

“DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF VINPOCETINE IN ITS BULK AND PHARMACEUTICAL DOSAGE FORM”

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ABSTRACT: The present study describes a simple, accurate, precise and cost-effective reverse phase High Performance Liquid Chromatographic method for estimation of vinpocetine in their pharmaceutical dosage form. The separation was carried on Kromasil, C18, 250 mm X 4.6 mm, 5 μ m. Detection was done using UV detector at isocratic point 228 nm. The developed method employed mobile Acetonitrile: Buffer (90:10 % v/v), with flow rate 1.0 ml/min. High linearity of the developed method was confirmed over concentration range 5-75 μ g/ml for vinpocetine with the correlation coefficient of 0.999. The Percentage RSD for precision of the method was found to be less than 2%. The percentage recoveries for vinpocetine were found to be in range 98.00-102.00 w/v. Peaks was obtained at retention time 6.4 min for Vinpocetine. By using all the above parameters, a simple, accurate, precise and cost-effective method were developed, optimize and validate. Some method by HPLC and UV, FTIR are already reported in vinpocetin drug .

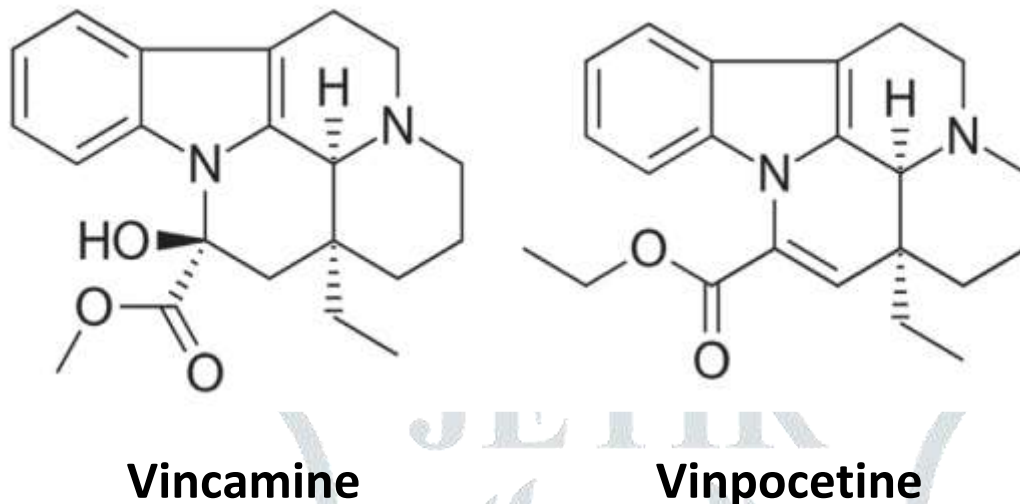
KEYWORDS: RP-HPLC, Method Optimization, development, Validation, vinpocetine.

INTRODUCTION

Pharmaceutical analysis, a branch of pharmacy, plays a very significant role in quality control of pharmaceuticals through a rigid check on raw materials used in manufacturing of formulation and on finished products. Analytical chemistry has since long, occupied an important place in the development of science and technology. It is primarily concerned about determining the qualitative and quantitative composition of material under study. The qualitative analysis gives us the information about the nature of sample by knowing about the presence or absence of certain components (<https://naturalmedicines.therapeuticresearch.com>). The quantitative analysis deals about the content present in the sample. The development in analytical sciences has been more significant and prominent in recent years than the past. This helped to develop new methods of analysis. In pharmacy analytical chemistry is responsible for developing sensitive, reliable and more accurate methods for the estimation of drug in pharmaceutical dosage form. Quality assurance is a wide-ranging concept covering all matters that

individually or collectively influence that quality of the product. It plays a central role in determining the safety and efficiency of medicines. Highly specific and sensitive analytical techniques hold the key role to the design, development, standard and quality control of medicinal product.

Fig -1 Chemical structure



Quality of the drug product is very vital, as it involves life. Proper manufacture and quality control of pharmaceuticals is the vital segment of strong primary healthcare programme worldwide. Quality is the total sum of all factors which contribute directly or indirectly to the safety; efficacy and acceptability of the product. Analytical Chemistry Analytical chemistry is an important part of pharmaceutical analysis. Analytical Chemistry may be defined as the science and art of determining the components of materials in terms of the elements or compound contained. Analytical Chemistry seeks ever improved means of measuring the chemical composition of natural and artificial materials. The techniques of this science are used to identify the substances which may be present in a material and to determine the exact amounts of the identified substances.

MATERIAL AND INSTRUMENTS:

1 Material:

1.1 Drugs:

Table No. 1.1 Procurement of Drug Samples of Vinpocetine

Sr. No.	Name of the Drug	Taken from
1	Vinpocetine	Alkem Laboratories Ltd.

1.2 Reagents:**Table No. 1.2 List of Reagents Used**

Sr. No.	Chemicals/ Reagents/ Solvents	Supplier	Grade
1	Methanol	Qualigens (Thermo fisher scientific)	HPLC grade
2	Acetonitrile	Qualigens (Thermo fisher scientific)	HPLC grade
3	Water	Siddhi Lab	HPLC grade
4	Ortho-phosphoric Acid (OPA)	Qualigens (Thermo fisher scientific)	HPLC grade
5	Ethanol	Merck	Analytical
6	DMF	Merck	Analytical
7	DMSO	Qualigens (Thermo fisher scientific)	Analytical
8	HCl	Merck	Analytical
9	HPLC Water	Moreshwar Enterprises	HPLC grade
10	Ammonium Acetate	Qualigens (Thermo fisher scientific)	Analytical

2. Instruments:**Table No. 2.1 List of Instruments used**

UV – Visible Spectrophotometer Double beam UV- Visible spectrophotometer	
Model	UV 550
Make	Jasco
Software	Spectra manager

HPLC System	
HPLC Binary Gradient System	
Model No.	1260 Infinity II
Make	Agilent
Pump	DEAX02386
Detector	DEACX16446
Column	Kromasil C18 column (250 mm X 4.6 mm. 5 μ m).
Software	Open lab EZ Chrome

a) Preparation of standard stock solutions:

- + Vinpocetine stock solution: Weighed 10 mg of Vinpocetine and dissolved in 10 mL of Methanol (1000 PPM of Vinpocetine)
- + **SOLUTION FOR UV SCAN:** Vinpocetine solution: Pipette out 0.4 mL of Vinpocetine stock solution and diluted up to 20 mL with Methanol. (20 PPM of Vinpocetine) Methanol as a blank and Vinpocetine drug solution were scanned from 400 nm to 200 nm.

b) Selection of analytical wavelength

The standard solution was scanned between 200-400 nm. The wavelength of maximum absorption was determined for the Vinpocetine was showed absorption maxima at 314, 272 and 228nm but 228 nm selected as a absorption maxima.

3. Fourier Transformation Infrared (FTIR) analysis

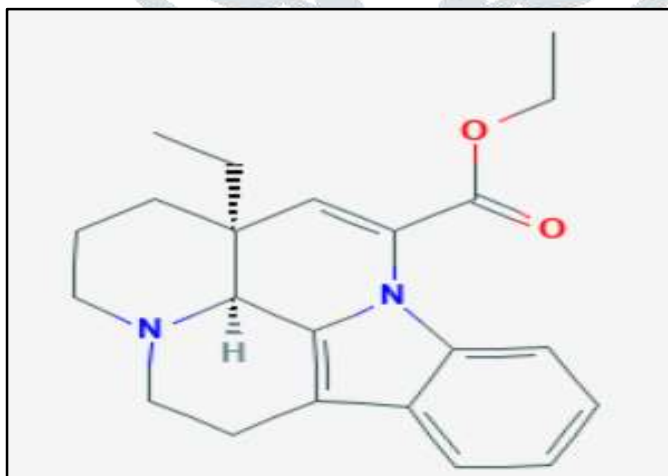


Fig 3.1 Chemical structure of Vinpocetine

4. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD FOR ANALYSIS OF VINPOCETINE:

4.1. Preparation of standard stock solution:

Weighed accurately 25 mg of Vinpocetine and transferred to 50 mL volumetric flask. Added 30-35 mL of Methanol, sonicated to dissolve it completely, make the volume up to the mark with Methanol. Filter the solution through suitable filter of 0.45 μ discarding 3-5 ml of filtrate. Dilute further 1 mL to 10 mL with Mobile phase.

4.2 API Sample Preparation: Weighed accurately 25 mg of Vinpocetine and transferred to 50 mL volumetric flask. Added 30-35 mL of Methanol, sonicated to dissolve it completely, make the volume up to the mark with Methanol. Filter the solution through suitable filter of 0.45 μ discarding 3-5 ml of filtrate. Dilute further 1 mL to 10 mL with Mobile phase.

4.3 Selection of analytical wavelength from the spectrophotometric method: Analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis. The standard solution was scanned between 200-400 nm. The wavelength of maximum absorption was determined for drug Vinpocetine and it was 228 nm.

Chromatographic Conditions:

Column: Kromasil C18,

Column Dimension: (250 mm X 4.6 mm i.d.) 5 μ m

Column oven temp: 35°C

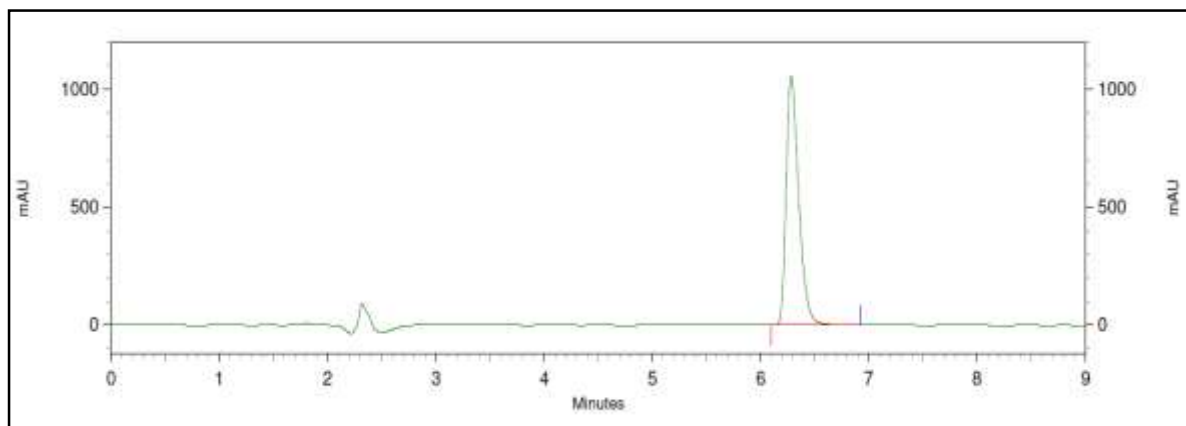
Detector: U.V. Detector

Wavelength: 228 nm

Flow Rate: 1.0 ml/min

Mobile phase: Acetonitrile: 50 mm Ammonium acetate solution (90:10)

Injection Volume: 20 μ l

Vinpocetine 100 PPM:**Fig. Typical chromatogram of Vinpocetine Trial 1**

Observation: Vinpocetine eluted and good chromatography observed.

Optimized Chromatographic Conditions

Parameter/condition	Description
Column name	Kromasil C18 (250 mm X 4.6 mm i.d.) 5 μ m
Detector	UV-3000-M
Injection Volume	20 μ L
Wavelength	228 nm
Mobile Phase	Acetonitrile : 50 mm Ammonium acetate solution (90:10)
Diluent	Methanol
Programme	Isocratic
Flow Rate	1.0 mL/min
Column Oven temp	35° C
Run time	15 min

Preparation of System suitability test (working standard solution):

STD wt. (mg)	Diluted to	Volume taken (mL)	Diluted to (mL)	Conc (μ g / mL)
25.2	50	1	10	50.40

Acceptance criteria:

1. RSD should not be more than 2.0 % for six replicate injections of standard.
2. USP Tailing Factor/ Asymmetry Factor is not more than 2.0.
3. The column efficiency as determined for Plate Count should be more than 2000.

5. VALIDATION OF RP-HPLC METHOD

1) **FILTRATION STUDY:** Filter study performed by using Centrifuged sample (Unfiltered), Sample passed through 0.45 μ PVDF filter and 0.45 μ Nylon filters, by discarding 5 mL of solution. (Performed on Tablet sample)

2) **STABILITY OF ANALYTICAL SOLUTION** Stability study will be performed at normal laboratory conditions. The solution will be stored at normal illuminated laboratory conditions and analysed after 12 hours and 24 hours. Test solution stability study will be performed by calculating the difference between results of the test solution at each stability time point to that of initial. Standard solution stability study will be performed by calculating the difference between results of standard solution at each stability time point to that of initial. Standard solution and sample solution injected at initial (0 Hr), after 12 Hrs and 24 hrs. Percent absolute difference calculated with respect to initial area.

3) **SPECIFICITY:** The following solution shall be prepared and injected to prove the specificity nature of the method. (Checked peak purity)

- | | |
|----------------------|-------------------------|
| I. Blank | III. standard solution, |
| II. Sample solution, | IV. placebo |

4) LINEARITY AND RANGE

Dilution table for linearity of vinpocetine

Level	mL of stock	Diluted to	Conc (μ g/mL)
10%	0.4	10	5.00
50%	2	10	25.00
100%	4	10	50.00
125%	5	10	62.50
150%	6	10	75.00

5. ACCURACY (% RECOVERY):

Level (%)	API (mg)	Diluted to (mL)	Volume taken	Diluted to (mL)	Conc (μ g/mL)
50	12.6	50	1	10	25.20
	12.6	50	1	10	25.20
	12.5	50	1	10	25.00
100	25.1	50	1	10	50.20
	25.2	50	1	10	50.40
	25.2	50	1	10	50.40

150	37.5	50	1	10	75.00
	37.6	50	1	10	75.20
	37.6	50	1	10	75.20

6. PRECISION Repeatability (Intraday Precision):

Acceptance Criteria:

% RSD = NMT 2% for test results.

Intermediate precision (Interday precision)

Acceptance criteria:

% RSD of 6 samples NMT 2.0% for test results.

% RSD of Total 12 samples NMT 2.0% for test results

(6 of Repeatability and 6 of Intermediate precision)

7. ROBUSTNESS: These samples were injected under different chromatographic conditions as shown below.

- Changes in flow rate. $\pm 10\%$
- Change in wavelength. ± 3 nm
- Change in Column oven temperature: $\pm 2^\circ\text{C}$

6. FORCE DEGRADATION STUDY

I. Physical degradation

- Photolytic
- Thermal

II. Chemical degradation

- Acid
- Base
- Peroxide

To achieve degradation in the range of 5 % to 20% of assay value.

1. PREPARATION OF DEGRADANT'S:

a) 5 N Hydrochloric acid:

42.5 mL of HCl diluted to 100 mL with water.

b) 5 N NaOH solution:

20 gm of NaOH dissolved in 100 mL of water.

Physical degradation:**1) Thermal degradation:**

Sufficient amount of API was kept in hot air oven at 105° C for 24 hrs. After 24 hours API was removed and kept in desiccators to reach at R.T.

Subjected sample prepared as per method of analysis and injected.

2) Photolytic degradation:

Sufficient amount of API was kept in direct sunlight for 72 hrs. After 72 hours API was removed and kept in desiccators to reach at R.T.

Subjected sample prepared as per method of analysis and injected.

Chemical degradation:**1) Acid degradation:**

Sample preparation: Weighed Vinpocetine API approx 25 mg and transferred in 50 mL volumetric flask. Added 30 mL of methanol sonicated to dissolve it completely. Added 5 mL of 5 N HCl and kept on bench for 24 Hours. After 24 Hours, reaction was neutralized by adding 5 ml of 5 N NaOH solutions. Volume made up to the mark with methanol. Solution filtered through 0.45µ PVDF syringe filter. Filtrate further diluted 1 mL to 10 ml with mobile phase.

2) Base/Alkali degradation:**Trial 1:**

Sample preparation: Weighed Vinpocetine API approx 25 mg and transferred in 50 mL volumetric flask. Added 30 mL of methanol sonicated to dissolve it completely. Added 5 mL of 5 N NaOH solutions and kept on bench for 24 Hours. After 24 Hours, reaction was neutralized by adding 5 ml of 5 N HCl solutions. Volume made up to the mark with methanol. Solution filtered through 0.45µ PVDF syringe filter. Filtrate further diluted 1 mL to 10 ml with mobile phase.

Trial 2:

Sample preparation: Weighed Vinpocetine API approx 25 mg and transferred in 50 mL volumetric flask. Added 30 mL of methanol sonicated to dissolve it completely. Added 5 mL of 5 N NaOH solution and kept

on bench for 1 Hours. After 1 Hours, reaction was neutralized by adding 5 ml of 5 N HCl solution. Volume made up to the mark with methanol. Solution filtered through 0.45 μ PVDF syringe filter. Filtrate further diluted 1 mL to 10 ml with mobile phase.

3) Peroxide degradation:

Sample preparation: Weighed Vinpocetine API approx 25 mg and transferred in 50 mL volumetric flask. Added 30 mL of methanol sonicated to dissolve it completely. Added 5 mL of 30% hydrogen peroxide solution and kept on bench for 24 Hours. After 24 Hours, reaction was neutralized by adding 5 ml of 30% Sodium sulfite solution. Volume made up to the mark with methanol. Solution filtered through 0.45 μ PVDF syringe filter. Filtrate further diluted 1 mL to 10 ml with mobile phase.

8. RESULT AND DISCUSSION

Preliminary Characterization and Identification of Drug, Table no. 8.1

Vinpocetine	
Parameters	Observation
Colour	White or slightly yellow
Appearance	crystalline powder

Melting Point Determination:

Table No.8.2. Melting Point (°C) of drugs

Vinpocetine	
Observed MP (°C)	Std. M.P(°C)
179-181	182
181-183	
180-183	

Solubility Study

Table No.8.3. Solubility study of drugs

Sr. No.	Name of Solvent	Solubility of vinpocetine
1	Water	Insoluble
2	Methanol	Slightly soluble
3	Acetonitrile	Slightly soluble
4	Phosphate buffer	Soluble
5	0.1 N HCl	Insoluble
6	ethanol	Soluble
7	DMF	Soluble
8	DMSO	Soluble

Determination of wavelength maxima and beers lamberts law study using UV- Spectroscopy

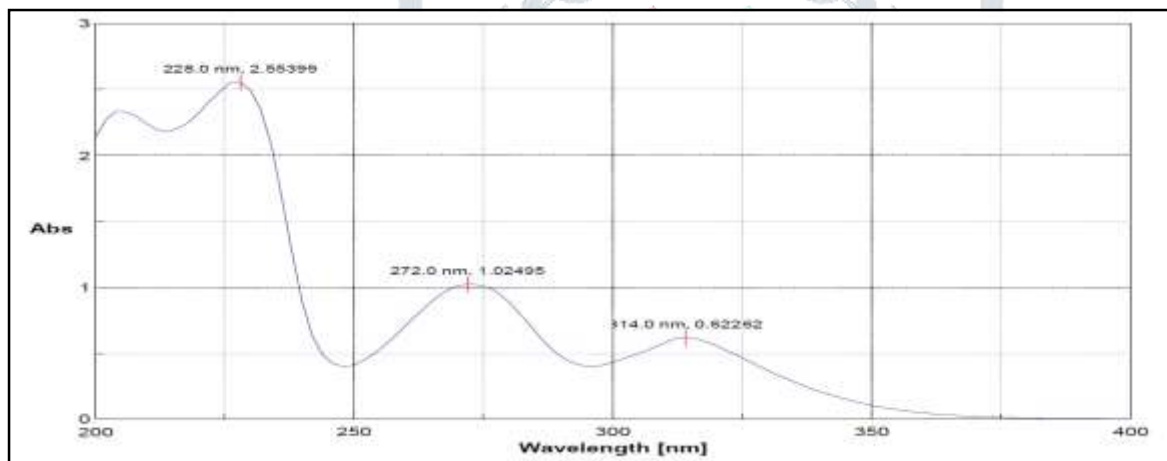
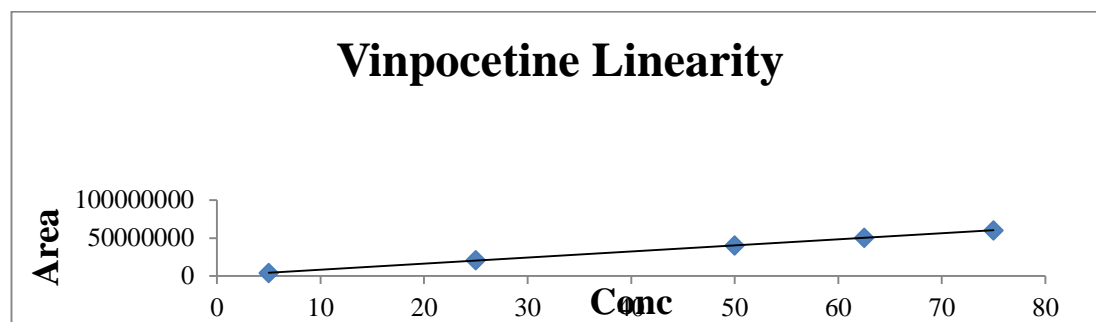


Fig. 8.4. UV spectrum of Vinpocetine in methanol

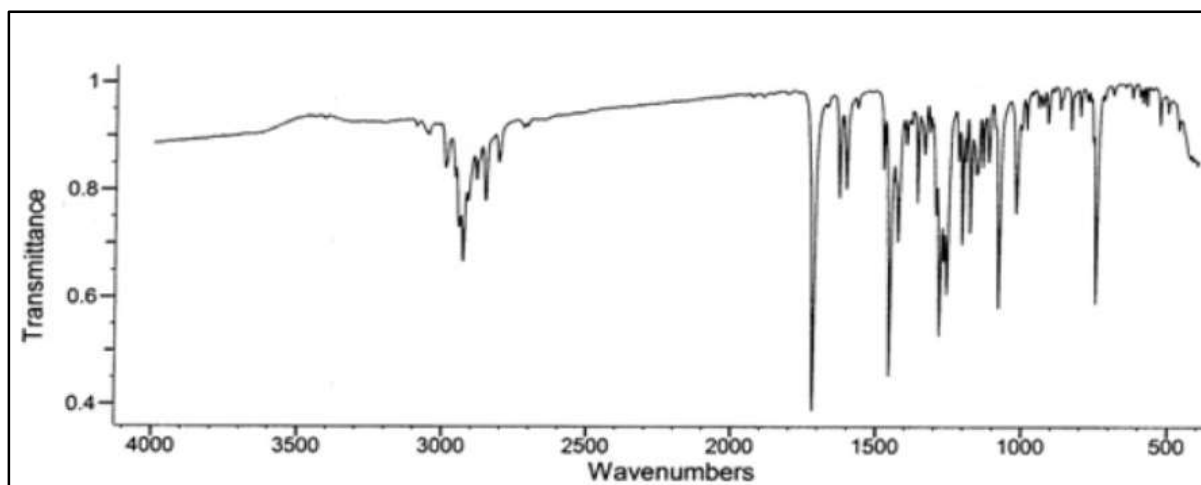
Observation: The standard solution was scanned between 200-400 nm. The wavelength of maximum absorption was determined for drugs. **vinpocetine** showed maximum absorbance at 228 nm

Study of Beers- Lambert's law:

Drugs	λ max
vinpocetine	228 nm



Fourier Transformation Infrared (FTIR) analysis:



FILTER TEST

Table no 8.5 , Analytical data of Filter Test for Vinpocetine

Sample	Area	% Absolute difference
Unfiltered	40069852	NA
0.45 μ PVDF filter	39848963	0.55
0.45 μ Nylon filter	39796846	0.68

Acceptance Criteria:

% Absolute difference NMT 2.0

Conclusion: Both filters PVDF and Nylon pass the criteria for filter study; hence both filters can be used.

ANALYSIS OF TEST SAMPLES (ASSAY):**a) Vinpocetine API: Assay Calculation**

$$\% \text{ Assay} = \frac{\text{sample area}}{\text{std mean area}} \times \frac{\text{std wt.}}{20} \times \frac{1}{50} \times \frac{50}{\text{sample wt.}} \times \frac{20}{1} \times 100$$

$$\frac{16170241}{16100420} \times \frac{10.2}{100} \times \frac{10}{20} \times \frac{100}{10.3} \times \frac{20}{10} \times 100$$

$$1.004336595 \times 0.102 \times 0.5 \times 9.70873786 \times 2 \times 100$$

% Assay =99.46%**ACCURACY(%RECOVERY):****Table no. 8.6 Result and statistical data of accuracy for Vinpocetine**

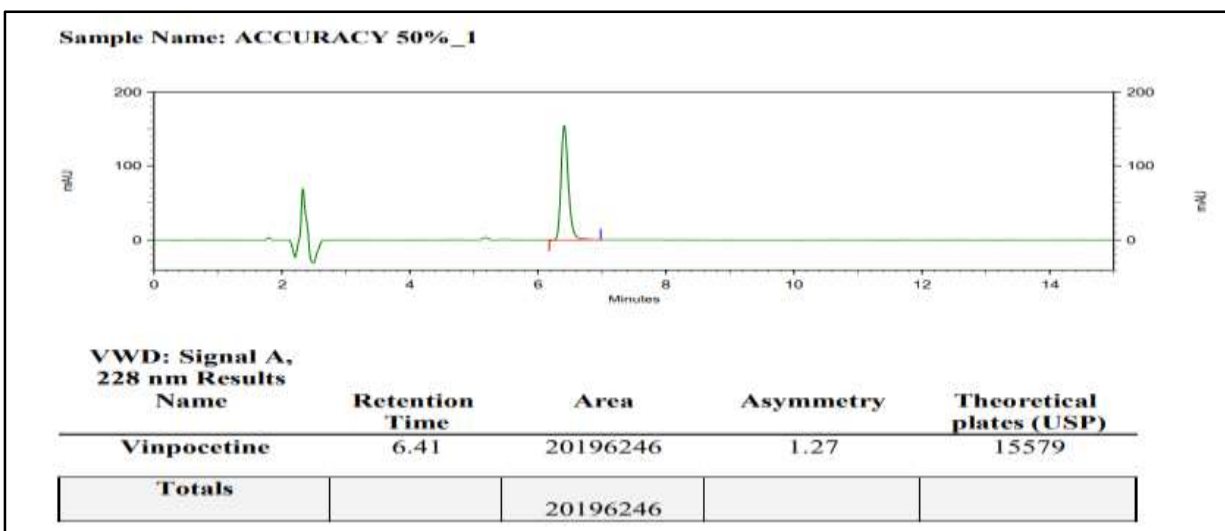
Level (50 %)	Area	Recovered conc	Added conc	% Recovery
50	20196246	25.42	25.20	100.87
	20156391	25.37	25.20	100.67
	20069864	25.26	25.00	101.04
100	39862492	50.18	50.20	99.96
	39796258	50.10	50.40	99.40
	40236986	50.65	50.40	100.50
150	59862418	75.36	75.00	100.48
	59712846	75.17	75.20	99.96
	59912578	75.42	75.20	100.29

Acceptance Criteria:

% Recovery: 98.00 % to 102.0 %

Conclusion:

% Recovery was found well within acceptance range at all three levels.

Chromatograms of Accuracy for drug:**PRECISION:****A) Intraday Precision:****Table No.8.7 Analytical data Intraday Precision of Vinpocetine**

Sample	Area	% Assay
Sample 1	39586248	99.66
Sample 2	40128706	100.63
Sample 3	39762584	98.92
Sample 4	40269814	100.58
Sample 5	39864712	99.96
Sample 6	40062586	100.86
Mean		100.10
STD DEV		0.734096
% RSD		0.733

Acceptance criteria:

% Assay value for individual sample must be within 98 % to 102% of Vinpocetine.

% RSD for 6 precision samples NMT 2.0 %.

Conclusion: Precision pass the criteria, no variation found by preparing six different samples. Results are good reproducible.

LINEARITY:**Table no.8.8 Result and statistical data of linearity of Vinpocetine**

Level	Cons ($\mu\text{g/mL}$)	Area	Mean	% RSD
10%	5	3799718	3779299	0.508
		3776595		
		3761584		
50%	25	20538687	20543883	0.247
		20495864		
		20597098		
100%	50	40058744	39951569	0.404
		40030158		
		39765806		
125%	62.5	50265800	50373849	0.317
		50556984		
		50298762		
150%	75	60098731	60098735	0.045
		60071628		
		60125845		

Conclusion:

From the calibration curve we had to conclude that the vinpocetine shows linear response in the range of 5-75 $\mu\text{g/mL}$. The Regression value was found well within the limit.

Chromatogram of linearity for drugs :

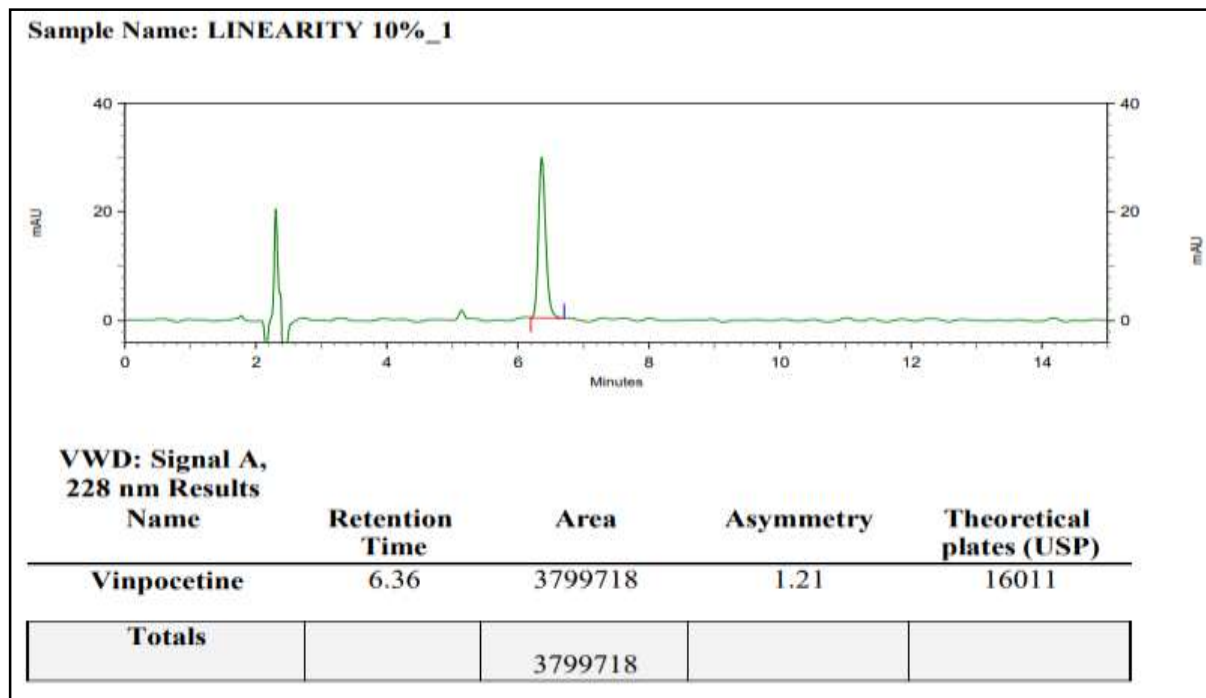


fig.8.9 Chromatogram of linearity for sample of 10% level-I

ROBUSTNESS:

Effect of variation in the flow rate of the mobile phase

1. Change in flow Rate

Table no. 8.10 Data for change in flow rate Vinpocetine

Sr. No.	System Suitability parameter	Vinpocetine		Limits
		As such+10%	As such-10%	
1	Peak area response	37168913	47042468	
2	Theoretical plates	13672	15012	NLT 2000
3	Asymmetry	1.27	1.32	NMT 2.0
4	% Assay	99.97	100.76	

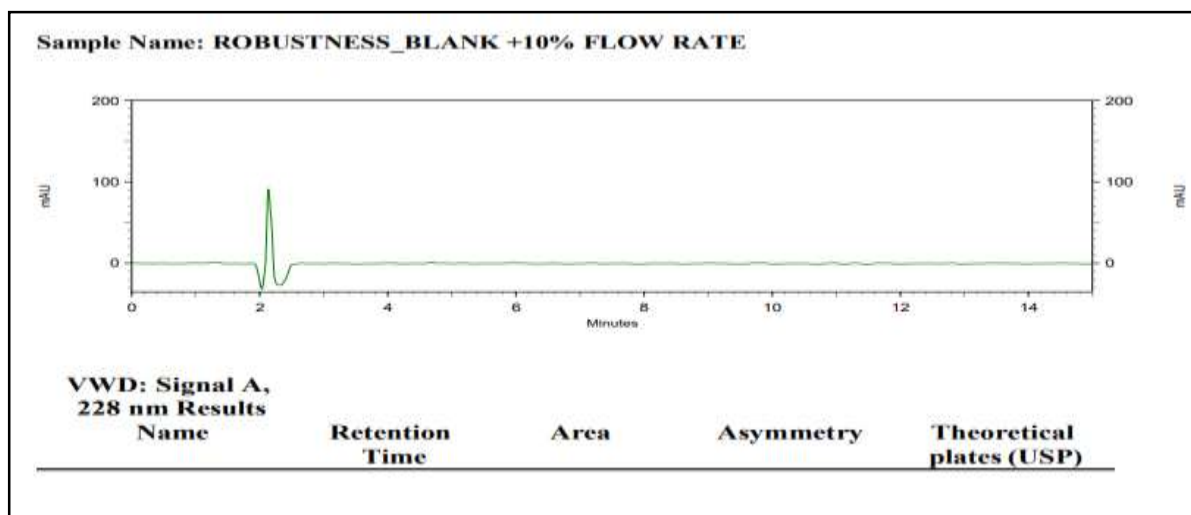
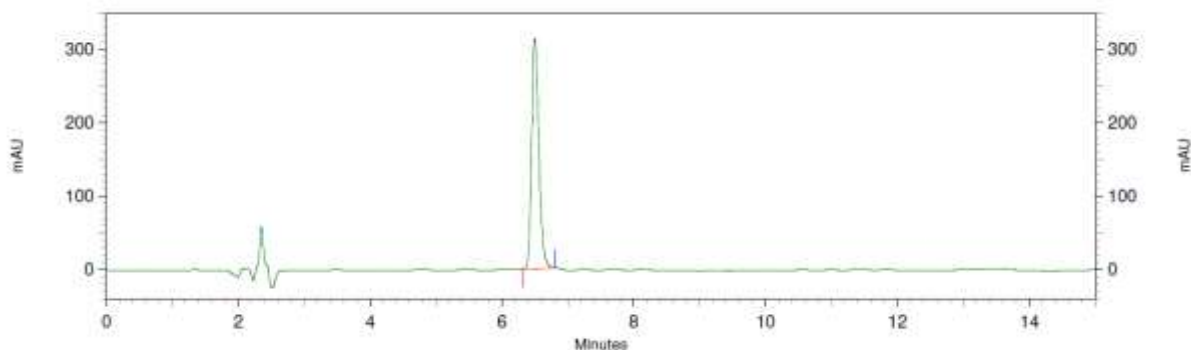
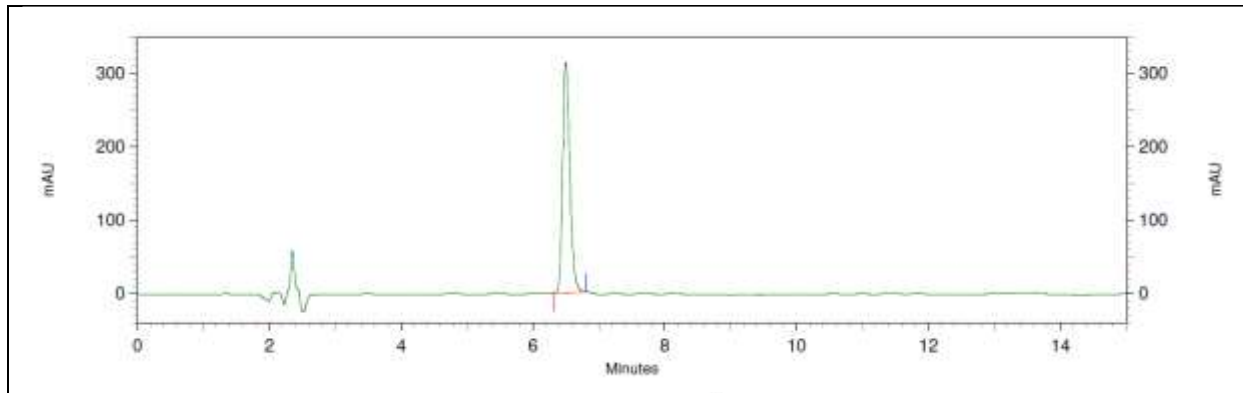
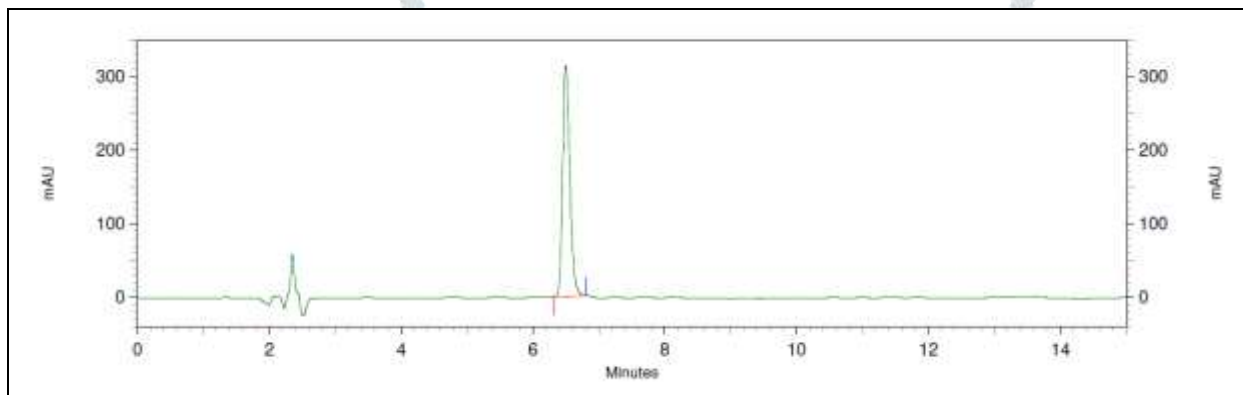
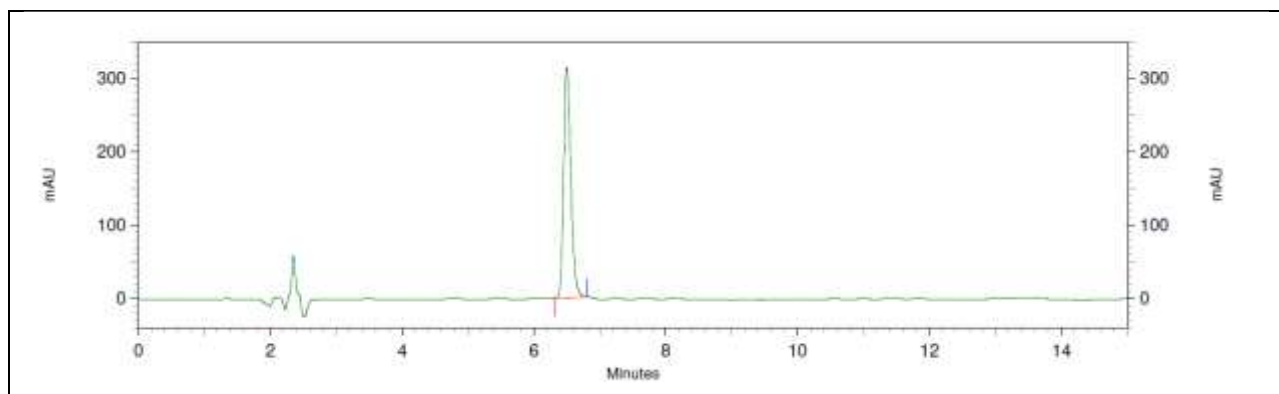
Chromatogram for robustness:

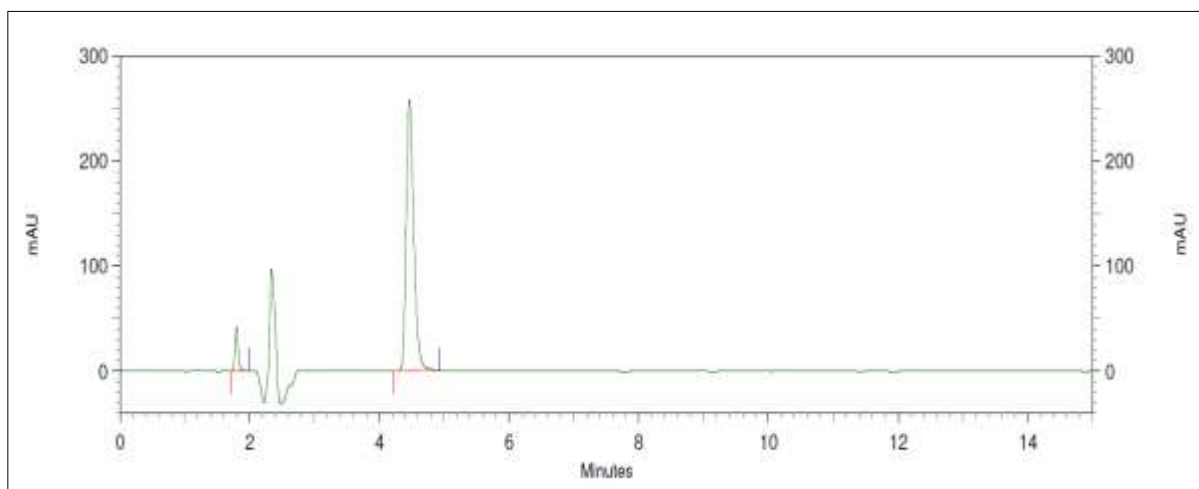
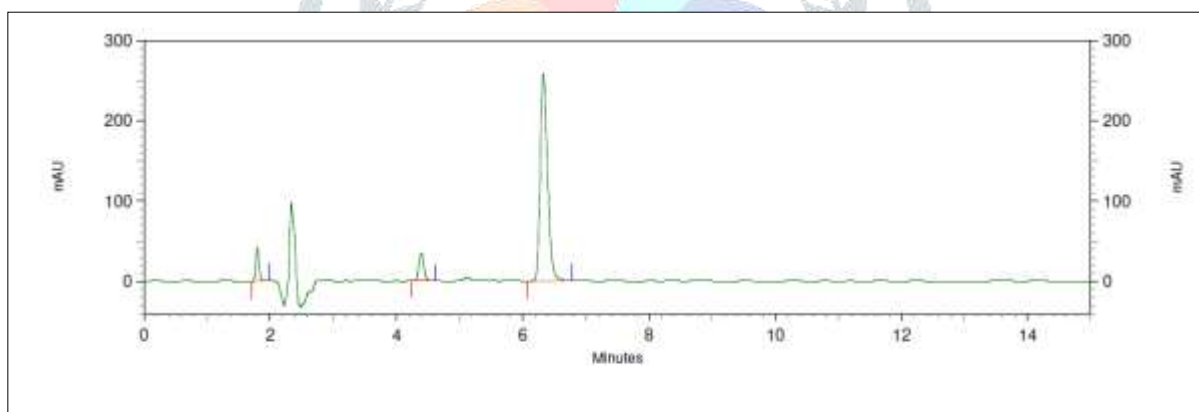
Fig.8.11 Chromatogram of blank for flow rate as such +10%

FORCED DEGRADATION STUDY:**1. PREPARATION OF DEGRADANT'S:**

- c) 5 N Hydrochloric acids:
42.5 mL of HCl diluted to 100 mL with water.
- d) 5 N NaOH solution:
20 gm of NaOH dissolved in 100 mL of water.
- e) 30% Hydrogen peroxide solution: Commercially ready made available.

Sample as such: Chromatogram:

Physical degradation:**1) Thermal degradation:Chromatogram:****Fig. 8.12 Typical Chromatogram of Thermal Degradation for forced degradation study****Observation:** No degradation found.**2) Photolytic degradation:Chromatogram:****Fig. 8.13 Typical Chromatogram of Photolytic Degradation for forced degradation study****Observation:** No degradation found.**3.Chemical degradation:****1) Acid degradation:Chromatogram:****Fig. 8.14 Typical Chromatogram of Acid Degradation for forced degradation study****Observation:** No degradation found.

2) Base/Alkali degradation:**Trial 1: Chromatogram:****Fig. 8.15 Typical Chromatogram of Base/ Alkali Degradation for forced degradation study Trial 1****Observation:** 100 % degradation found.**Trial 2: Chromatogram:****Fig. 8.16 Typical Chromatogram of Base/ Alkali Degradation for forced degradation study Trial 2****Observation:** 10.66 % degradation found.

3) Peroxide degradation: Chromatogram:

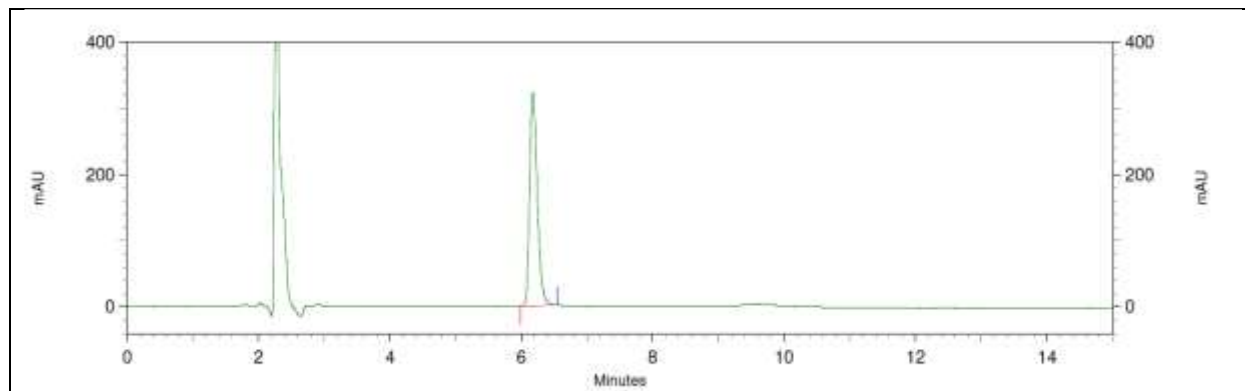


Fig. 8.17 Typical Chromatogram of Peroxide Degradation for forced degradation study

Observation: No degradation found. (Peak at 2.2 Min is of Hydrogen peroxide)

SUMMARY AND CONCLUSION:

- A literature survey revealed that several methods have been reported for the determination of vinpocetine in bulk drug or pharmaceutical dosage forms separately, but no one method was developed for estimation of vinpocetine by using RP-HPLC method. Hence, in the present study, a new, sensitive, and suitable reversed-phase high-performance liquid chromatography method was developed and validated for the determination of vinpocetine in bulk drug and pharmaceutical dosage form.
- In the developed RP-HPLC method, the analyte was resolved by using an isocratic program, and the mobile phase was used Acetonitrile: Buffer (90:10 % v/v) at a flow rate of 1.0 mL/min, on an HPLC system containing UV- visible detector with Openlab EZ-Chrome Software and Kromasil C18 column (250 mm X 4.6 mm i.d. 5 μ m). The detection was carried out at 228nm. The retention time for vinpocetine was found to be 6.4 min.
- A system suitability test includes Asymmetry, number of Theoretical Plates, Area, etc. The results of all system suitability parameters were acceptable in their limits defined by official guidelines for analytical method development and validation is working properly and can give accurate and precise results.
- The results of the analysis in the developed method were validated in terms of linearity, accuracy, precision, and robustness etc.
- The developed method has several advantages, including reproducibility of results, rapid analysis, simple sample preparation, and improved selectivity as well as sensitivity. The regression coefficient (r^2) for each analyte is not less than 0.999 which shows good linearity. The % recovery was in the acceptable range in the tablet dosage form. The % percent RSD was also less than 2.0 % showing a high degree of precision of the proposed method.

- Since the developed method is robust and reproducible and also less time consuming, it can be performed for routine analysis in the pharmaceutical industry for the bulk drug of vinpocetine and also in the pharmaceutical dosage form.

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