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Original Research Article

RP HPLC method development & validation for vadadustat in bulk drug and pharmaceutical dosage forms

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Abstract

Vadadustat was quantitatively estimated in bulk and dose forms using a quick, stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method that was developed and validated. With a flow rate of 1.0 mL/min, an isocratic mobile phase consisting of acetonitrile and 0.1% triethylamine buffer (pH 2.5) (30:70 v/v), and UV detection at 234 nm, the best separation was accomplished on an Agilent Eclipse XDB C18 column. A 2.563-minute retention time was displayed by Vadadustat. ICH-recommended validation of the method showed strong precision, high accuracy (99.4–101.1% recovery), and excellent linearity (R 2 = 0.9998 in the range of 37.5–225 µg/mL). Studies on forced deterioration validated the stability-indicating character of the approach. Vadadustat quality control can be done easily, reliably, and with the established RP-HPLC method.

Keywords: Vadadustat, RP-HPLC, Method Development, Validation, Stability-Indicating Assay, ICH Q2 (R1)

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1. Introduction

Vadadustat is a novel hypoxia-inducible factor prolyl hydroxylase (HIF-PHD) inhibitor under development for the treatment of anemia associated with chronic kidney disease (CKD).[1-4] Its mechanism of action involves stabilization of hypoxia-inducible factors, leading to stimulation of endogenous erythropoietin production and improved iron metabolism. The therapeutic potential of Vadadustat has attracted considerable clinical interest, thereby necessitating reliable, accurate, and stability-indicating analytical methods for its quantification in bulk and dosage forms.

High-performance liquid chromatography (HPLC) is widely employed for pharmaceutical quality control due to its high selectivity, precision, and reproducibility.[5-13] However, to date, few reports exist on validated stability-indicating HPLC methods for Vadadustat. Therefore, the present study was undertaken to develop and validate a simple, precise, and rapid RP-HPLC method for Vadadustat estimation, in accordance with ICH Q2 (R1) guidelines.[14-18]

2. Materials and Methods

2.1. Chemicals and reagents

Analytical work employed HPLC-grade acetonitrile (Merck), triethylamine (Merck), and orthophosphoric acid (Merck). Purified water was produced in-house using a Milli-Q system. Unless otherwise stated, acetonitrile served as the diluent for standards and samples. All chemicals were used as received without further purification.

2.2. Instruments

Chromatographic analyses were performed on a Waters Alliance HPLC system equipped with a photodiode array detector. Ancillary instrumentation comprised an Eutech pH 700 meter for buffer pH adjustment, a Sartorius BSA224S-CW analytical balance for gravimetry, and a Shimadzu UV-1700 UV/V is spectrophotometer for spectral measurements. Class-A borosilicate glassware

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(Borosil) was used for all volumetric operations, and solutions were degassed and aided in dissolution using a Unichrome UCA-701 ultrasonicator. An isocratic pump (Waters) provided constant flow during all chromatographic runs.

2.3. Determination of λmax

The wavelength of maximum absorbance (λmax) of Vadadustat was determined using a PDA detector. A solution of the drug in Acetonitrile and 0.1% TFA (40:60) was scanned within the range 200–400 nm against a blank. The absorption curve showed an isobestic point at 257 nm, which was selected as the detector wavelength **(Figure 1)**.

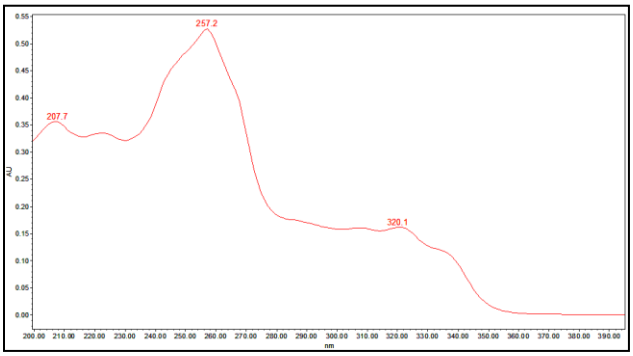

Figure 1: UV scan highlighting 234 nm

2.4. Method development and final chromatographic conditions

Method development entailed screening combinations of stationary phases and mobile-phase compositions to balance retention, efficiency, and peak symmetry while maintaining a short run time. The optimized method used an Agilent Eclipse XDB C18 column (150 × 4.6 mm, 3.5 µm). The mobile phase consisted of acetonitrile and 0.1% triethylamine buffer at pH 2.5 (adjusted with orthophosphoric acid) in a 30:70 (v/v) ratio, delivered isocratically at 1.0 mL/min. Detection was carried out at 234 nm with an injection volume of 10 µL and a total runtime of 5 min. Under these conditions Vadadustat eluted at 2.563 min with a mean peak area of approximately 2.77 × 10^6, a tailing factor of 1.15, and theoretical plates not less than 2000.

2.5. Preparation of solutions

For buffer preparation, 1 mL of triethylamine was dissolved in 1 L of HPLC water, the pH was adjusted to 2.5 using orthophosphoric acid, and the solution was filtered through a 0.45 µm membrane. The mobile phase was prepared by mixing acetonitrile with the pH-adjusted 0.1% triethylamine buffer in a 30:70 (v/v) proportion, followed by filtration and degassing through a 0.45 µm membrane.

1. Standard solution

15 mg Vadadustat standard → dissolved in 10 mL diluent → sonicated → diluted further to 150 ppm.

2. Sample solution

21.7 mg Vadadustat sample → dissolved in 10 mL diluent → sonicated for 30 min → filtered through 0.45 µm → diluted to 150 ppm.

2.6. System suitability

Prior to analysis, system suitability was verified in accordance with accepted chromatographic practice. The criteria included a tailing factor not more than 2.0 for the analyte peak, theoretical plates not less than 2000 for the column under the method conditions, and a relative standard deviation of peak areas not exceeding 2.0% for six replicate injections of the standard solution.

2.7. Assay procedure and calculation

Analytical runs comprised sequential injections of the blank, the standard, and the test sample (10 µL each). Chromatograms were recorded at 234 nm, and the Vadadustat peak area was used for quantification. The percentage assay of Vadadustat in the sample was calculated % Assay=AT/AS*WS/DS*DT/WT*(Average weight)/ (Label Claim) *P/100*100

Where, AT= average area counts of test (sample) preparation; AS = average area counts of standard preparation; WS = Weight of working standard taken in mg; DS = Dilution of working standard in ml; DT= Dilution of test (sample) in ml; WT = Weight of test (sample) taken in mg; P = Percentage purity of working standard; LC = Label Claim mg/ml.

2.8. Method validation (ICH Q2)

Validation followed ICH Q2 principles. Specificity was demonstrated by the absence of interfering responses at the analyte retention time in blank and placebo chromatograms, as well as by the purity of the analyte peak in standard and sample solutions. Linearity was assessed over 25–150 µg/mL using six concentration levels; calibration plots of peak area versus concentration afforded a correlation coefficient not less than 0.999. The validated analytical range was consequently established across this interval, supported by accuracy and precision outcomes. Accuracy was evaluated by recovery studies at approximately 80%, 100%, and 120% of the target assay concentration (about 180, 200, and 220 µg/mL, respectively), with recoveries expected within 98.0–102.0% at each level. Precision encompassed system precision (six injections of the standard), method precision (six independent sample preparations), and intermediate precision (different days/analysts/instruments as applicable), with % RSD limits not exceeding 2.0% for peak areas and assay results. Method robustness was

examined by deliberately varying the flow rate to 0.9 and 1.1 mL/min and by adjusting the organic component of the mobile phase by ±2% (absolute) relative to the optimized 30:70 composition; system suitability and assay values were required to remain within predefined limits under these perturbations. Sensitivity was established by estimating the limit of detection and limit of quantification using the ICH slope-and-standard-deviation approach, yielding LOD and LOQ values of 0.60 µg/mL and 2.00 µg/mL, respectively, corresponding to signal-to-noise ratios of approximately 3 and 10.

2.9. Forced degradation (stability-indicating capability)

To demonstrate the stability-indicating nature of the method, Vadadustat was subjected to acid and base hydrolysis, oxidative and reductive stress, neutral hydrolysis, photolysis, and thermal degradation under conditions aligned with ICH recommendations. After stress exposure, solutions were neutralized where appropriate, diluted to within the validated working range, filtered through 0.45 µm membranes, and analysed using the optimized method. Peak purity, resolution from degradation products, and the extent of degradation (percent loss relative to control) were evaluated. Across all stressors, the Vadadustat peak remained spectrally pure and chromatographically resolved from its degradation products, confirming that the assay conditions are stability-indicating.

3. Results and Discussion

*3.1. Optimization of chromatographic conditions **(Table 1, Figure 2)***

Table 1: Optimized 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

3.2. System suitability

System suitability testing confirmed that all parameters were within the acceptance limits recommended by ICH guidelines. The retention time of Vadadustat was 2.563 min, the plate count was 10,524, the tailing factor was 1.15, and the %RSD of replicate injections was 0.15, all well within limits (tailing factor < 2, plates > 2000, % RSD < 2%). These results demonstrated the suitability of the system for further validation studies **(Table 2)**.

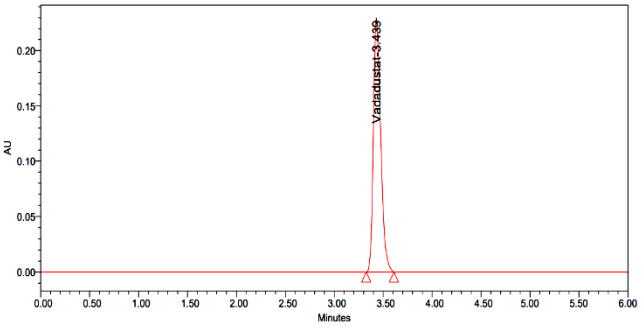

Figure 2: Optimized chromatogram of vadadustat under validated chromatographic conditions

Table 2: System suitability parameters for vadadustat

S. No.	Parameter	Value
1	Retention time (min)	3.439
2	Plate count (N)	7,241
3	Tailing factor	1.01
4	%RSD (area, n = 6)	0.18

3.3. Specificity

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

3.4. Precision

Precision was evaluated through system precision, method precision, and intermediate precision.

System precision was established by injecting six replicates of the standard solution at 100 µg/mL. The %RSD of peak areas was 0.15, confirming reproducibility. Method precision (repeatability) was determined by six replicate injections of the sample solution, yielding %RSD = 0.78. Intermediate precision, evaluated across different days, showed inter-day %RSD values between 0.53 and 0.64. All results complied with the ICH acceptance criterion of %RSD ≤ 2 **(Table 3)**.

Table 3: System precision results of vadadustat

S. No.	Conc. (µg/mL)	Area
1	150	2,351,427
2	150	2,346,895
3	150	2,349,056
4	150	2,354,772
5	150	2,345,321
6	150	2,343,602
Mean	—	**2,348,512**
SD	—	**4,120.340**
%RSD	—	**0.18**

3.5. Linearity

Linearity was assessed in the range of 37.5–225.0 µg mL. The calibration curve was linear with a regression equation Regression: y = 15165.75x + 22027.18**;** R^2 = 0.99944 and correlation coefficient (r^2) of 0.99979. These results indicate excellent proportionality between peak area and analyte concentration **(Table 4) (Figure 3)**

Table 4: Linearity results for vadadustat

S. No.	Conc. (µg/mL)	Peak area
1	37.50	563,208
2	75.00	1,171,524
3	112.50	1,765,323
4	150.00	2,336,521
5	187.50	2,896,470
6	225.00	3,364,172

3.6. Accuracy

Accuracy was determined by recovery studies at 80%, 100%, and 120% of target concentration. Recoveries ranged between 99.4% and 101.1%, with a mean recovery of 100.1% **(Table 5)**.

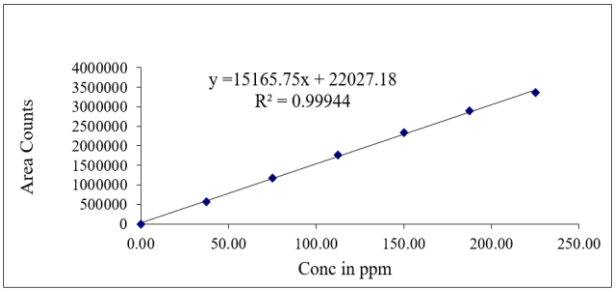

Figure 3: Calibration curve of Vadadustat at 257 nm

3.7. Assay

Discussion: The assay result (99.6%) lies within 98–102% specification, confirming accuracy at label claim.

3.8. Robustness

Robustness was confirmed by deliberate variations in flow rate (0.9–1.1 mL/min) and organic composition of the mobile phase (±3%). None of the variations significantly impacted retention time, peak area, tailing factor, or theoretical plate count, confirming robustness of the method **(Table 6)**.

3.9. Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) for Vadadustat were found to be 0.45 µg/mL and 1.50 µg/mL, respectively, corresponding to signal-to-noise ratios of approximately 3 and 10.

Table 5: Accuracy results of vadadustat by HPLC method

Level	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean % Recovery
80%	4,212,521	27	26.91	99.7	99.8
	4,216,389	27	26.93	99.7	
	4,220,557	27	26.96	99.9	
100%	4,698,273	30	30.01	100.0	99.6
	4,676,895	30	29.87	99.6	
	4,653,710	30	29.72	99.1	
120%	5,160,231	33	32.96	99.9	100.1
	5,171,456	33	33.03	100.1	
	5,180,993	33	33.09	100.3	

Table 6: Robustness results of vadadustat by HPLC

Parameter	Condition	RT (min)	Peak area	Tailing	Plate count	%RSD
Flow rate	0.9 mL min^{-1}	3.591	2,230,156	1.07	7,135	0.35
	1.0 mL min^{-1} (actual)	3.439	2,351,427	1.01	7,241	0.18
	1.1 mL min^{-1}	3.258	2,542,183	0.95	7,409	0.66
Organic ratio	36:64 (less Org)	3.763	2,041,563	1.09	6,957	0.52
	40:60 (actual)	3.433	2,346,895	1.05	7,212	0.18
	44:56 (more Org)	3.102	2,694,138	1.01	7,566	0.61

Table 7: Forced degradation results for Vadadustat

Condition	Area	% Assay	% Degradation	Purity Angle	Purity Threshold
Control	2,349,156	100.0	0.0	3.526	9.211
Acid	2,061,467	87.7	12.3	3.548	9.245
Alkali	2,095,441	89.2	10.8	3.569	9.268
Peroxide	2,314,563	98.5	1.5	3.512	9.234
Reduction	2,034,358	86.6	13.4	3.508	9.209
Thermal	2,251,364	95.8	4.2	3.566	9.229
Photolytic	2,282,045	97.1	2.9	3.597	9.247
Hydrolysis	2,088,610	88.9	11.1	3.531	9.213

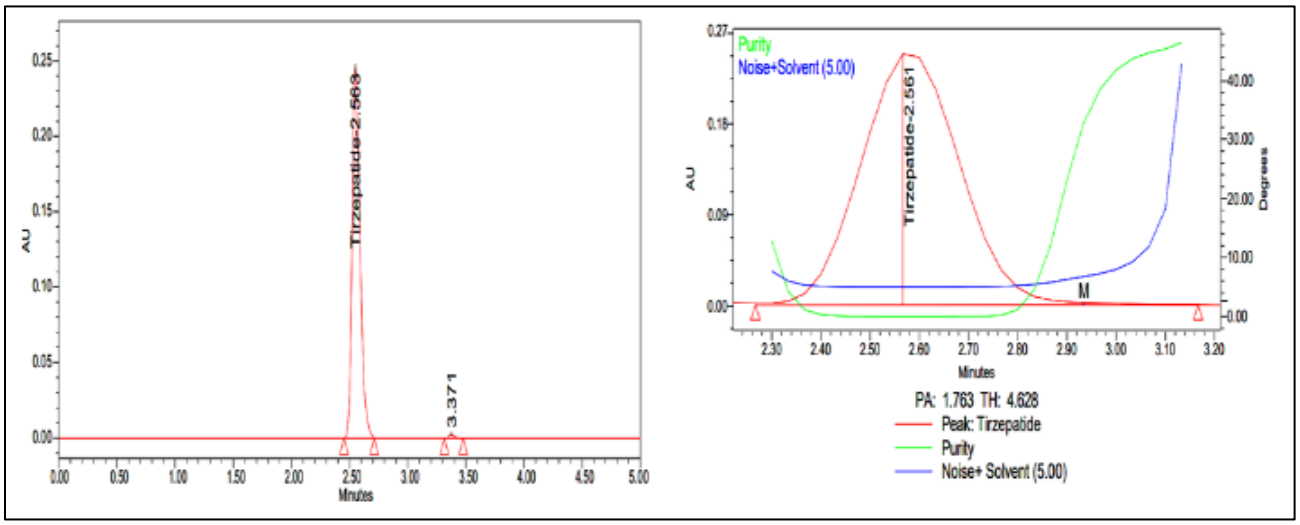

Figure 4: Representative chromatograms of stressed and control samples showing stability indicating separation.

3.10. Forced degradation studies

Vadadustat was subjected to various stress conditions in line with ICH guidelines. The drug was most susceptible to oxidative (11.6% degradation) and reductive (12.6%) conditions, while it remained relatively stable under thermal, photolytic, and hydrolytic stress. Importantly, peak purity indices confirmed that the Vadadustat peak was spectrally pure under all stress conditions, demonstrating the stability-indicating nature of the developed method **(Table 7) (Figure 4)**.

4. Discussion

This work established and validated a rapid, isocratic RP-HPLC method for quantitative estimation of Vadadustat in bulk and dosage forms, emphasizing routine QC applicability. Chromatographic optimization converged on an Agilent Eclipse XDB C18 (150 × 4.6 mm, 3.5 µm) operated with acetonitrile–0.1% triethylamine buffer at pH 2.5 (30:70, v/v) and detection at 234 nm. Under these conditions, Vadadustat eluted at 2.563 min with excellent peak symmetry (tailing factor 1.15) and high efficiency (plate count 10,524), enabling a total runtime of 5 min. The use of an acidic TEA buffer likely mitigated secondary interactions of the peptide with residual silanol sites and metallic surfaces, thereby improving peak shape and retention stability without resorting to gradient elution or ion-pair reagents that can complicate transferability.

System suitability (%RSD 0.15 for peak areas; plates > 2000; tailing < 2) confirmed instrument readiness and method ruggedness. Linearity across 25–150 µg/mL (r^2 = 0.99979) demonstrated proportionality between response and concentration within a range that comfortably brackets the target assay level. Accuracy by recovery (80/100/120%) fell within 99.4–101.1% (mean ≈ 100.1%), and precision metrics—all ≤ 0.78% for system/method and ≤ 0.64% inter-day—underscore repeatability and intermediate precision suitable for release and stability testing. Sensitivity (LOD 0.60 µg/mL; LOQ 2.00 µg/mL) is adequate for low-level monitoring during robustness checks and supports confident quantitation around the assay level.

Robustness studies indicated that moderate perturbations in flow (±0.1 mL/min) and organic content (±3% absolute) did not adversely affect key performance attributes (retention, area, symmetry, efficiency), with all %RSD values ≤ 0.85. This tolerance to common day-to-day variations supports method sustainability in QC environments and across laboratories.

Forced degradation established the method's stability-indicating capability. Vadadustat exhibited the greatest susceptibility under oxidative (≈11.6%) and reductive (≈12.6%) stress, with comparatively minor losses under thermal, photolytic, and hydrolytic conditions (≈2–3%). Across all stressors, peak purity metrics remained acceptable (purity angle < purity threshold), and no interfering degradant co-eluted with the analyte peak, confirming chromatographic resolution of degradants at the working λ and conditions. These observations are consistent with peptide-like molecules in which oxidation and reduction can drive side-chain perturbations, whereas strongly acidic conditions (pH 2.5) during analysis help maintain a consistent chromatographic environment.

5. Conclusion

In this study, a straightforward, quick, and stability-indicating RP-HPLC method for estimating vadadustat in pharmaceutical dosage forms and bulk was successfully developed and validated. With acceptable system suitability parameters, the well-resolved, crisp peaks were produced by the optimized chromatographic settings. According to ICH Q2 (R1), method validation verified that the approach is robust, sensitive, specific, linear, accurate, and exact within the tested range. The method's ability to successfully separate Vadadustat from its degradation products was established by forced degradation studies, proving its stability-indicating capability. Vadadustat can be routinely tested for quality control, assay determination, and stability in both research and pharmaceutical manufacturing environments using the developed RP-HPLC method because of its dependability, repeatability, and adherence to regulatory standards.

6. Source of Funding

None.

7. Conflict of Interest

None.

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