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A Comparative Study for Method Development and Validation of Daprodustat in Bulk and Pharmaceutical Tablet Dosage Form by UV-visible and RP-HPLC.

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ABSTRACT

This research introduces a straightforward, rapid, sensitive, and selective analytical method for measuring daprodustat in both bulk powder and tablet forms. The techniques used include UV-Visible spectrophotometry and reversed-phase high-performance liquid chromatography (RP-HPLC). For the UV-Visible method, a Systonic AU-2702 double-beam spectrophotometer was used to measure Daprodustat 's absorbance at 265 nm. The RP-HPLC procedure employed a Shimadzu SPD-10A C18 column (250×4.6 mm, 5 µm). The mobile phase consisted of a mixture of acetonitrile and distilled water (70:30, v/v), with 0.1% formic acid added. Key operational parameters included a flow rate of 1.2 mL/min, an injection volume of 10 µL, and a column temperature of 30 °C. The method's linearity ranged from 10 to 100 µg/mL. The entire procedure was validated following ICH Q2 (R1) guidelines, confirming its specificity, linearity, sensitivity, precision, accuracy, robustness, and system suitability. The technique yields highly sensitive results, with Limits of Detection (LODs) and Limit of Quantification (LOQs) of 0.6174 µg/mL and 1.87µg/mL for daprodustat. Recovery rates ranged from 98% to 102%, and both intra-day and inter-day precision (measured by % RSD) were below 2%. The method demonstrated excellent specificity, avoiding interference from excipients or formulation matrices. Overall, this study provides a complete analytical framework applicable for routine pharmaceutical analysis, ensuring quality control, safety assessment, and regulatory compliance for daprodustat.

Keywords: Daprodustat, UV VIS Spectrophotometer, RP-HPLC, Mobile phase, Stationary Phase, C-18 Column, Retention time, Retention factor, Regression coefficient, ICH guidelines.

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INTRODUCTION

Daprodustat is an orally available, specific inhibitor of hypoxia-inducible factor prolyl hydroxylase used to treat patients with anaemia and chronic renal disease on dialysis. Hypoxia-inducible factor 1α and 1β are subunits of a heterodimeric transcription factor called HIF1, which is produced at low levels under normal conditions. In response to hypoxia, they dimerize and induce the expression of genes that increase erythropoietin production and, consequently, hemoglobin levels. Under normal oxygen tension, prolyl hydroxylase inactivates HIF1α, leading to its degradation and preventing dimerization with HIF1β. This stops erythropoiesis from increasing. By inhibiting prolyl hydroxylase, daprodustat mimics hypoxic conditions, resulting in increased erythropoietin and erythropoiesis. In several animal models of chronic renal disease and anaemia, daprodustat increased erythropoietin and haemoglobin levels. In an open-label trial involving adult patients with chronic renal disease and anaemia on dialysis, daily oral doses of daprodustat raised haemoglobin levels similarly to injections of recombinant erythropoietin. A comparable outcome was observed in renal disease patients with anaemia not on dialysis; however, in this group, there was a slight but significant increase in cancers, tumour progression, and recurrences with daprodustat compared to recombinant erythropoietin. Based on these findings, daprodustat received accelerated approval as a therapy for adults with renal disease and anaemia, but only for those on dialysis.

Method development and validation are crucial steps in the discovery, development, and production of pharmaceuticals, which are complex and diverse processes. A review of existing literature shows that few comparative studies are using UV-Visible spectroscopy and reversed-phase high-performance liquid chromatography to measure daprodustat in tablet formulations. This research aimed to create a fast, inexpensive, accurate, and reliable method for detecting daprodustat in its bulk and tablet forms.

MATERIALS AND METHOD

Chemicals and Reagents

Drug substances were provided as a gift sample by Dr Reddy`s Laboratories Ltd, Hyderabad. The commercial tablet form, Jesduvroq, was bought from a local market and contains 8 mg of daprodustat per tablet. All the reagents and chemicals used in this study were of analytical reagent grade and HPLC grade. These included methanol, acetonitrile, isopropyl alcohol, and distilled water. The accuracy and reliability of HPLC analysis are enhanced by using high-quality reagents, solvents, and filters. Solutions of formic acid (0.1%) were prepared in double-distilled water and used for the studies.

Instrumentation

Spectroscopy methods involved measuring the absorbance of Daprodustat at 265 nm using a Systronic AU-2702 double-beam UV-visible spectrophotometer. Chromatographic separation was carried out with a Shimadzu liquid chromatography system, comprising an LC-10AD pump, an SPD–10A photodiode array detector, and an injector with a 10 µL loop. All data were collected and processed via the LC solution data station (Shimadzu, Japan). A Phenomenex C18 column (250 mm × 4.6 mm, 5 µm) was used for analysis.

Method Development

Different volumes (0.0, 0.1, 0.2, 0.3, 0.4, 0.5 mL) of a standard Daprodustat solution (100 µg/mL) were accurately transferred into a series of 10 mL volumetric flasks and then filled up to the mark with diluents. The absorbance of each solution was measured at 265 nm using a diluent as a blank. Calibration curves were plotted based on these measurements, and the concentration of unknown samples was determined from the calibration graph, which was calculated using data obtained from Beer's law. Various mobile phases were tested to develop the RP-HPLC method for analyzing daprodustat. The most suitable mobile phase was selected based on its selectivity and sensitivity.

Chromatographic Conditions

A mobile phase consisting of acetonitrile: distilled water (70:30, v/v) containing 0.1% formic acid was prepared. The mobile phase was filtered through a 0.45-micron membrane filter and sonicated for 30 minutes. The flow rate was set to 1.2 mL/min, the injection volume was 10 µL, the column temperature was maintained at 30°C, and daprodustat was detected at 265 nm.

Selection of wavelength

Standard solutions of daprodustat at 10 ppm were prepared and scanned with a UV spectrophotometer over the range 200–400 nm. The UV spectra obtained are shown in Figure 1. Based on the spectral data, a wavelength of 265 nm was selected for detecting daprodustat.

Preparation of Standard Stock Solution (1mg/mL)

One hundred mg of the drug (daprodustat) was accurately weighed and dissolved in diluent, using sonication in a 100 mL volumetric flask. The solution was made up to the mark with diluent. This resulted in a stock solution (Stock solution-I) of 1 mg/mL or 1000 µg/mL.

Preparation of Working Standard Solution

One mL of the above stock solution was transferred to a 10 mL volumetric flask and diluted with diluent to the mark, resulting in a concentration of 100 µg/mL for the drug. Then, 1 mL of the above solution was transferred to another 10 mL volumetric flask, diluted with diluent to the mark,

resulting in a final concentration of 10 µg/mL. Working standard mixtures were created by performing serial dilutions of the 10 µg/mL stock solution using the mobile phase/diluent over a concentration range of 2–10 µg/mL.

Calibration curve

A calibration curve was prepared by measuring different volumes of standard stock solutions of daprodustat in 10 mL volumetric flasks and diluting them with the mobile phase up to the mark. This produced final concentrations of 2, 4, 6, 8, and 10 µg/mL for daprodustat. Five standard solutions were injected using the loop injection system, and chromatograms were recorded. The effluent was analyzed at 265 nm. The calibration curve was plotted by graphing the average peak area against concentration, and a regression equation was derived from this data.

Method Validation

The method was validated for linearity, accuracy, specificity, limit of detection, limit of quantification, intra-day and inter-day precision, repeatability, robustness, ruggedness, and system suitability parameters.

Assay of market formulation by UV-Visible Spectroscopy / RP-HPLC

Twenty tablets were accurately weighed and ground into a fine powder. An accurately measured amount of the tablet powder, equivalent to 100 mg of daprodustat, was transferred into two 100 mL volumetric flasks (or calibrated flasks). The volume was adjusted to the 100 mL mark with the respective diluent or mobile phase. The solution was filtered using Whatman No. 42 filter paper or 0.45-micron Whatman filter paper. Then, 1 mL of the solution was further diluted to 10 mL with the respective diluent or mobile phase to obtain the final sample concentration (100 µg/mL). The findings from the assay of the marketed formulation are presented in Table 3 and Figure 5.

RESULTS AND DISCUSSION:

A reliable, precise, and suitable method using UV-Visible Spectroscopy and RP-HPLC was developed to measure daprodustat in bulk and tablet forms. In the UV-spectrophotometric approach, the diluent served as the solvent, and measurements were taken at a wavelength of 265 nm. The optimal conditions and data for the regression equation of daprodustat are summarized in Table 1. Various combinations of mobile phases were tested, and the chosen chromatographic conditions were found to be appropriate for accurately determining the compound. Chromatograms of the standard solutions of daprodustat are shown in Figure 2. The optimized chromatographic conditions are summarized in Table 2.

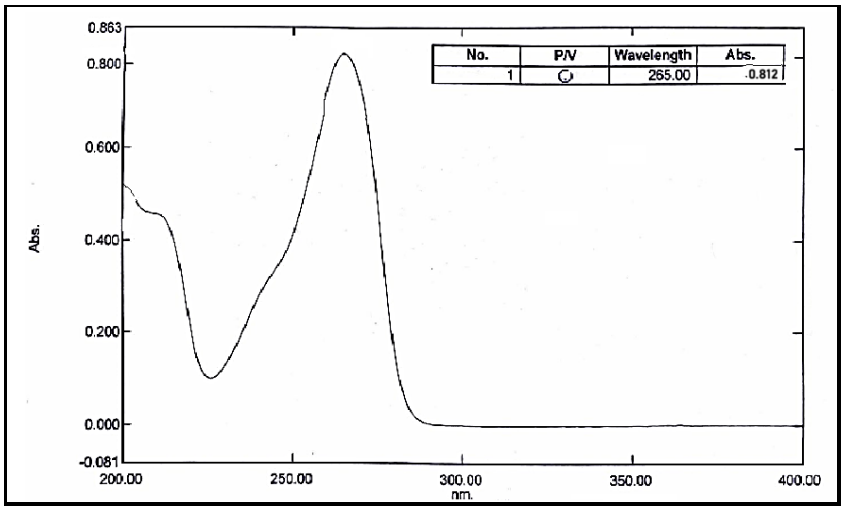

Figure 1: UV Spectrum of Daprodustat

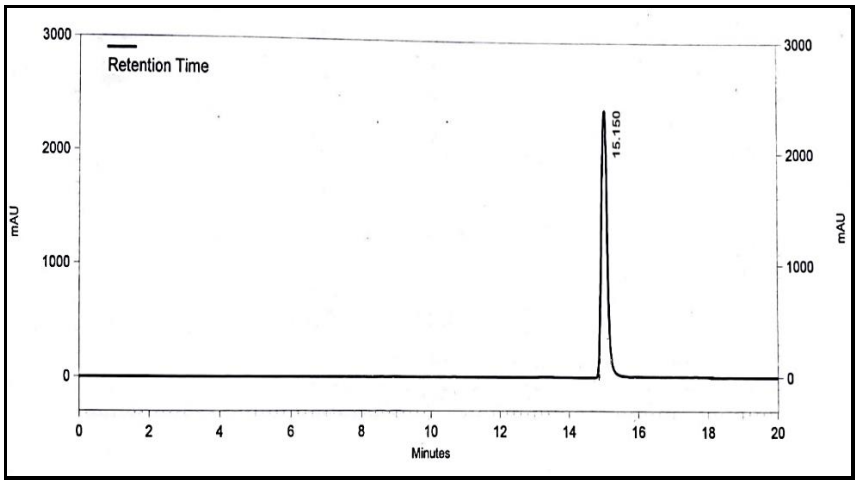

Figure 2: Chromatogram of Daprodastat Standard

Table 1: Optimum conditions and Statistical data for the regression equation of Daprodustat

Sr. No	Parameters	Optimized conditions
1.	λ max	265 nm
2.	Linearity (Beer's law limit in ug/mL)	2 – 10 ug/mL
3.	Regression Equation	y = 0.0641x - 0.008,
4.	Slope	0.06411
5	Correlation coefficient (R2)	0.9998

Table 2: Optimized chromatographic conditions for Daprodustat

Sr. No	Parameters	Optimized conditions
1.	Column	Shimadzu C18 SPD-10A (250×4.6, 5µm)
2.	Mobile phase	Acetonitrile and distilled water (70: 30 v/v)
3.	Diluent	Acetonitrile and distilled water
4.	Column temperature	30°C
5.	Wavelength	265 nm
6.	Flow rate	1.2 mL/min
7.	Injection volume	10 µl
8.	Run time	20 min
9.	Retention time	15.150

Linearity studies UV-visible spectroscopy / RP-HPLC

A direct relationship was observed between the absorbance at the maximum wavelength (λ max) and the concentration of daprodustat within the ranges provided in Table 4. The linearity range for daprodustat was established as 2–10 µg/ml for both methods. The regression equations for daprodustat using UV-Visible spectroscopy were y = 0.0641x - 0.008, with correlation coefficients (R^2) of 0.9998 (Table 4 & Figures 3). Similarly, for RP-HPLC, the regression equations were y = 27590x − 1932, with a correlation coefficient (R^2) of 0.9995 (Table 4 & Figures 4).

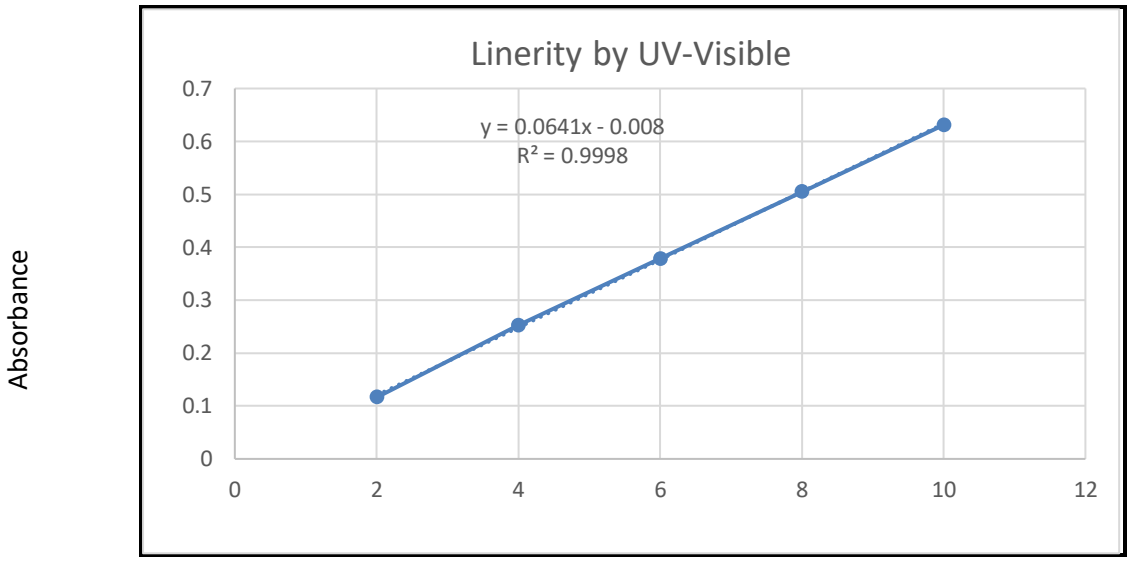

Figure 3: Calibration curve of Daprodastat by UV-Visible Spectroscopy

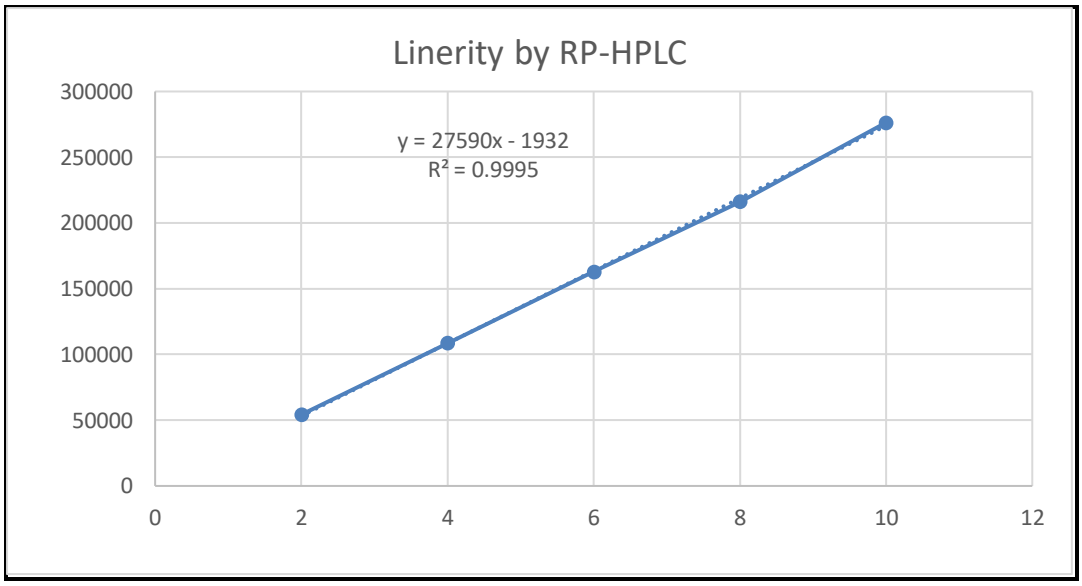

Figure 4: Calibration curve of Daprodastat HCl by RP-HPLC

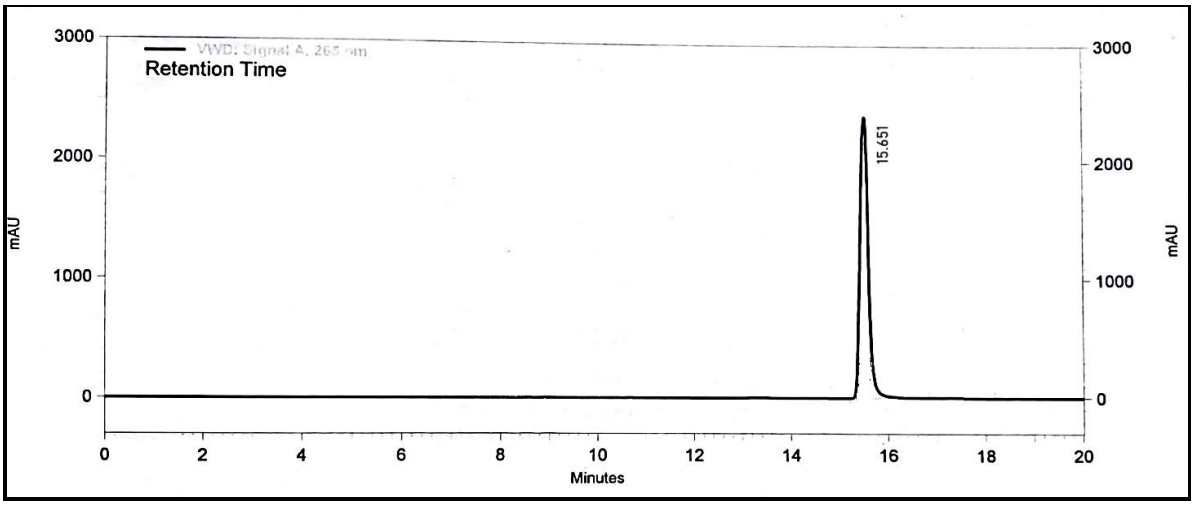

Figure 5: Chromatogram of Daprodastat Dosage Form

Table 3. Assay of Daprodustat Tablet Dosage Form

Estimation of Terbinafine HCl			
Dosage form	Labelled claim (mg)	Amount estimated (mg)	% Purity
Daprodustat By UV-visible spectroscopy	8 (mg)	7.87 (mg)	98.4 %
Daprodustat By RP-HPLC	8 (mg)	7.84 (mg)	98.0 %

Table 4: Linearity studies of Daprodustat

S.No	Concentration ug/ml	Absorbance / Area*(n=5)		Concentration ug/mL found	
		UV-Spectroscopy	RP-HPLC	UV-Spectroscopy	RP-HPLC
1.	2	0.1162	54232	1.7	1.90
2	4	0.2524	108544	3.8	3.86
3.	6	0.3786	162816	5.8	5.83
4.	8	0.5048	216088	7.8	7.76
5	10	0.6311	276360	9.7	9.95

Precision studies by UV-visible spectroscopy / RP-HPLC

We evaluated the precision and the agreement between repeated measurements by preparing and analyzing five separate samples according to the established method. The method proves precise, as the percentage relative standard deviation (% RSD) for both intra-day and inter-day variations was below 2%. Tables 5 and 6 list the detailed results.

Table 5. Precision Data of Daprodustat by UV – Spectroscopy

Absorbance Intraday Precision							Absorbance Inter day Precision		
S.No	Conc. (ug/ml)	0 hrs	2 hrs	4 hrs	6 hrs	8 hrs	Day 1	Day 2	Day 3
1.	10	0.6395	0.6333	0.6373	0.6343	0.6313	0.6753	0.6833	0.6763
2.	10	0.6389	0.6319	0.6389	0.6399	0.6399	0.6659	0.6799	0.6799
3.	10	0.6333	0.6363	0.6372	0.6373	0.6343	0.6763	0.6843	0.6753
4.	10	0.6323	0.6343	0.6343	0.6333	0.6333	0.6873	0.6863	0.6843
5.	10	0.6343	0.6333	0.6353	0.6333	0.6333	0.6783	0.6753	0.6773

6.	10	0.6313	0.6343	0.6323	0.6323	0.6313	0.6763	0.6743	0.6763
Mean		0.6349	0.6339	0.6359	0.6351	0.6339	0.6766	0.6806	0.6806
Standard D		0.0035	0.0015	0.0024	0.0029	0.0032	0.0068	0.0049	0.0049
% RSD		0.54	0.23	0.38	0.46	0.50	1.01	0.73	0.73

Table 6. Precision Data of Daprodustat by RP-HPLC

Peak Area Intraday Precision							**Peak Area Interday Precision**		
S.No	**Conc. (ug/ml)**	**0 hrs**	**2 hrs**	**4 hrs**	**6 hrs**	**8 hrs**	**Day 1**	**Day 2**	**Day 3**
1.	10	275456	275356	277351	275452	275436	275356	265456	275451
2.	10	276448	266458	267452	266443	276448	276348	276447	276442
3.	10	276437	276447	277444	276434	276447	276337	276437	266434
4.	10	266456	276356	277355	276455	276456	266356	276456	276456
5.	10	275451	275451	277452	275451	275551	275351	275458	275458
6.	10	276451	266451	277453	276452	266451	276351	276451	276453
Mean		274450	272753	275751	274448	274465	274350	274451	274449
Standard D		3946	4899	4066	3952	3954	3946	4424	3957
% RSD		1.4	1.8	1.5	1.4	1.4	1.4	1.6	1.4

Accuracy studies by UV-visible spectroscopy / RP-HPLC

To assess accuracy, we conducted recovery studies in triplicate using the standard addition method. We spiked known amounts of daprodustat into pre-analyzed samples at 80%, 100%, and 120% of the expected concentration. The subsequent analysis using the proposed UV-visible / HPLC method yielded the results presented in Tables 7 and 8.

Table 7. Accuracy Data of Daprodustat by UV – Spectroscopy

S.No	**Level of Recovery**	**Amount Added (ug/ml) Mean (n=3)**		**Total Amount Recovered (ug/ml)**	**% Recovery**
		Test	**Standard**		
1.	80 %	10	8	17.55	97.5
2.	100 %	10	10	19.20	96.0
3	120 %	10	12	21.10	98.0
Mean Recovery = 97.16 %					

Table 8. Accuracy Data of Daprodustat by RP-HPLC

S.No	**Level of Recovery**	**Amount Added (ug/ml) Mean (n=3)**		**Total Amount Recovered (ug/ml)**	**% Recovery**
		Test	**Standard**		
1.	80 %	10	8	17.81	98.94
2.	100 %	10	10	19.50	97.50
3	120 %	10	12	21.75	98.86
Mean Recovery = 98.43 %					

Specificity studies by RP-HPLC

The purity of the daprodustat peaks was checked by comparing their retention times with those of

standard pure compounds. A strong correlation was found between the retention times of the standards and the samples.

System Suitability parameters studies by RP-HPLC

To verify the validity of the analytical method, a system suitability test was conducted. Six replicates of a standard solution (10 µg/mL) were injected. Parameters like the number of theoretical plates (N), tailing factor, and retention time were calculated. These parameters are detailed in Table 9.

Table 9: System suitability & specificity Parameters of Daprodustat by RP-HPLC

Parameters	Daprodustat
Retention Time (min)	15.150
Resolution (Rs)	1.5
Tailing Factor (T)	1.01
Theoretical Plates (N)	45810
Range (ug/ml)	10 - 50

Robustness & Ruggedness of method studies UV-visible spectroscopy / RP-HPLC

Robustness refers to the ability of a method to consistently deliver accurate and precise results despite variations in experimental conditions. To assess the robustness of the method, the most important parameters were deliberately altered while keeping all other parameters constant. At the same time, the chromatographic profile was closely observed and documented. To evaluate the robustness of the developed RP-HPLC method, small, controlled changes were introduced to the optimized parameters. The effects of variations in flow rate, wavelength, and the ratio of the mobile phase on retention time and tailing factor were evaluated. Despite these changes, the method showed consistent performance, which indicates a high level of robustness and ruggedness (Tables 10 & 11). The results confirm that the process is reliable and robust.

Table 10: Robustness studies of Daprodustat by RP-HPLC

Parameter (n=5)	Condition	Level	Retention time	Tailing Factor
Flow rate(mL/min)	1.0	-2	15.125	1.04
(± 0.2mL)	1.2	0	15.150	1.01
	1.4	+2	15.155	1.05
	Mean(n=3)		15.143	1.03
Detection wavelength	264	-1	15.135	1.04
(nm)	265	0	15.150	1.03
(± 1nm)	266	+1	15.145	1.05
	Mean(n=3)		15.143	1.04
Mobile phase composition	63:27	-10	15.128	1.04
(v/v)	70:30	0	15.150	1.05
(± 10% of organic phase)	77:33	+10	15.142	1.04
	Mean(n=3)		15.140	1.04

Table 11. Ruggedness studies of Daprodustat by UV– Spectroscopy / RP-HPLC

S.No	Conditions	Conc. (ug/ml)	Absorbance UV - Spectroscopy	Peak Area RP-HPLC
1.	Analyst-1	10	0.6395	275456
2.		10	0.6389	276448
3.		10	0.6333	276437
		Mean	0.6372	276114
		SD	0.003	569.6
		% RSD	0.54	0.21
4.	Analyst-2	10	0.6843	276456
5.		10	0.6773	275458
6.		10	0.6763	276451
		Mean	0.6793	276122
		SD	0.004	574.8
		% RSD	0.64	0.21

LOQ and LOD studies by UV-visible spectroscopy / RP-HPLC

LOD refers to the lowest concentration that can be detected, while LOQ is the lowest amount that can be measured accurately and precisely. These values were calculated using the formulas: LOQ = 10s/m and LOD = 3.3s/m, where s is the standard deviation and m is the slope of the calibration curve. For daprodustat using UV-Visible spectroscopy, the LOD was 0.6174 µg/mL, and the LOQ was 1.87 µg/mL. For RP-HPLC, the LOD and LOQ were also 0.617 and 1.870 µg/mL, respectively.

CONCLUSION:

The developed RP-HPLC and UV-Visible spectroscopy methods established a validated, precise, and highly accurate approach for the quantitative analysis of daprodustat in bulk and pharmaceutical formulations. Our findings confirmed robust method validity, with all parameters consistently showing a % RSD below 2. This research makes a significant contribution by providing a straightforward and reliable tool for routine quality control, thereby ensuring product consistency and safety. Further studies could explore the application of these methods for analyzing daprodustat in biological matrices, further expanding their utility.

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REFERENCES:

1. Mahar KM, Caltabiano S andrews S. Clinical pharmacokinetics of daprodustat: results of an absorption, distribution and excretion study with intravenous microtracer and

concomitant oral doses for bioavailability determination. Clin Pharmacol Drug Dev. 2021; 10(12): 1419-31.

2. Meadowcroft AM, Cizman B, Holdstock L, Biswas N, Johnson BM, Jones D, et al. Daprodustat for anemia: a 24-week, open-label, randomized controlled trial in participants on hemodialysis. Clin Kidney J. 2019; 12(1): 139-48. Doi: http://dx.doi. org/10.1093/ckj/sfy014.
3. Ariazi JL, Duffy KJ, Adams DF. Discovery and preclinical characterization of GSK1278863 (Daprodustat), a small molecule hypoxia-inducible factor-prolyl hydroxylase Inhibitor for Anemia. J Pharmacol Exp Ther. 2017; 363(3): 336-47. Doi:1 0.1124/jpet.117.242503.
4. Caltabiano S, Cizman B, Burns O, Mahar KM, Jonson BM, Ramanjineyulu B, et al. Effect of renal function and dialysis modality on daprodustat and predominant metabolite exposure. Clin Kidney J. 2019; 12(5): 693-701. Doi:http://dx.doi.org/10.1093/ckj/sfz 013.
5. Mazzarino M, Perretti I, Stacchini C, Fabio Comunità F, de la Torre X, Botrè F. UPLC-MS-Based Procedures to Detect Prolyl-Hydroxylase Inhibitors of HIF in Urine, J Anal Toxicol. 2021; 45(2): 184-94. Doi: https://doi.org/10.1093/jat/bkaa055.
6. International Conference on Harmonization (ICH), Draft revised Guidance on Validation of Analytical Procedure: Text and Methodology. Federal Register, step 4 version [cited 1/6/2023]. Available from: https://www.ich.org/page/ quality-guidelines. Vol. Q2A; 2005. (p. R1).
7. Bi Y-A, Jordan S, King-Ahmad A, West MA, Varma MVS. Mechanistic determinants of daprodustat drug-drug interactions and pharmacokinetics in hepatic dysfunction and chronic kidney disease: Significance of OATP1B-CYP2C8 interplay. Clin Pharmacol Ther [Internet]. 2024 [cited 2024 May 19];115(6): 1336-45. Available from: https://go.drugbank.com/drugs/DB11682.
8. Enmark M, Glenne E, Leśko M, Weinmann AL, Leek T, Kaczmarski K, et al. Investigation of robustness for supercritical fluid chromatography separation of peptides: isocratic vs gradient mode. J Chromatogr A. 2018 Sep 21; 1568:177-87.
9. Ishii T, Tanaka T, Nangaku M. Profile of daprodustat in the treatment of renal anemia due to chronic kidney disease. Ther Clin Risk Manag. 2021 Feb 17; 17:155-63.
10. Desai AM, Andreae M, Mullen DG, Holl MM, Baker Jr JR. Acetonitrile shortage: Use of isopropanol as an alternative elution system for ultra/high performance liquid chromatography. Anal Methods. 2011;3(1):56-8.

11. Thekkudan DF, Rutan SC, Carr PW. A study of the precision and accuracy of peak quantification in comprehensive two-dimensional liquid chromatography in time. J Chromatogr A. 2010 Jun 25;1217(26):4313-27.
12. Žuvela P, Skoczylas M, Jay Liu J, Bą czek T, Kaliszan R, Wong MW, Buszewski B. Column characterization and selection systems in reversed-phase high-performance liquid chromatography. Chem Rev. 2019 Jan 3;119(6):3674-729.