IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
March 2022 Vol.:23, Issue:4

Quantitative Determination of Vidarabine in Tablet Formulation by RP-HPLC

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Pavan Kumar Kasu*, B.Satynarayana

**Department of chemistry, Osmania University, Hyderabad-500007, Telangana, India*

Submitted: 23 February 2022
Accepted: 28 February 2022
Published: 30 March 2022

www.ijppr.humanjournals.com

Keywords: Vidarabine, RP-HPLC, Method Validation, optimized.

ABSTRACT

A simple, stability indicating and robust reverse phase RP-HPLC method has developed and validated for the simultaneous estimation of Vidarabine in bulk and pharmaceutical dosage form with forced degradation studies. Stationary phase used for the separation is STD Ascentis C18 (150mm×4.6mm 5µm), and the Mobile phase is 0.1per OPA and Acetonitrile in the ratio of 70:30 at a flow rate 1.0ml/min was maintained, maximum wave length at 250nm, Temperature was set to 30°C. The average retention time of Vidarabine were found to be 2.540 min respectively. By injecting the standard six times, the system suitability characteristics were evaluated, and the results were significantly below the acceptance requirement (Limit of less than 2). A linearity analysis was conducted between 25% and 150% levels, and the R2 value was found to be 0.999. Several validation criteria, including precision, accuracy, LOD, LOQ, and robustness, were determined to be within accepted limits. % recovery was obtained as 99.56% for Vidarabine respectively. The approach was discovered to be simple, accurate, sensitive, quick, and cost effective, with a runtime of less than 5 minutes. In practice, this approach may also be used to determine assay in tablet formulation.

INTRODUCTION:

Apparently, the medicine was meant to be used as an anti-cancer treatment [1]. Vidarabine is an adenosine analogue with antiviral activity in vitro against herpes viruses, poxviruses, rhabdoviruses, and certain RNA tumour viruses (see Fig.1). Most HSV and VZV strains are totally suppressed by vidarabine at 3 g/mL or less, and it also inhibits idoxuridine-resistant and acyclovir-resistant strains. Vidarabine is an antiviral treatment for herpes, poxviruses, rhabdoviruses, hepadnaviruses, and certain RNA tumour viruses.[3] Vira-A, a 3% ophthalmic ointment, is used to treat HSV-1 and HSV-2-induced acute keratoconjunctivitis and recurrent superficial keratitis.[2] Vidarabine (adenine arabinoside) is a purine nucleoside used to treat herpes simplex virus encephalitis and herpes zoster in immunocompromised patients. However, vidaribine's potential utility is restricted by its low solubility, which necessitates continual infusion of vast amounts of intravenous fluid. Vidarabine 5'-monophosphate is highly soluble and has the benefit of being able to be given intramuscularly or intravenously on a regular basis[4].It is an adenosine stereoisomer. It has a half-life of 60 minutes, is 0.05 percent soluble, and can cross the blood–brain barrier (BBB) when converted to its active metabolite [5].

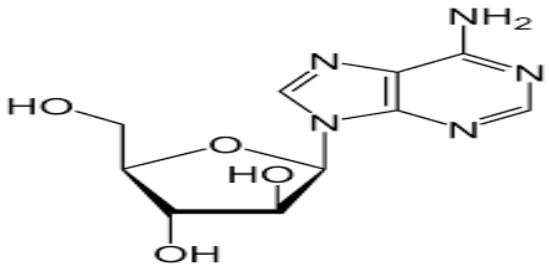


Figure-1: Structures of Vidarabine

One of the most effective separation analytical methods for determining drug estimation is high performance liquid chromatography. Some RP-HPLC [6 7 8 9] methods for estimating Vidarabine drugs and a few other anti retroviral drugs individually or in combination dosage forms have been described in the literature. More economical methods were discovered in the literature review, so a simple, cost-effective stability-indicating simultaneous estimation of Vidarabine by RP-HPLC in pharmaceutical dosage form must be developed and validated in accordance with ICH (Q2 specification) [10].

MATERIALS AND REAGENTS

Vidarabine pure drugs were obtained from Spectrum Pharma research solutions. The HPLC grade methanol and acetonitrile procured from Rankem chemical division, India. Sodium hydrogen phosphate procured from Rankem, India and Pure milli-Q water is used with the help of 0.45µ Millipore filters (Rankem, India).

Instrumentation and Chromatographic Conditions

WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector was used for the development and method validation, with an automated sample injector. Std Ascentis (150mm×4.6mm 5µm) column was used for the separation. 0.1per OPA used as mobile phase A and Acetonitrile is used as mobile phase B (70:30 Ratio). Analysis was carried out in isocratic mode with flow rate of 1.0 mL/min and injection volume was 10 µL. The column temperature was 30°C; the run time was 5 min. The data was acquired at detection wavelength 250nm and using the software Empower 2.

Preparation of Solutions

Diluent: Mixed Water and Acetonitrile in the ratio of 50:50v/v.

Preparation of buffer

Buffer (OPA 0.1 per): Accurately take 1ml of OPA solution in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Preparation of Standard solution: Accurately Weighed and transferred 30mg of Vidarabine into a 50 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents.(600µg/ml vidarabine). 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml. (60µg/ml Of Vidarabine)

Preparation of Sample stock solution: Accurately weighed equivalent weight of the combination powder sample transfer into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by 0.45µ milli-Q filters (600µg/ml vidarabine), 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (60µg/ml of Vidarabine)

Method Validation

The validation of HPLC method was carried out for the simultaneous estimation of Vidarabine drug substance as per the ICH guidelines to demonstrate that the method is proposed for the routine analysis.

System suitability: The system suitability was performed for every validation parameters by injecting of system suitability solution containing Vidarabine 60µg/ml. System suitability chromatogram was shown in figure 2 and values are mentioned in the table 1.

Specificity (Selectivity): Checking of the interference in the optimized method. We should not found interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific. Representative chromatogram is shown in Figure 3 and experimental data is given in Table 1.

Table 1: Specificity data

Sample name	**Retention time(mins)**
Vidarabine	2.538

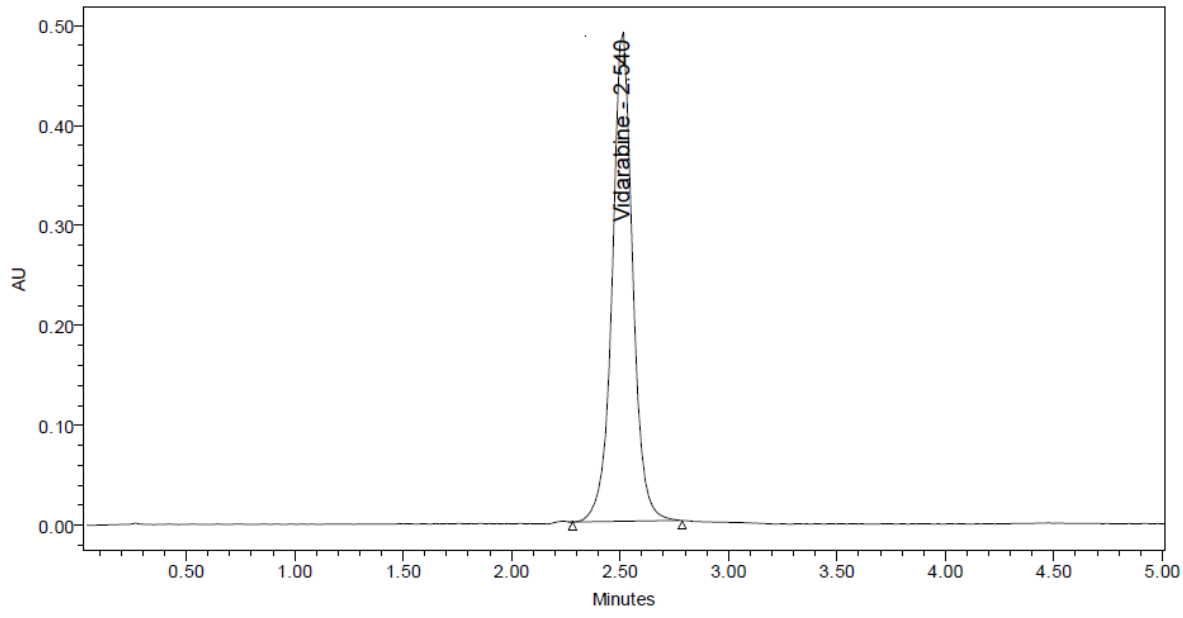


Figure 2: Typical representation of HPLC Chromatogram of Vidarabine

Table 2: system Suitability Parameter

S no	**Vidarabine**		
Inj	RT(min)	USP Plate Count	Tailing
1	2.513	4400	1.25
2	2.538	4470	1.26
3	2.541	4437	1.25
4	2.549	4449	1.25
5	2.551	4373	1.25
6	2.557	4469	1.24

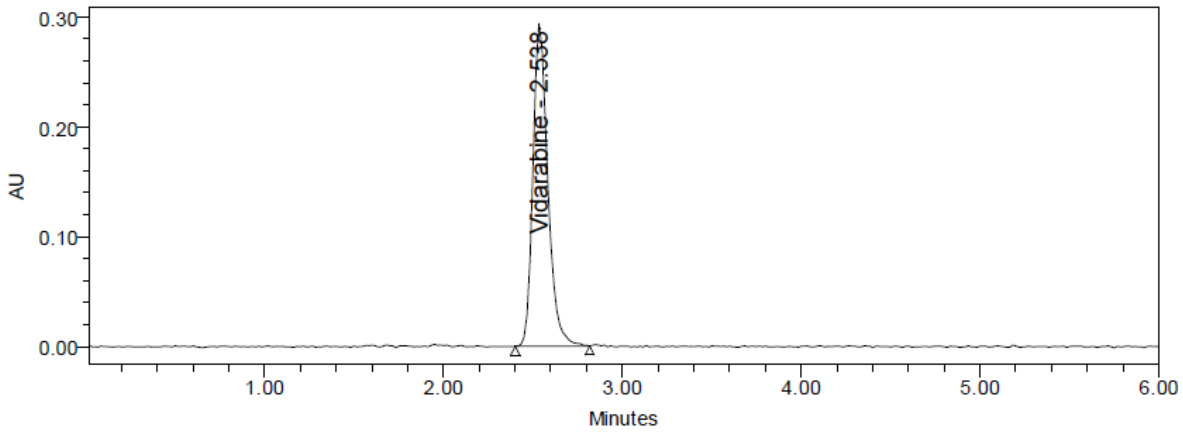


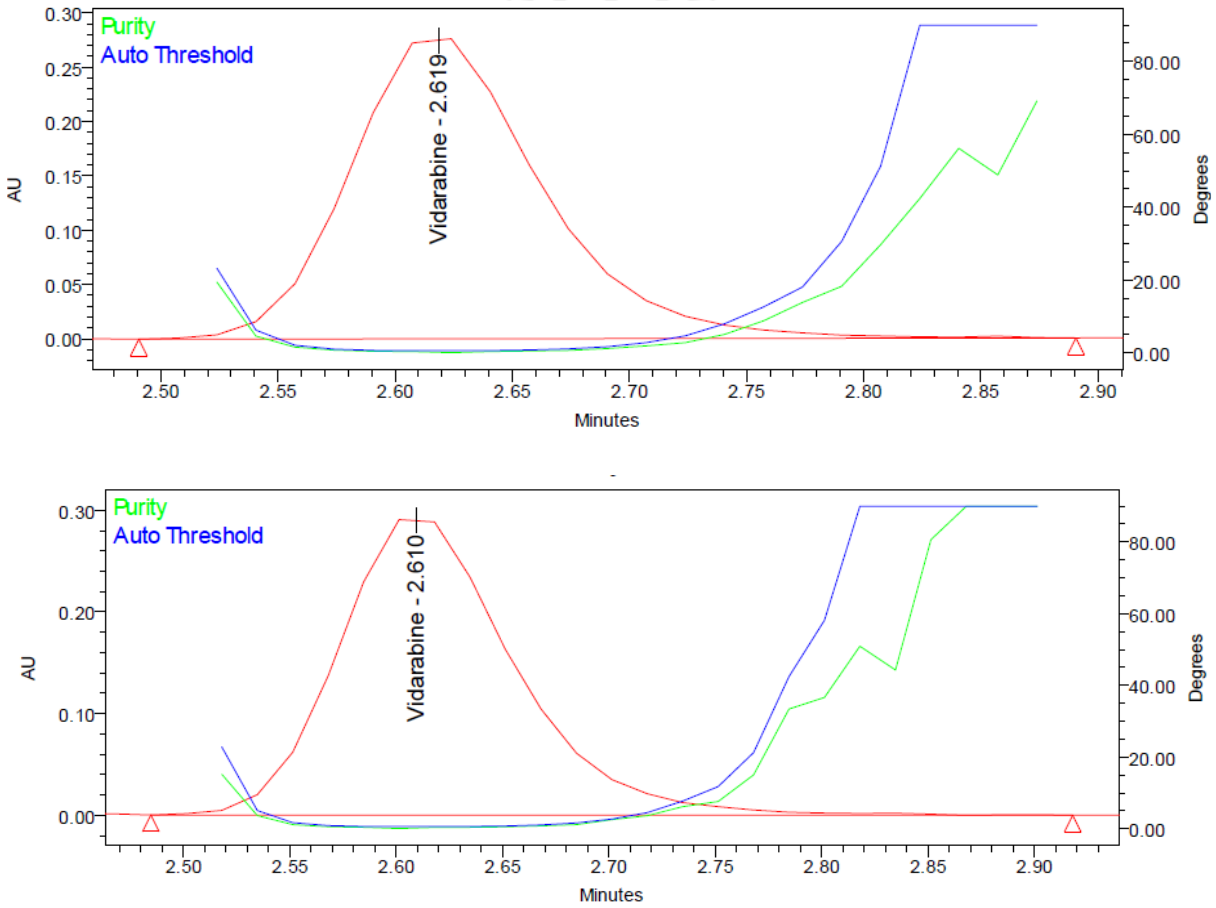
Figure 3: System suitability Chromatogram of Vidarabine

Vidarabine, samples were stressed by acid, base, oxidation, thermal, light, and water to assess the stability indicating nature of the HPLC method. A photodiode-array detector was used to analyse the degraded samples. Vidarabine was purified to its highest purity. Table 3 lists the forced degradation conditions, while Table 4 lists the results.

Table 3: Forced degradation conditions for Vidarabine.

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxdation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

From the results, no degradation was observed when the samples were exposed to acid, base, hydrolysis, thermal, light and water. According to the stress study, none of the degradant co-eluted with the active drug peaks formed. Here are the purity plots shown in figure 4.



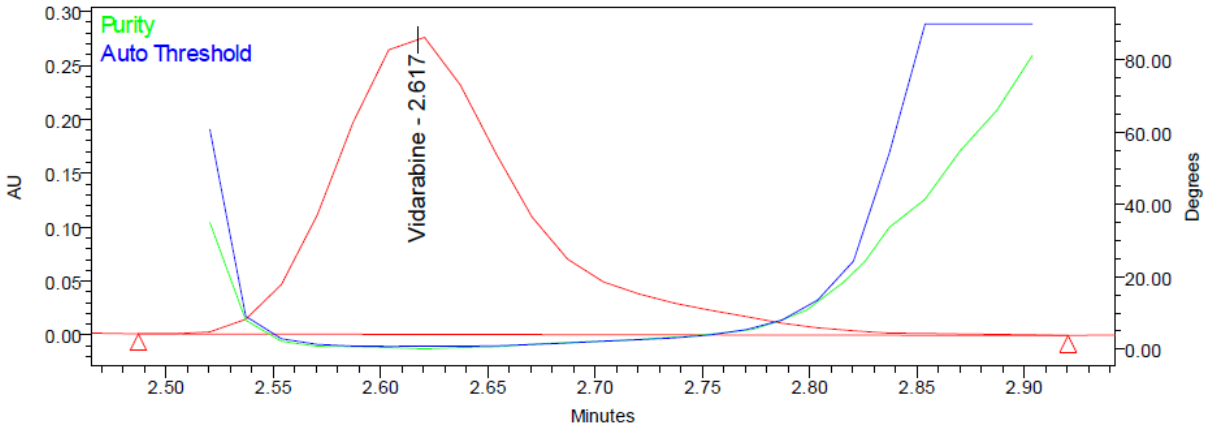


Figure 4: Typical representation of HPLC purity Chromatogram of forced degradation. Based on above results of each component was within the limit.

Table 4: Degradation profile results

Degradation condition	**Vidarabine % Undegraded**	**Vidarabine % Degraded**
Acid	96.63	3.37
Base	96.72	3.28
Oxidation	91.16	8.84
Thermal	97.62	2.38
Photolytic	97.66	2.34
Hydrolytic	99.28	0.72

Limit of detection (LOD) and Limit of quantitation (LOQ): The detection limit is defined as a very low level of analyte concentration in a sample that can be detected but not necessarily quantitated. The limit of quantitation is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and method accuracy. Table 5 shows the LOD values obtained for Vidarabine, and Figure 4 shows a representative chromatogram.

Table 5: Summary of limit of detection

Sample	Conc. (µg/ml)	Peak area	S/N Ratio
Vidarabine	0.10	33883	14.9

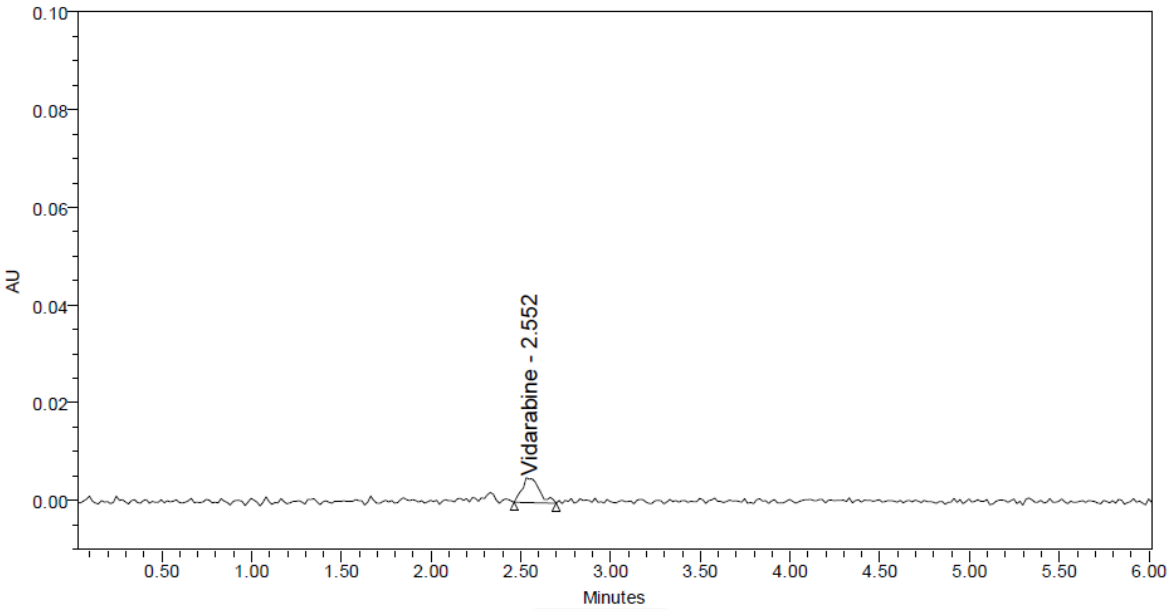


Figure 5: Typical representation of HPLC Chromatogram of LOD Solution. Based on above results for LOD, S/N ratio of each component was within the limit.

The LOQ values obtained for Vidarabine are listed in Table 6 and corresponding representative chromatogram is shown in Figure 4.

Table 6: Summary of limit of Quantification

Sample	Conc (µg/ml)	Peak area	S/N Ratio
Vidarabine	0.32	91523	25.2

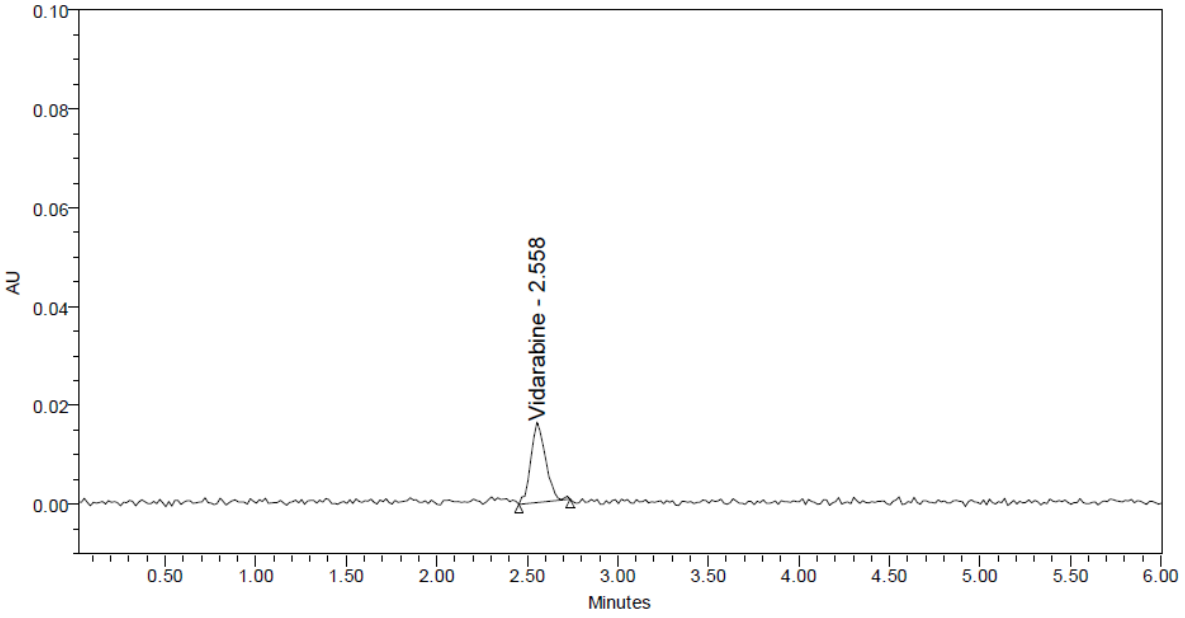


Figure 6: Typical representation of HPLC Chromatogram of LOQ Solution. Based on above results for LOD, S/N ratio of each component was within the limit.

Linearity: The method's linearity was demonstrated for Vidarabine by analyzing solutions ranging from 25% to 150% of the specification limit (Table 7). Vidarabine had a correlation coefficient of 0.999. This demonstrates good linearity (Figures 5-7).

Linearity Level (%)	Concentration (ppm)
0	0
12.5	584405
25	1167767
37.5	1749356
50	2313833
62.5	2858432
75	3468562

Table 7: Linearity data

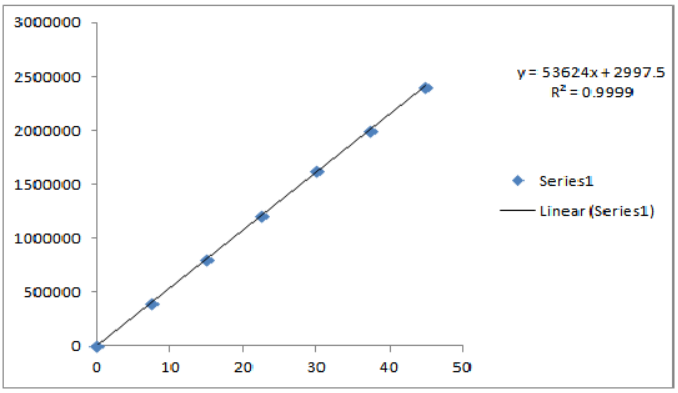


Fig 7: Linearity plot

Accuracy: The accuracy of the method was determined by using solutions containing spiked samples of Vidarabine at 50%, 100% and 150% of the working strength. All the solutions were prepared in triplicate and analyzed. The percentage recovery results obtained for each impurity was listed in Table 8.

Table 8: %Recovery data

%Level	**%Recovery**
50% Level	**Vidarabine**
	99.99
	99.05
	99.37
100% Level	99.11
	99.93
	99.72
150% Level	99.12
	100.41
	99.36
Mean%	99.56

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Vidarabine. Results of peak area are summarized in Table 9.

Table 9: System precision data

Injection	**Vidarabine**
1	1645195
2	1637236
3	1636731
4	1626242
5	1668773
6	1606265
Avg	1636740
Std dev	20672.6
%RSD	1.3

The % RSD for the peak areas of Vidarabine obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Vidarabine. (Six individual sample preparations). Data obtained is summarized in Table 10.

Table 10: Method precision data

Injection	**Vidarabine**
1	1633421
2	1624639
3	1625542
4	1631995
5	1626827
6	1633223
Avg	1629275
Std dev	4039.7
%RSD	0.2

From the above results, the % RSD of method precision study was within the limit for Vidarabine.

Robustness: The chromatographic conditions were deliberately changed to evaluate the robustness of the existing method. To determine the robustness of method, system suitability solution is prepared as per methodology and injected into HPLC at different altered conditions to check the method's ability like flow rate (± 10%), column oven temperature (± 5°C) and Mobile phase (± 10%) from actual method conditions. No significant change is observed by changing flow, temperature, Mobile phase and system suitability also complied as per methodology. The robustness results are summarized in Table 11.

Table 11: Robustness results

Chromatographic condition	**Vidarabine (RSD)**
Flow(-)	0.9
Flow(+)	0.8
Temp(-)	0.6
Temp(+)	0.4
Mobile phase(-)	0.3
Mobile phase (+)	0.7

From the robustness study, system suitability criteria comply with the results.

CONCLUSION

According to the results of the preceding tests, the newly proposed technique for simultaneous estimation of Vidarabine was discovered to be simple, precise, and accurate, with high resolution, a shorter retention period, and separated degradants. The proposed method is inexpensive and could be used for routine evaluations in the pharmaceutical industry.

ACKNOWLEDGMENTS:

I wish to express my warm regards and sincere thanks to Osmania university & Spectrum Pharma Research my work. I consider myself lucky to work under the guidance of Dr. B.Satynarayana Solution, Hyderabad for the enthusiasm and support laid by him during all stages of, I am highly indebted to spectrum labs for their valuable guidance**.**

REFERENCES

1. Sneader W et all.., Drug discovery: a history. New York: Wiley. p. 258. ISBN 0-471-89979-8.
2. Vidarabine.drug.com, HMDB0014340, 2022-03-07.
3. John E. Bennett MD, in Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 2020.
4. RICHARD J. WHITLEY ET all.., Pharmacology, Tolerance, and Antiviral Activity of Vidarabine Monophosphate in Humans, antimicrobial agents and chemotherapy, Nov, Vol. 18.
5. Waterson AP (1980). Recent advances in clinical virology (No. 2 ed.). Edinburgh: Churchill Livingstone. ISBN 978-0-443-02094-0
6. Mohan Goud and Srinivasa Rao Avanapu. Method development and validation of rp-hplc for assay of vidarabine in pharmaceutical dosage form 2014.

7. Jiang, li-sha; ma et al.., a new rp-hplc method for determining vidarabine. Chinese journal of pharmaceutical analysis 2001, 21(4); 247-248.
8. W H Hong, D H Szulczewski. Stability-indicating assay for vidarabine, J Pharm Sci 68(4):499-503.
9. L. M. L. Stolk, W. Huisman et al.., Formulation of a stable vidarabine infusion fluid, Pharmaceutisch Weekblad 1983, 5; 57–60.
10. Harmonised Tripartite Guideline Ich Validation Of Analytical Procedures: Text and Methodology Current Step 4 Version, November.