Journal of Applied Pharmaceutical Research
Volume 14 Issue 2, Year of Publication 2026, Page 213 – 219
DOI: https://doi.org/10.69857/joapr.v14i2.2014

Check for updates

Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com *ISSN: 2348 – 0335*

LC-MS/MS BASED BIO ANALYSIS OF DESIDUSTAT: FROM DEVELOPMENT TO APPLICATION

Shaik Ayesha Ameen[1]*, Nagaraju Pappula[2]

Article Information
Received: 30th October 2025
Revised: 15th December 2025
Accepted: 29th January 2026
Published: 15th March 2026

Keywords
Liquid Chromatography-Mass Spectrometry, Multiple Reaction Monitoring (Mrm), Bioanalytical Method, Pharmacokinetics, Quantification.

ABSTRACT
Background: Desidustat's potential as a medicinal treatment for anaemia has generated considerable interest. For pharmacokinetic analysis, therapeutic monitoring, and drug development research, precise quantification of Desidustat in biological matrices is crucial. Hence, more advanced methods are necessary for detection at minute concentrations (ng/mL level) in plasma. **Methodology:** A reverse-phase C18 column with an isocratic elution technique utilising acetonitrile and an aqueous buffer as mobile phase to accomplish chromatographic separation. To produce characteristic ion transitions for quantification, after electrospray ionization (ESI), tandem mass spectroscopy in positive ion mode was chosen for detection. The technique's linearity, sensitivity, precision, and accuracy were systematically improved and verified, and the validated method was successfully applied to pharmacokinetic studies. **Results and Discussion:** The developed method's validation results confirmed good selectivity, sensitivity (LLOQ 6 ng/ml), excellent linearity, and acceptable precision and accuracy, with a remarkably low lower limit of quantification (LLOQ). Recovery and matrix effect studies showed minimal ion suppression and reproducible extraction efficiency. The stability of desidustat is demonstrated in rat plasma. Intra-day and inter-day precision and accuracy values were well within the acceptable limits prescribed by regulatory guidelines, confirming the method's reliability and reproducibility. The optimized LC–MS/MS conditions ensured high sensitivity and selectivity, making the method suitable for bioanalytical applications. **Conclusion:** A novel, highly sensitive, and reliable LC–MS/MS bioanalytical method for estimation of Desidustat in rat plasma was successfully developed and validated. The method provides a robust analytical tool to support pharmacokinetic studies, therapeutic monitoring, and future research related to Desidustat.

INTRODUCTION

Desidustat, a potent hypoxia-inducible factor prolyl hydroxylase inhibitor, has notable recognition as a capable therapeutic for anaemia [1,2], especially in patients with chronic kidney disease. By modulating the hypoxia-inducible factor (HIF) pathway,

[1]*ANU College of Pharmaceutical Sciences, Department of Pharmaceutical Analysis, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India, 522510.*
[2]*Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur, Andhra Pradesh, India, 522002.*

For Correspondence:** **skayeshaameen@gmail.com

Desidustat promotes red blood cell production [3,4] in response to low oxygen levels, offering a potentially effective alternative to traditional erythropoiesis-stimulating agents (ESAs). To support its development, a brief understanding of Desidustat pharmacokinetics, including its absorption, distribution, metabolism, and excretion (ADME) [5,6], is crucial. Quantification of Desidustat in biological matrices, particularly plasma, is necessary to examine its pharmacokinetic properties [7]. Plasma samples are characteristically used in preclinical studies to monitor drug concentrations and systemic exposure. While methods such as UV-Vis spectroscopy or basic HPLC have been employed, they often lack the sensitivity needed to detect minute concentrations of Desidustat in plasma. The existing literature on this topic is largely clinical in nature. As Desidustat progresses through its preclinical and clinical stages, the reliable bioanalytical method for precise quantification in biological matrices becomes increasingly scarce. Unlike existing RP-HPLC methods that use UV detection for formulation analysis, the present LC-MS/MS method provides highly sensitive and selective estimation of Desidustat in rat plasma, with an LLOQ of 6 ng/mL using MRM detection. Its successful pharmacokinetic application underscores its superiority and novel bioanalytical relevance. A comparative study of pre-existing and current research data is summarized in Table 1. LC-MS/MS is a highly sensitive and selective analytical technique that allows accurate identification and quantification of compounds in complex biological samples. This method's capability to detect low concentrations of Desidustat in plasma is vital for pharmacokinetic studies [8], where precise measurements are essential. It also offers significant benefits in speed and reliability, making it ideal for large-scale preclinical and clinical studies.

Table 1: Comparative performance of reported methods for desidustat to the current LC-MS/MS method.

Parameter	Reported RP-HPLC Methods	Present LC-MS/MS Method
Detection technique	UV absorbance	Tandem mass spectrometry (MRM)
Typical LLOQ	50–200 ng/mL	6 ng/mL
Selectivity	Limited (co-eluting matrix interference possible)	Highly specific (precursor → product ion monitoring)
Sample matrix	Pharmaceutical formulation	Rat plasma (biological matrix)
Sample preparation	Dilution/filtration	Protein precipitation
Run time	8–15 min	Short (rapid elution)
Bioanalytical application	Not applied	Successfully applied to a pharmacokinetic study
Sensitivity suitability for PK	Moderate	Excellent for low plasma levels

MATERIAL AND METHOD

Instrumentation and Analytical Conditions

A Waters HPLC system equipped with an X-Terra RP-18 column (150mm x 4.6mm) was selected for chromatographic separation, featuring a column oven, high-speed autosampler, and a degasser to ensure optimal analyte preparation. The HPLC system was seamlessly interfaced with the SCIEX QTRAP 5500 mass spectrometer, inbuilt with an electrospray ionization (ESI) source, providing the requisite sensitivity and selectivity. This setup, along with the Empower-2 software, ensured efficient data acquisition and precise analysis in a systematic and effective manner. The mass spectrometer was operated in positive ion electrospray ionization (ESI) and Multiple Reaction Monitoring (MRM) modes to enhance the specificity and precision of Desidustat quantification.

Mobile phase preparation: Chromatographic separation was achieved by using an isocratic elution of acetonitrile and aqueous buffer. A buffer solution was prepared by dissolving 6.30g of Ammonium formate in 1 L of HPLC water, and its pH was adjusted to 3.0 with formic acid. The mobile phase used was a 20:80 acetonitrile-buffer mixture, followed by filtration through a 0.45 µm membrane filter.

Extraction

Protein precipitation was chosen to extract the drug from the matrix because of its consistent extraction efficiency and minimal endogenous interference. 800 µL of acetonitrile was added to 200 µL of plasma, vortexed thoroughly, and centrifuged at 4000 RPM for 15–20 minutes. The resulting supernatant was gently transferred to an HPLC vial for analysis.

Solutions preparation

Desidustat Parent Stock Solution (48,000 ng/mL): Six mg of Desidustat was dissolved in diluent and made up to 10 mL. From this, 0.8 mL was further diluted to 10 mL with diluent.

Desidustat Stock Solution: An aliquot of 0.1 mL of the parent stock solution was diluted to 10 mL with diluent.

Standard Solution (120 ng/mL Desidustat and 100 ng/mL Daprodustat): To 500 µL of Desidustat stock solution, 200 µL

of plasma, 500 µL of internal standard solution, and 800 µL of acetonitrile were added. The mixture was centrifuged for 20 min, and the supernatant was filtered and transferred to an HPLC vial.

Linearity Solutions Preparation: Using the same method as previously described, linearity solutions containing Desidustat concentrations ranging from 6 ng/mL to 240 ng/mL were prepared.

Selection of internal standard: Daprodustat was picked as an internal standard based on its structural similarity and molecular polarity resembling desidustat, resulting in comparable extraction recovery and ionisation response in ESI-MS/MS.

Internal Standard Stock Solution (400 ng/mL): Five mg of Daprodustat was dissolved in diluent and made up to 10 mL. From this, 0.8 mL was diluted to 10 mL, and 0.1 mL of the resulting solution was further diluted to 10 mL with diluent.

Optimisation of chromatographic conditions: The instrumental parameters were finely tuned to confirm optimal performance and are presented in Tables 2 and 3 and in the chromatogram (Figure 1).

Table 2: Optimised chromatographic and mass spectrometric conditions

Optimised Chromatographic Conditions:	Mass spectrometer conditions
Column: Waters X-Terra RP-18 column, 150mm x 4.6mm, 3.5µm	Collision Energy: 14 V
Injection Volume: 10µL	Ion Spray Voltage: 5500 V
Column Temperature: Ambient	Source Temperature: 550°C
Flow Rate: 1.0 mL/min	Drying Gas Temperature: 120-250°C
Sample Temperature: Ambient	Collision Gas: Nitrogen
Run Time: 5 min	Drying Gas Flow Rate: 5 mL/min
	De-clustering Potential: 40 V
	Entrance Potential: 10 V
	Exit Potential: 7 V
	Dwell Time: 1 sec

Table 3: Optimised MRM transitions in positive ionisation mode

Compound	Precursor ion (m/Z)	Product ion (m/Z)
Desidustat	333.3964	230.5241
Daprodustat	394.5557	181.8564

METHOD VALIDATION

The developed analytical method was validated in accordance with ICH M10 and ICH Q2(R2) bioanalytical method validation guidelines to ensure its suitability for its intended use. Validation parameters included system suitability, linearity, accuracy, precision, sensitivity, recovery & stability. The method demonstrated acceptable performance within predefined limits [11,12], confirming its reliability, reproducibility & compliance with regulatory requirements for quantitative analysis.

RESULTS AND DISCUSSION

System suitability: It is a preliminary check to ensure the chromatographic system meets specified requirements and produces accurate, reproducible results. It helps identify issues such as column degradation, instrument variability, or sample preparation errors, thereby preventing inaccurate interpretation of data. Compliance with system suitability parameters confirms that the method operates within acceptable limits for scientific and regulatory requirements.

Key Parameters Evaluated

☑ Retention Time (RT) Consistency

☑ Peak Area Ratio (Analyte/ISTD

☑ Peak Shape & Resolution:

☑ Mass Accuracy & Sensitivity ☑ Carryover Check

Decision: The %CV for Desidustat and internal standard area ratio was found to be 0.21. Hence, it passed the system suitability. The results are tabulated in Table 4.

Sensitivity: The method's sensitivity was evaluated by determining the lower limit of quantification (LLOQ). The LLOQ was defined as the lowest concentration of Desidustat that could be quantified with acceptable accuracy and precision. LLOQ samples were analysed in replicates, and the lower limit of quantification (LLOQ) for Desidustat was established at 6 ng/mL. At this concentration, the method demonstrated excellent precision (%CV = 1.65%) and acceptable accuracy (93.22%), fulfilling the bioanalytical validation acceptance criteria. The analyte signal at LLOQ exceeded 10:1, confirming adequate sensitivity for quantitative analysis.

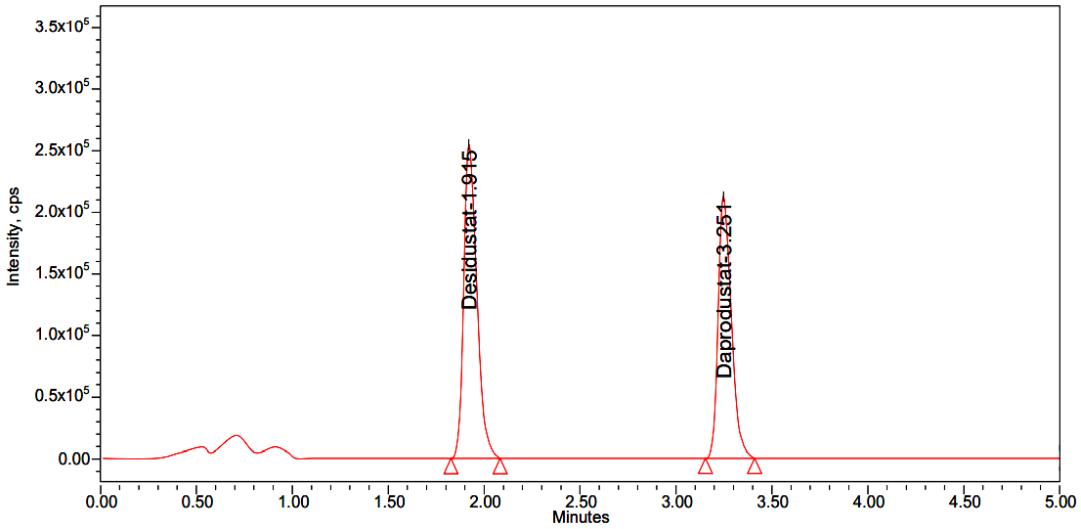

Figure 1: Optimised chromatogram showing separation of Desidustat and Daprodustat

Minor early-eluting peaks corresponding to the solvent front and endogenous plasma components were observed between 0.5 and 1.0 min in the chromatograms. These peaks were well resolved from the retention times of Desidustat and the internal standard and did not interfere with analyte quantification, confirming the selectivity of the developed LC-MS/MS method.

Table 4: System suitability results of desidustat

Sample Name MQC(120ng/ml)	Analyte Area (cps)	Analyte RT (min)	ISTD Area (100ng/ml)	ISTD RT (min)	Area Ratio
MQC-1	$2.559x10^5$	1.915	$2.125x10^5$	3.251	1.2042
MQC-2	$2.551x10^5$	1.917	$2.119x10^5$	3.259	1.2039
MQC-3	$2.547x10^5$	1.913	$2.121x10^5$	3.256	1.2008
MQC-4	$2.555x10^5$	1.915	$2.129x10^5$	3.250	1.2001
MQC-5	$2.549x10^5$	1.911	$2.127x10^5$	3.254	1.1984
MQC-6	$2.557x10^5$	1.918	$2.130x10^5$	3.256	1.2005
Mean	$2.553x10^5$	1.915	$2.125x10^5$	3.254	1.2013
SD	0.00473	0.00256	0.00440	0.00339	0.00228
%CV	0.19	0.13	0.21	0.10	0.19

Matrix effect: Matrix effect was evaluated by calculating the matrix factor (MF) for Desidustat at HQC (180 ng/mL) and LQC (18 ng/mL) levels across six plasma lots by comparing post-extraction plasma responses to neat standards. The MF was normalized using the internal standard (IS-normalized MF). The %CV values were 0.16% for HQC and 1.35% for LQC, indicating negligible ion suppression or enhancement. Matrix effect data is given below in Table 5.

Linearity: The standard curves were linear over the concentration range of 6.0-240.0 ng/mL of Desidustat. The mean correlation coefficient was 0.999. Samples were quantified using the analyte-to-IS peak area ratio. Peak area ratios were plotted against plasma concentrations. Figure 2 illustrates the related outcome.

Precision and accuracy: Intra-assay precision and accuracy were estimated by analysing six replicates containing Desidustat at four QC levels. The inter-assay precision was determined by analysing the four QC levels across four runs. The criteria for data acceptability include accuracy within 85–115% of the actual values and a precision of within ±15% relative standard deviation (RSD), except for LLQC, where it should be within 80–120% for accuracy and <20% RSD. The resultant data is given in Table 6.

Recovery of analyte: The recovery of the drug and IS was evaluated at three concentration levels, namely low, medium, and high-quality control. Recovery was calculated by comparing its response in replicate samples with that of the neat standard solution responses. Analyte recovery from a sample matrix (extraction efficiency) is a comparison of the analytical response from an amount of analyte added to that determined from the sample matrix. Because of Desidustat's basic properties, extraction was carried out using the mobile-phase solvent. The % CV of recovery at each QC level and for ISTD was found to be ≤ 15.00 %. The overall mean recovery % CV for all QC levels was ≤ 20.00 %, shown in Table 7.

Table 5: Matrix effect Results of Desidustat (HQC-180ng/ml, LQC-18ng/ml)

S.No.	Plasma Lot No.	HQC	LQC
		Nominal Concentration(ng/ml)	
		180	18
		Analyte peak area	
1.	Lot 1	$3.759x10^5$	$0.369x10^5$
		$3.751x10^5$	$0.363x10^5$
		$3.755x10^5$	$0.367x10^5$
2.	Lot 2	$3.766x10^5$	$0.368x10^5$
		$3.762x10^5$	$0.375x10^5$
		$3.765x10^5$	$0.372x10^5$
3.	Lot 3	$3.754x10^5$	$0.370x10^5$
		$3.767x10^5$	$0.368x10^5$
		$3.757x10^5$	$0.366x10^5$
4.	Lot 4	$3.752x10^5$	$0.362x10^5$
		$3.748x10^5$	$0.359x10^5$
		$3.749x10^5$	$0.361x10^5$
5.	Lot 5	$3.756x10^5$	$0.364x10^5$
		$3.758x10^5$	$0.371x10^5$
		$3.763x10^5$	$0.365x10^5$
6.	Lot 6	$3.755x10^5$	$0.377x10^5$
		$3.753x10^5$	$0.374x10^5$
		$3.766x10^5$	$0.366x10^5$
N		18	18
Mean		$3.758x10^5$	$0.368x10^5$
SD		0.00607	0.00496
%CV		0.16	1.35
% Mean Accuracy		98.13%	96.10%
No. of QC Failed		0	0

Stability studies: Stability was evaluated under various processing and storage conditions for Desidustat. Short-term stability of Desidustat in rat plasma was evaluated by storing LQC and HQC samples at room temperature for 6 hours prior to analysis. Long-term stability was assessed by storing plasma samples at −20 °C for 30 days. Autosampler stability was evaluated by keeping processed samples at 10 °C for 24 hours. Freeze–thaw stability was examined over three cycles between −20 °C and room temperature. Wet extract stability was evaluated by storing processed plasma samples in the autosampler at 10 °C for 24 h prior to analysis. Dry extract stability was assessed by evaporating the extracted samples to dryness, storing them at −20 °C for 24 h, then reconstituting and analyzing. In all cases, measured conc. remained within ±15% of nominal values, confirming adequate stability under the tested conditions.

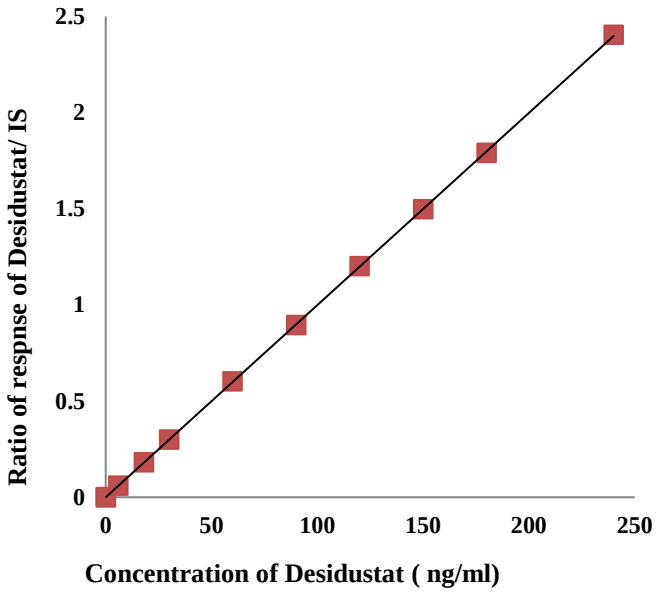

Figure 2: Calibration plot for concentration v/s Area ratio of Desidustat

Table 6: Precision and accuracy Results of Desidustat

Acquisition Batch ID	Date	HQC	MQC	LQC	LLQC
		Nominal Concentration (ng/ml)			
		180	120	18	6
		Analyte peak area			
		$3.759x10^5$	$2.488x10^5$	$0.369x10^5$	$0.122x10^5$
		$3.752x10^5$	$2.484x10^5$	$0.366x10^5$	$0.120x10^5$
		$3.753x10^5$	$2.487x10^5$	$0.372x10^5$	$0.118x10^5$
		$3.758x10^5$	$2.482x10^5$	$0.368x10^5$	$0.123x10^5$
		$3.751x10^5$	$2.486x10^5$	$0.373x10^5$	$0.119x10^5$
		$3.757x10^5$	$2.481x10^5$	$0.371x10^5$	$0.124x10^5$
N		6	6	6	6
Mean		$3.755x10^5$	$2.485x10^5$	$0.370x10^5$	$0.121x10^5$
SD		0.00341	0. 00280	0.00264	0.00237
% CV		0.09	0.11	0.71	1.96
% Mean Accuracy		98.05%	97.34%	96.62%	94.79%

Table 7: Recovery data of desidustat and daprodustat

QC level	Conc. ng/ml	Mean recovery (%)	% CV
LQC	18	97.0	<1
MQC	120	97.18	0.13
HQC	180	97.98	<1
IS	100	97.04	0.14

APPLICATION

Pharmacokinetic studies

Blood samples (300 µL) were collected from rats by administering 50 mg desidustat tablets at various time points (0.5 to 12.0 hours) post-dose, as well as a Predose sample. Plasma was obtained, stored at −70 ± 10°C, and spiked with an internal standard (IS) for processing QC samples. Pharmacokinetic parameters were calculated using WinNonlin software. Stability was assessed using acquired sample reanalysis (ISR), in which samples collected near Cmax and in the elimination phase were considered stable if the % difference did not exceed 20%. Data related to the pharmacokinetics of Desidustat are provided in Tables 8 and 9, and the drug's recovery plot in rat plasma is shown in Figure 3.

Table 8: Pharmacokinetic studies data

Time Intervals (Hrs)	Desidustat (ng/ml)
0.5	41.457
1	80.243
2	113.702
4	93.749
6	66.510
8	44.097
10	21.347
12	0

Table 9: Pharmacokinetic parameters of Desidustat

Pharmacokinetic parameters	Desidustat
AUC_{0-t}	703±39ng-hr/ml
C_{max}	113.7±7.1ng/ml
$AUC_{0-\infty}$	703±42ng-hr/ml
t_{max}	2.0±0.2 Hrs
$T_{1/2}$	10.0±0.8 Hrs

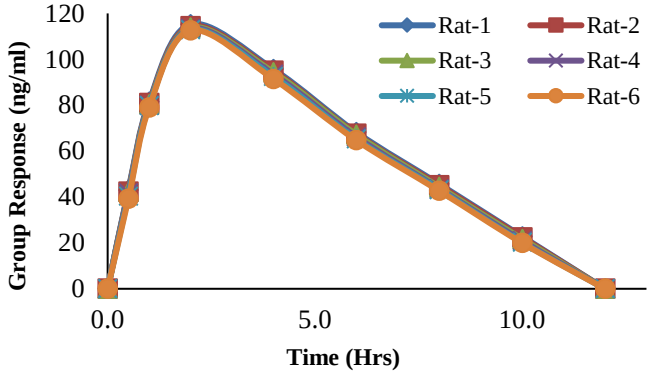

Figure 3: Recovery plot for Desidustat in Rat plasma

CONCLUSION

In summary, the validated LC-MS/MS method in this study offers exceptional sensitivity, specificity, and reliability for quantifying Desidustat in biological matrices. Its robustness and accuracy met the highest bioanalytical standards, making it an ideal tool for preclinical pharmacokinetic studies and bioanalytical quantification in biological matrices. The method's success supports its potential for use in Desidustat development and quality control. Additionally, this approach sets forth the groundwork for analysing similar compounds in future pharmaceutical research.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Ayesha Ameen conceptualized the study and designed the methodology, conducted data collection and performed the analysis, contributed to data interpretation and visualization, and drafted the original manuscript. Nagaraju Pappula reviewed and edited the manuscript. All authors read and approved the final manuscript.

REFERENCES

[1] Dhillon S. Desidustat: first approval. *Drugs*, **82**, 1207–1212 (2022) https://doi.org/10.1007/s40265-022-01744-w

[2] Parmar DV, Kansagra KA, Patel JC, Joshi SN, Sharma NS, Shelat AD, Patel NB, Nakrani VB, Shaikh FA, Patel HV. Outcomes of desidustat treatment in people with anemia and chronic kidney disease: a phase 2 study. *Am. J. Nephrol.*, **49**, 470–478 (2019) https://doi.org/10.1159/000500232

[3] Akizawa T, Nobori K, Matsuda Y, Taki K, Hayashi Y, Hayasaki T, et al. Molidustat for the treatment of anaemia in Japanese patients undergoing peritoneal dialysis: a single-arm, open-label, phase 3 study. *Ther. Apher. Dial.*, **26**, 368–377 (2022) https://doi.org/10.1111/1744-9987.13713

[4] Philip M, Karakka Kal AK, Subhahar MB, Karatt TK, Mathew B, Perwad Z. In vitro studies of hypoxia inducible factor–prolyl hydroxylase inhibitors daprodustat, desidustat, and vadadustat for equine doping control. *Drug Test. Anal.*, **14**, 317–348 (2022) https://doi.org/10.1002/dta.3188

[5] Jain M, Joharapurkar A, Patel V, Kshirsagar S, Sutariya B, Patel M, et al. Pharmacological inhibition of prolyl hydroxylase protects against inflammation-induced anemia via efficient erythropoiesis and hepcidin downregulation. *Eur. J. Pharmacol.*, **843**, 113–120 (2019) https://doi.org/10.1016/j.ejphar.2018.11.023

[6] Sharma S, Jain S. Bioanalytical perspectives in drug metabolism studies. *Drug Metab. Bioanal. Lett.*, **16**, 133–139 (2023) https://doi.org/10.2174/2949681016666230828150206

[7] Solanki J, Parikh PK, Chauhan P, Parikh N. Optimizing quality and sustainability in drug development: a comprehensive desidustat stability study using QbD and green chemistry. *J. Liq. Chromatogr. Relat. Technol.*, **48**, 637–652 (2025) https://doi.org/10.1080/10826076.2025.2569880

[8] Reddy GN, Palepu MSK, Sonti R. LC–MS/MS-based investigation of pharmacokinetic interactions between CYP3A4 substrates enzalutamide and repaglinide in rats. *Anal. Methods*, **17**, 9565–9574 (2025) https://doi.org/10.1039/D5AY01451D

[9] Viswanathan CT, Bansal S, Booth B, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. *Pharm. Res.*, **24**, 1962–1973 (2007) https://doi.org/10.1007/s11095-007-9291-7

[10] Zhou S, Chan E, Li SC. Critical review of development, validation, and transfer for high throughput bioanalytical LC–MS/MS methods. *Curr. Pharm. Anal.*, **1**, 3–14 (2005) https://doi.org/10.2174/1573412052953346

[11] Van Eeckhaut A, Lanckmans K, Sarre S, Smolders I, Michotte Y. Validation of bioanalytical LC–MS/MS assays: evaluation of matrix effects. *J. Chromatogr. B*, **877**, 2198–2207 (2009) https://doi.org/10.1016/j.jchromb.2009.01.003

[12] Patel H, Modi N, Chaudhari J, et al. Nonclinical pharmacokinetic evaluation of desidustat: a novel prolyl hydroxylase inhibitor for the treatment of anemia. *Eur. J. Drug Metab. Pharmacokinet.*, **47**, 725–740 (2022) https://doi.org/10.1007/s13318-022-00788-3