Robustness of the method was determined. The results obtained by deliberate variation in method parameters are summarized below in Table 13 .

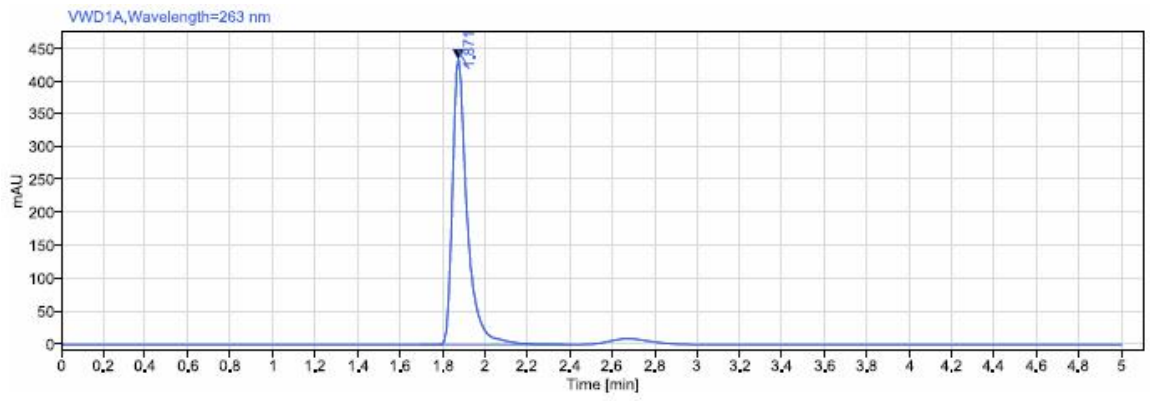


Fig.10.1 *:* Chromatogram of flow rate at 0.8mL/min.

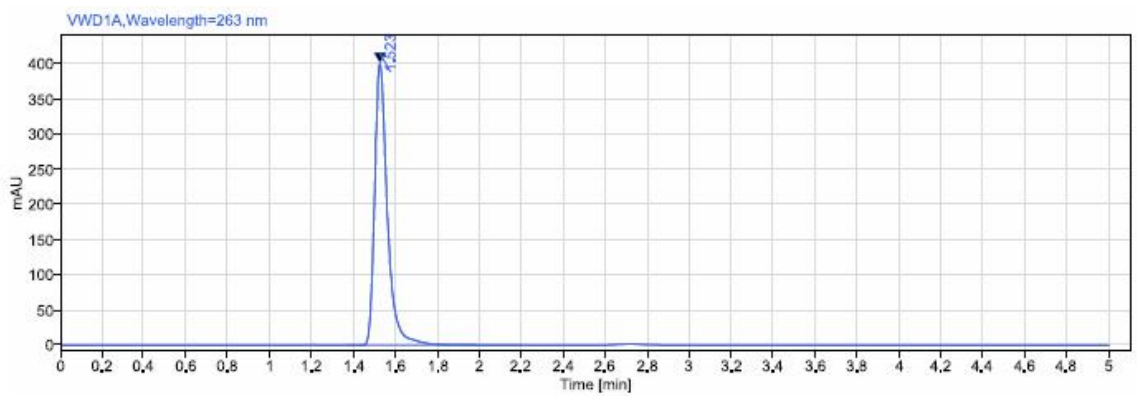


Fig.10.2**:** Chromatogram of flow rate at 1.2mL/min.

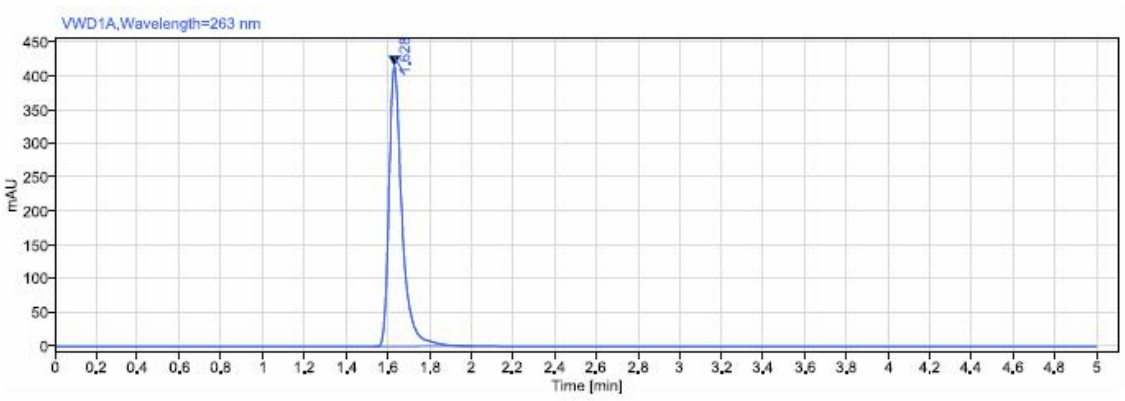


Fig.11.1 **:** Chromatogram of Temperature at 35°C

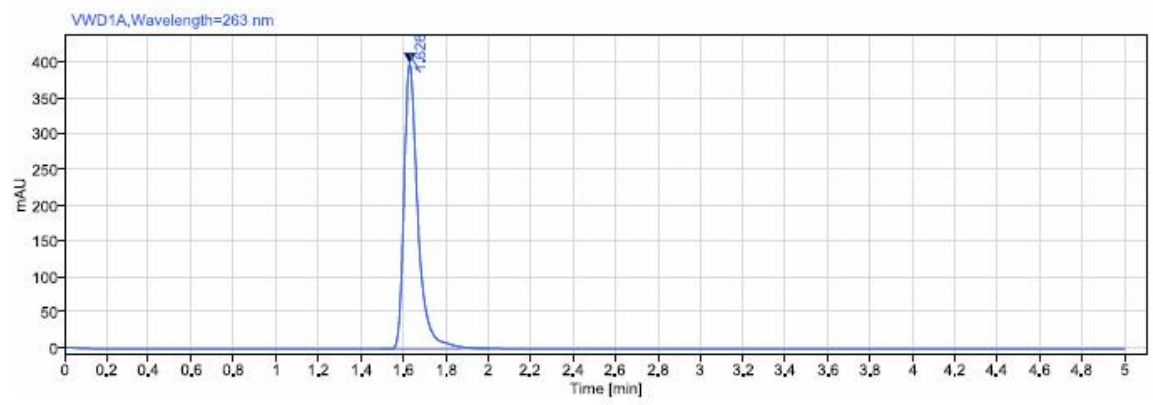


Fig 11.2 **:** Chromatogram of Temperature at 45°C

Table 13 **:** Results for Robustness of PHENTERMINE

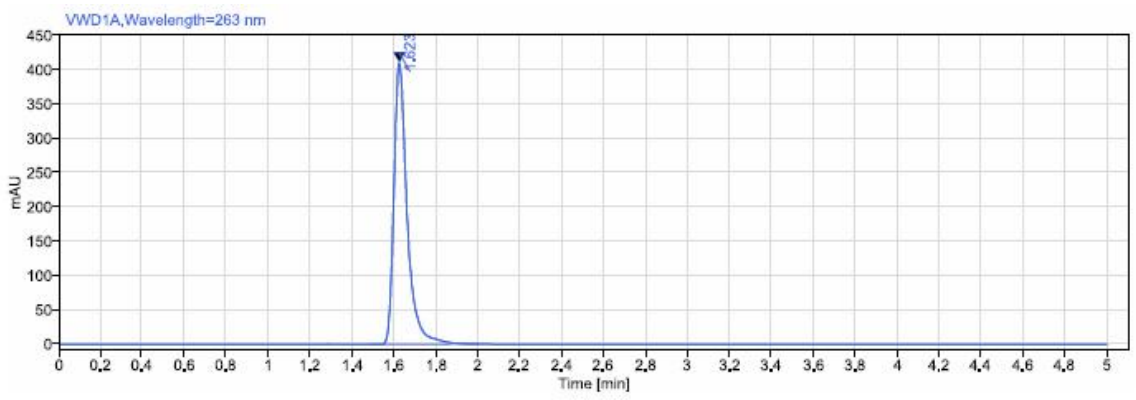
Chromatographic changes		**Rt(min)**	**Tailing Factor**	**Theoretical Plates**	**%RSD for Standard areas**
Flow rate (mL/min)	0.4	1.871	1.63	4171	0.03
	0.6	1.523	1.43	3459	0.49
Temperature	25	1.628	1.48	3786	0.05
	35	1.626	1.52	3434	0.42

Result

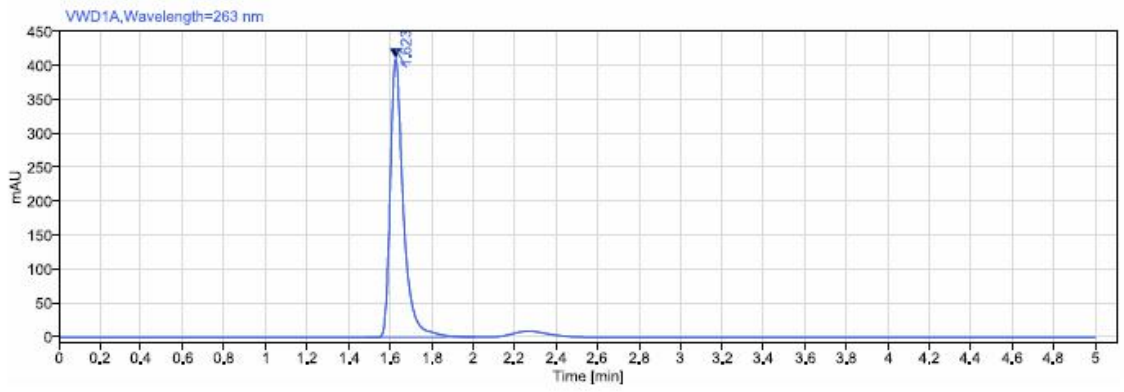
The tailing factor was found to be within the limits on small variation of flow rate and wavelength.

Intermediate Precision (Ruggedness):

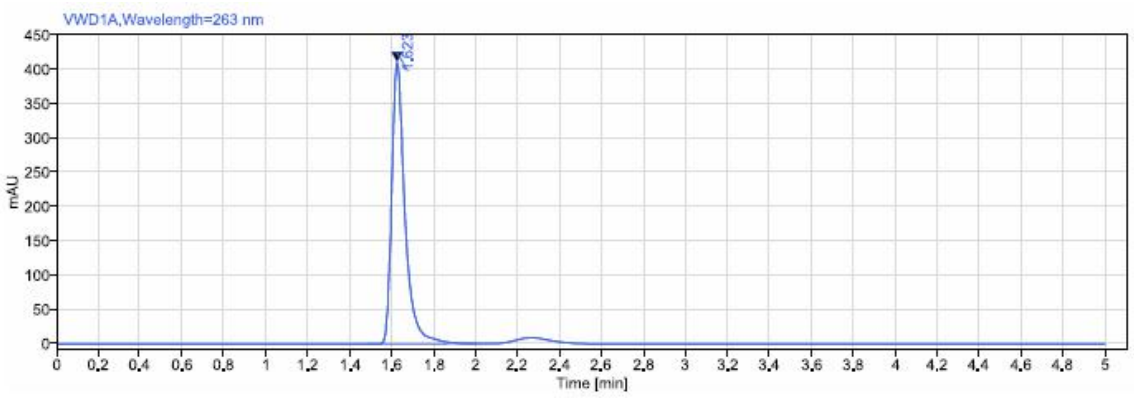
Intermediate precision (also called within-laboratory or within-device in different days, different analysts) is a measure of precision under a defined set of conditions: same measurement procedure, same measuring system, same location, and replicate measurements on the same or similar objects over an extended period of time.



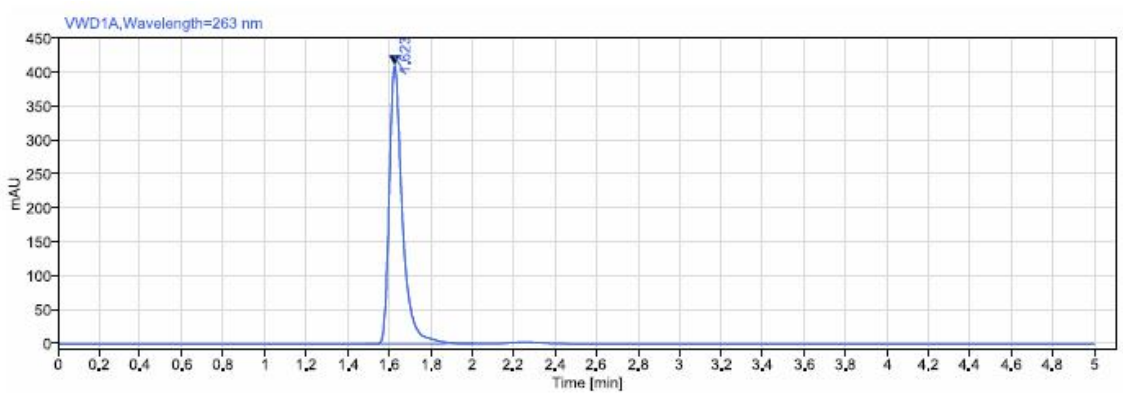
Chromatogram of Analyst-01 standard



Chromatogram of Analyst-01 sample



Chromatogram of Analyst-02 standard



Chromatogram of Analyst-02 sample

Table 14**:** Ruggedness Results of PHENTERMINE

PHENTERMINE	**%Assay**
Analyst 01	100.22

Analyst 02	100.17
%RSD	0.14

Results:

From the above results % Assay and %RSD obtained acceptance criteria so method is rugged.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of phentermine was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

BIBILOGRAPHY

1. Chatwal**,** R**.** G**.;** Anand**,** K**.** S**.** High performance liquid chromatography**.** *Instrumental methods of chemical analysis***,** 5th ed**.;** Himalaya publishers**:** Mumbai, 2010**;** 2.570-2.629**.**
2. Sharma**,** B**.** K**.** High performance liquid chromatography**.** *Instrumental methods of chemical analysis***,** 24th ed**.;** Goelpublishers**:** Meerut**,** 2005**;** 295 - 300**.**
3. Dong,W. M. HPLC instrumentation and trends. *Modern HPLC for practicing scientists*, USA, 2006; 5-10, 78-110.
4. UHPLC solvent properties *www.sanderkok.com/techniques/hplc/eluotropic_series_extended.htm*
5. Swartz, M. E.; Ira Krull, S, Analytical method development. *Analytical method development and validation*, 1st ed.; Marcel Dekker, Inc: New York, 2009; 17-80.
6. Satinder, A.; Dong, M. W. Method development and validation. *Pharmaceutical analysis by HPLC*, 15th ed.; New York, 2005; 16-70.
7. Snyder, R. L.; Kirkland, J. J.; Glajch, L. J. Getting Started. *Practical HPLC Method Development*, 2nd ed.; New York, 1997; 30-100.
8. Selection of buffers http://www.sigmaaldrich.com/etc/medialib/docs/Aldrich/General_Information/labbasics_pg144.Par.0001.File.tmp/labbasics_pg144.pdf.
9. ICH, *Text on Validation of Analytical Procedures,* ICH – Q2A, International Conference on Harmonisation, IFPMA, Geneva, 1995, 2-3, A–1 to A–3.
10. ICH, *Validation of Analytical Procedures: Methodology,* ICH – Q2B, International Conference on Harmonisation, 1996, 1-3.
11. ICH Guidelines, Q2 (R1) - Validation of Analytical Procedures:Text and Methodology,2005, 1-6.
12. https://en.wikipedia.org/wiki/Phentermine
13. https://www.drugbank.ca/categories/DBCAT001351