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## STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF VADADUSTAT BY USING HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORM.

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ARTICLE HISTORY	ABSTRACT
Received on: 18-12-2025 Revised on: 03-01-2026 Accepted on: 04-02-2026	<p>A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Vadadustat in bulk and pharmaceutical dosage form. Chromatographic separation of Vadadustat was achieved on Waters Alliance-e2695, by using Waters Luna Phenyl Hexyl (150x4.6mm, 3.5µm) column and the mobile phase containing Acetonitrile and 0.1% TFA in the ratio of 40:60% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 257nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Vadadustat were NLT 2000 and should not be more than 2, respectively. % Relative standard deviation of peak areas of all measurements is always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be a simple, economical, suitable, precise, accurate &amp; robust method for quantitative analysis of Vadadustat.</p> <p><b>Keywords:</b> Vadadustat, RP-HPLC, Method Development, Method Validation, Quantitative Analysis, Pharmaceutical Dosage Form.</p>
	

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### INTRODUCTION

Vadadustat is an orally administered, small-molecule hypoxia-inducible factor prolyl hydroxylase (HIF-PH) inhibitor indicated for the treatment of anemia associated with chronic kidney disease (CKD). It is a low-molecular-weight synthetic compound (molecular formula: C<sub>14</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>3</sub>; molecular weight: 290.70 g/mol) characterised by moderate lipophilicity and limited aqueous solubility, factors that influence its formulation design and absorption behavior[1]. Mechanistically, vadadustat acts by selectively and reversibly inhibiting HIF prolyl hydroxylase enzymes, preventing degradation of HIF-α under normoxic conditions. Stabilised HIF translocates to the nucleus and activates transcription of hypoxia-responsive genes, including erythropoietin (EPO), thereby enhancing endogenous EPO production, stimulating erythropoiesis, and improving iron homeostasis via regulation of iron-metabolism proteins [2,3]. Pharmacokinetically, vadadustat is absorbed following oral administration,

undergoes hepatic metabolism, and is eliminated through renal and faecal pathways. Therapeutically, it provides an oral alternative to injectable erythropoiesis-stimulating agents (ESAs), representing a novel strategy that leverages physiological hypoxia signalling to correct CKD-related anaemia [4]. Till today, there is no Literature review on the method development and validation of Vadadustat.

### MATERIALS AND METHODOLOGY

#### Reagents & Chemicals

Vadadustat purchased from Riverx Labs. Analytical reagent (AR) grade Acetonitrile, Tri Fluoro acetic acid, and ortho-phosphoric acid (Merck) were utilised for pH adjustment and buffer preparation.

#### Instrumentation

The chromatographic analysis was performed using a Waters Alliance HPLC system equipped with an isocratic pump. UV/VIS measurements were obtained with a Shimadzu UV-1700 spectrophotometer, and all volumetric glassware used was Class-A grade (Borosil).

#### Preparation of standard solution

Accurately weigh and transfer 15mg of Vadadustat working standard into a 10 ml volumetric flask, add Diluent and sonicate to dissolve it completely and make the volume up to the mark with the same solvent. (Stock solution) Further

pipette 1ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (150ppm of Vadadustat)

#### Sample Solution Preparation

Accurately weigh and transfer 21.7mg of Vadadustat sample into a 10mL volumetric flask, add Diluent, and sonicate it for up to 30 min to dissolve it completely and make the volume up to the mark with the same solvent. (Stock solution) .Further pipette 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Then it is filtered through a 0.45 micron Injection filter. (150ppm of Vadadustat)

#### Determination of Working Wavelength ( $\lambda_{max}$ )

In the estimation of drug maximum absorbance was used. So, this wavelength was used to accurately estimate the Vadadustat drug. The wavelength of maximum absorption of the solution of the drug in a mixture of Acetonitrile and 0.1% TFA (40:60) were scanned using a PDA Detector within the wavelength region of 200–400 nm against Acetonitrile and 0.1% TFA (40:60) as blank.

#### Method Validation

##### System Suitability

Six duplicate injections containing Vadadustat 150  $\mu\text{g}/\text{mL}$  were produced to ascertain System Suitability [5].

##### Specificity

To assess specificity, an analytical method must be capable of identifying a target analyte exclusively among controls and known impurities. To accomplish this, chromatograms were acquired for standards, blanks, and samples [6].

##### Linearity and LOD, LOQ

The developed method's linearity was evaluated by injecting 37.50-225  $\mu\text{g}/\text{mL}$  of Vadadustat. Triplets are produced and administered into the chromatographic system. The linearity graph plots the peak area against concentration, where concentration- X- axis and Peak area -Y-axis are represented in  $\mu\text{g}/\text{mL}$ .

The minimum number of analytes that were identifiable, nevertheless not quantified in a sample, is the lowest analyte number that an analytical technique can detect but not necessarily quantify. This is the point at which the analyte or compound's instrument response can be separated from the instrument noise. LOQ was a specification in analytical chemistry that specifies the lowest possible concentration of analytes which are measurable with adequate accuracy and precision [7-9].

##### Accuracy

This method's accuracy was evaluated using the % recovery method. Solutions at 80, 100 and 120 % concentration levels were prepared, with three replicates of each concentration [10,11].

##### Precision

Method precision indicates the precision observed over identical experimental conditions (same instrument in the same lab on the same day) over a short period. The study of precision under varied experimental conditions, like different equipment, labs, and days, was termed intermediate precision. Precision was expressed as % RSD and should be < 2 per ICH guidelines [12,13].

##### Robustness

To assess the robustness of a method, variations in specifications, for instance, such as the % of organic solvent, pH value, ionic strength, and temperature, are applied. The changing components that make up robustness research are usually parameters that the technique states. Variability in the resultant data is noted in the approach procedure so that the analyst may take steps to control these variables closely. This would ensure that the analysis yielded consistent findings each time [14,15].

##### Assay

The HPLC system was injected with standard and sample solutions containing Vadadustat. The % purity was computed using the peak area response values obtained [16].

##### Stability studies

Degradation studies of Vadadustat were performed to evaluate the stability-indicating capability of the developed RP-HPLC method under various stress conditions. The sample was subjected to acid, alkali, oxidative, reductive, hydrolytic, photolytic, and thermal degradation. For acid and alkali degradation, the drug sample was treated with 1 N HCl or 1 N NaOH and heated at 60°C for 1 hour, followed by neutralisation and dilution with diluent. Oxidative degradation was carried out using 10% w/v hydrogen peroxide, while reductive degradation employed 10% w/v sodium bisulphite, both maintained at 60°C for 1 hour. Hydrolytic degradation was performed using HPLC-grade water under similar thermal conditions. Photolytic degradation was evaluated by exposing the sample to UV light in a photostability chamber for 3 hours, and thermal degradation was conducted by placing the standard drug in a hot air oven at 105°C for 3 hours. After stress treatment, all samples were appropriately diluted, filtered through 0.45  $\mu\text{m}$  syringe filters, and analyzed by RP-HPLC. The method effectively separated Vadadustat from its degradation products. The acceptance criterion was satisfied, as the purity threshold was consistently greater than the purity angle, confirming the specificity and stability-indicating nature of the method [17,18].

## RESULTS AND DISCUSSION

### Determination of Working Wavelength ( $\lambda_{max}$ )

The wavelength of maximum absorption of the solution of the drug in a mixture of Acetonitrile and 0.1% TFA (40:60) was scanned using a PDA Detector within the wavelength region of 200–400 nm against Acetonitrile and 0.1% TFA (40:60) as blank. The absorption curve shows at 257nm. Thus, 257nm was selected as the detector wavelength for the HPLC chromatographic method (Figure 01).

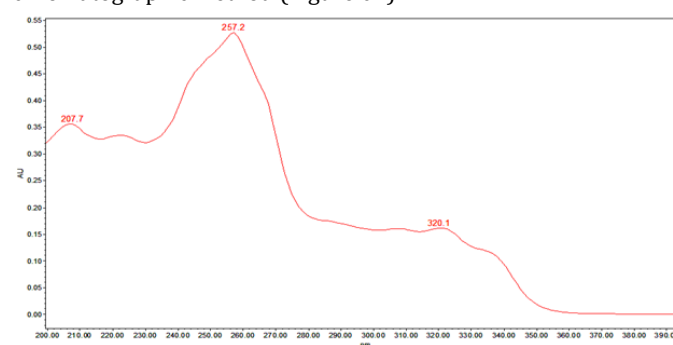


Figure 01: PDA Spectrum of Vadadustat

**Optimisation of Chromatographic Conditions**

A series of chromatographic trials were performed to establish optimal RP-HPLC conditions for the analysis of Vadadustat. Initial experiments using a Waters X-Terra RP-18 (150 × 4.6 mm, 3.5 μm) column with an Acetonitrile:0.1% OPA (80:20, v/v) mobile phase and PDA scanning (200–400 nm) resulted in a retention time outside acceptable limits. Modification of the mobile phase to Acetonitrile:0.1% OPA (70:30, v/v) with detection at 257 nm produced an unknown peak, indicating possible interference or inadequate selectivity. Further adjustment to Acetonitrile:0.1% TFA (10:90, v/v) did not yield satisfactory system suitability, as plate count and tailing factor were not within prescribed limits. Subsequently, a Luna Phenyl Hexyl (150 × 4.6 mm, 3.5 μm) column was evaluated. Trials with Acetonitrile:0.1% TFA (20:80 and 30:70, v/v) showed the presence of unknown peaks and an unstable baseline, respectively. Finally, optimisation with Acetonitrile:0.1% TFA (40:60, v/v) at a flow rate of 1.0 mL/min, detection wavelength of 257 nm, and injection volume of 10 μL provided a symmetrical peak with acceptable retention time, theoretical plates, tailing factor, and baseline stability. The optimised conditions were therefore selected as suitable for method validation (Figure 02 and Table 01).

**Table 01: Optimised chromatographic conditions**

Parameters	Observation
Instrument used	Waters Alliance e-2695 HPLC
Injection volume	10μl
Mobile Phase	Acetonitrile and 0.1% TFA (40+60)
Column	Luna Phenyl Hexyl (150x4.6 mm, 3.5μm)
Detection Wave Length	257nm
Flow Rate	1 mL/min
Runtime	6min
Temperature	Ambient(25° C)
Mode of separation	Isocratic mode

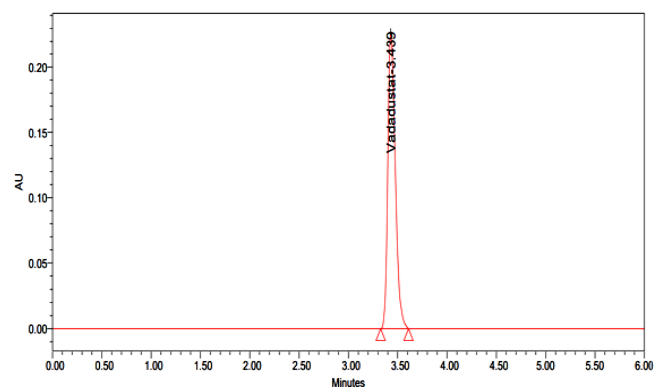


Figure 02: Optimised chromatogram of Vadadustat.

**Method Validation**

ICH criteria validated the optimised procedure, and the results are shown below.

**System suitability**

According to ICH guidelines plate count should be more than 2000, the tailing factor should be less than 2, and the resolution must be more than 2. All the system's suitable parameters were passed and were within the limits (Table 02).

**Table 02: System suitability parameters for Vadadustat**

Parameter	Vadadustat
Retention time	3.439
Plate count	7241
Tailing factor	1.01
%RSD	0.18

**Specificity**

Retention times of Vadadustat were 3.439 min. (Figure 4) We did not find any interfering peaks in blank and placebo at the retention times of these drugs in this method (Figure 3). So, this method was said to be specific.

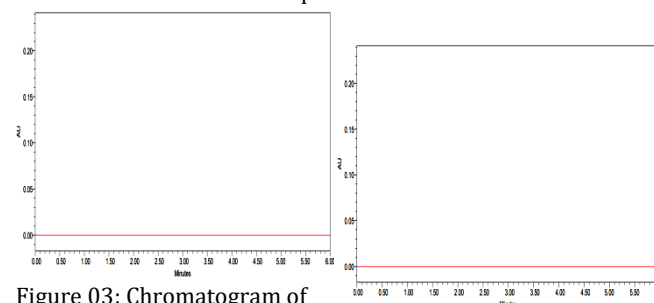


Figure 03: Chromatogram of blank and Placebo

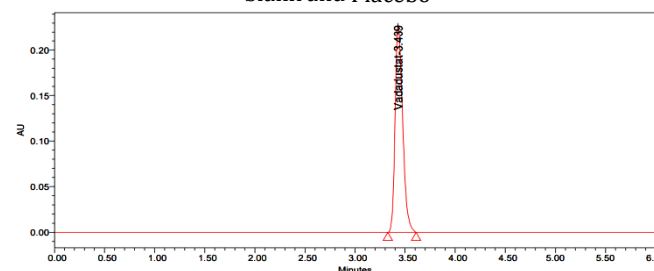


Figure 04: Optimized chromatogram of Vadadustat

**Linearity, LOD & LOQ**

The linearity of the RP-HPLC method for Vadadustat was evaluated across a concentration range of 37.50–225.00 μg/mL. A strong proportional relationship between concentration and peak area was observed, as presented in Table 3 and illustrated in Figure 5. The calibration curve demonstrated excellent linearity with the regression equation  $y = 15165.75x + 22027.18$  and a correlation coefficient ( $R^2$ ) of 0.99944, indicating a high degree of fit.

The LOD was found to be 0.45 μg/mL with a signal-to-noise ratio of 3, while the LOQ was 1.50 μg/mL with a signal-to-noise ratio of 10.

Table 03: Results of linearity for Vadadustat

S.NO	Vadadustat	
	Conc.(μg/ml)	Peak area
1	37.50	563208

2	75.00	1171524
3	112.50	1765323
4	150.00	2336521
5	187.50	2896470
6	225.00	3364172
<b>Regression equation</b>	$y = 15165.75x + 22027.18$	
<b>Slope</b>	15165.75	
<b>Intercept</b>	22027.18	
<b>R<sup>2</sup></b>	0.99944	

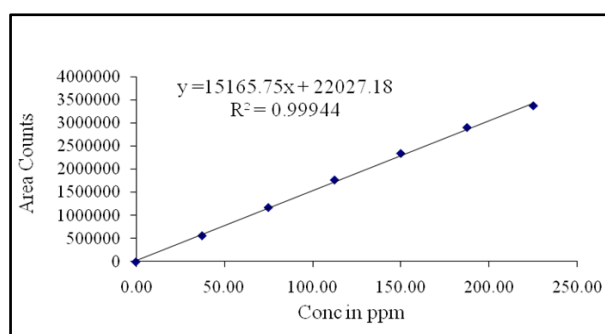


Figure 05: Calibration curve for Vadadustat at 257 nm

**Accuracy**

The accuracy of the developed RP-HPLC method was assessed by recovery studies at 80%, 100%, and 120% of the specification level. The percentage recovery values ranged from 99.1% to 100.3%, as shown in Table 4, demonstrating excellent trueness of the method. The mean % recovery at each level (99.8%, 99.6%, and 100.1%) confirms that the method is accurate and free from interference by excipients.

Table 04: Accuracy results of Vadadustat by the HPLC method

Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean % Recovery
80%	4212521	27	26.91	99.7	99.8
	4216389	27	26.93	99.7	
	4220557	27	26.96	99.9	
100%	4698273	30	30.01	100.0	99.6
	4676895	30	29.87	99.6	
	4653710	30	29.72	99.1	
120%	5160231	33	32.96	99.9	100.1
	5171456	33	33.03	100.1	

	5180993	33	33.09	100.3	
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**Precision**

System precision of the RP-HPLC method was evaluated by six replicate injections of Vadadustat at 150 µg/mL. The %RSD of peak areas was found to be 0.18%, indicating excellent instrument repeatability. Method precision results, summarized in Table 5, showed a %RSD of 0.87%, confirming good reproducibility of the analytical procedure. Both values were well within the acceptable limit of NMT 2.0%, demonstrating that the method is precise and reliable.

Table 05: System and Method Precision of Vadadustat

		System	Method
S. No	Vadadustat Concentration (µg/ml)	Area of Vadadustat	Area of Vadadustat
1.	150	2351427	2346152
2.	150	2346895	2318695
3.	150	2349056	2327406
4.	150	2354772	2340189
5.	150	2345321	2373648
6.	150	2343602	2360584
Mean		2348512	2344446
S. D		4120.340	20433.795
%RSD		0.18	0.87

**Assay of Vadadustat**

The assay of Vadadustat in the Vafseo formulation showed consistent chromatographic responses with minimal variation between replicate injections. The calculated amount found was 14.94 µg/mL, corresponding to a % assay of 99.6%. This value falls within acceptable pharmacopeial limits (typically 98–102%), confirming the accuracy and reliability of the developed RP-HPLC method.

**Robustness**

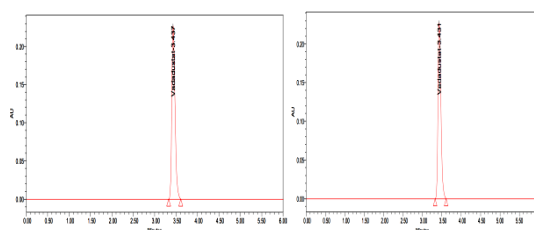
Robustness of the developed RP-HPLC method was evaluated by deliberately varying critical chromatographic parameters, including flow rate (±0.1 mL/min) and organic phase composition (±4%). The results indicated that minor changes in flow rate (0.9–1.1 mL/min) produced slight shifts in retention time without affecting peak symmetry, plate count, or precision. Similarly, variations in mobile phase composition (36:64 and 44:56) did not significantly influence chromatographic performance. In all cases, the tailing factor remained within acceptable limits, theoretical plates were satisfactory, and %RSD values were below 2.0%. These findings confirm that the method is robust and reliable under small, intentional variations in analytical conditions.

**Forced degradation studies**

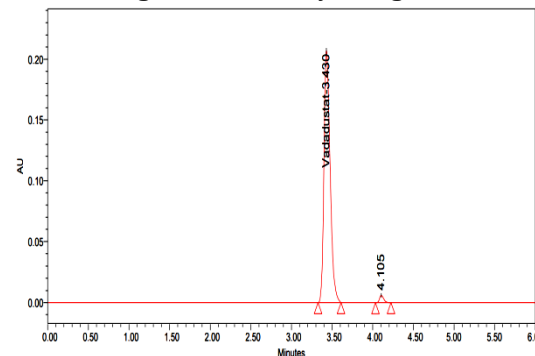
Forced degradation studies were performed to demonstrate the stability-indicating nature of the RP-HPLC method for Vadadustat. The drug exhibited notable degradation under acid (12.3%), alkali (10.8%), reduction (13.4%), and hydrolytic (11.1%) conditions, while relatively lower degradation was observed under peroxide (1.5%), thermal (4.2%), and photolytic (2.9%) stress. Peak purity analysis confirmed specificity, as the purity angle was consistently lower than the purity threshold in all stress conditions (Table 6 & Figure 6). These findings indicate that the method effectively separates Vadadustat from its degradation products and is suitable for stability studies.

**Table 06: Forced Degradation results for Vadadustat**

Degradation Condition	Vadadustat				
	Area	% Assay	% Degradation	Purity Angle	Purity Threshold
Control	2349156	100	0	3.526	9.211
Acid	2061467	87.7	12.3	3.548	9.245
Alkali	2095441	89.2	10.8	3.569	9.268
Peroxide	2314563	98.5	1.5	3.512	9.234
Reduction	2034358	86.6	13.4	3.508	9.209
Thermal	2251364	95.8	4.2	3.566	9.229
Photolytic	2282045	97.1	2.9	3.597	9.247
Hydrolysis	2088610	88.9	11.1	3.531	9.213



**Thermal degradation Photolytic degradation**



**Hydrolysis degradation**

**Figure 08: Chromatograms under different stress conditions**

**CONCLUSION**

A simple, rapid, precise, sensitive, and reproducible RP-HPLC method was successfully developed and validated for the quantitative estimation of Vadadustat in bulk and pharmaceutical dosage forms. The optimized chromatographic conditions provided good peak symmetry, acceptable retention time, and satisfactory system suitability parameters. The method exhibited excellent linearity, accuracy, precision, robustness, and sensitivity in accordance with ICH Q2(R1) guidelines. Forced degradation studies confirmed the stability-indicating capability of the method, as Vadadustat was effectively separated from its degradation products with peak purity maintained under all stress conditions. The assay results demonstrated that the marketed formulation complies with the labeled claim. Overall, the developed method is reliable, economical, and suitable for routine quality control and stability analysis of Vadadustat.

**AUTHOR CONTRIBUTIONS**

Venkata Lakshmanan Sagar Dantinapalli: Conceptualisation, methodology, investigation, data curation, writing – original draft. Shaileshkumar Bhingaradiya: Formal analysis, validation, visualisation, writing – review & editing. Mylsamy Palanisamy and Selvaraja Elumalai: Supervision, resources, project administration, writing – review & editing.

**FUNDING STATEMENT**

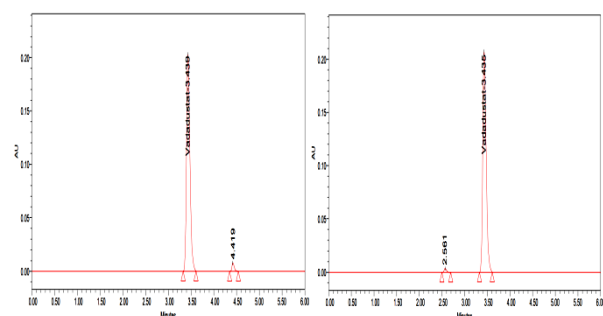
Nil

**CONFLICT OF INTEREST**

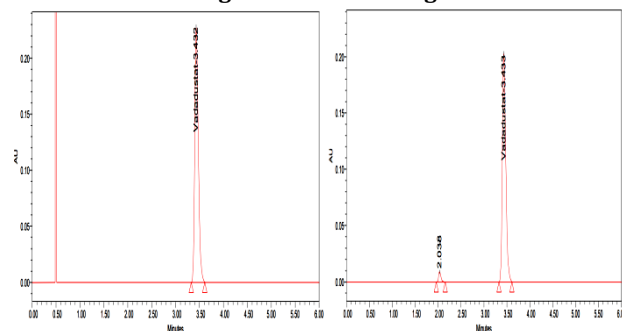
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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**Acid degradation Alkali degradation**



**Peroxide degradation Reduction degradation**

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