https://doi.org/10.48047/AFJBS.6.11.2024. 326-344

African Journal of Biological Sciences

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF DESIDUSTAT IN BULK DRUG AND FORMULATION

Rajendra D. Dighe[1], Gaurav S. Deore[1*], Ganesh B. Sonawane[2] and Vinod A. Bairagi[3]

[1]*Department of Pharmaceutical Quality Assurance, K.B.H.S.S. Trust's Institute of Pharmacy Malegaon, Nashik-423105, Maharashtra, India*
[2]*Department of Pharmaceutical Chemistry, Divine College of Pharmacy, Satana, Nashik-423301, Maharashtra, India*
[3]*Department of Pharmacology, K.B.H.S.S. Trust's Institute of Pharmacy Malegaon, Nashik-423105, Maharashtra, India*
***Corresponding Author-** gauravdeore1907@gmail.com

Article History
Volume 6, Issue 11, 2024
Received: 02 Jun 2024
Accepted: 15 Jun 2024
doi: ***10.48047/AFJBS.6.11.2024. 326-344***

ABSTRACT:

Desidustat is Zydus Cadila's low-dose oral drug designed to treat chronic kidney disease (CKD), coronavirus, and anemia. Prolyl hydroxylase (HIF-PH) is a hypoxia-inducible nuclear factor. Desidustat inhibits the catalytic domain of prolyl hydroxylase and modulates erythropoietin growth and hypoxia-stimulated erythropoiesis-stimulated variable. A specific, sensitive, accurate, and reliable isocratic RP-HPLC method was validated for determination using a thermos fisher C18 particle size analysis column (250 mm x 4.6 mm). A mobile phase of methanol and water (30:70 v/v) at a 0.1 mL/min flow rate was used to prepare the sample. The column temperature and vehicle model are set to ambient conditions. The temperature was 25 3 °C, the injection volume was 20 microliters, and the detector wavelength was 232 nm. The forced degradation of multiple species and drug doses is necessary for this method to demonstrate stability. According to the International Conference on Harmonization (ICH), the developed method was validated for specificity, linearity, precision, accuracy, reliability, limit of detection, and limit of quantification. Stability studies show that major mass degradation occurs under thermal and oxidative conditions. The correlation coefficient of the decidustat calibration curve is 0.999. RSD was less than 2 for precision and robustness.

KEYWORDS: Desidustat, Chronic Kidney Disease, Stability, Forced degradation study, Validation, etc

1. INTRODUCTION:

Chronic kidney disease, also known as chronic kidney failure, is a condition in which the kidneys cease to function properly. Urine is produced by the kidneys and is excreted as waste products and excess blood water [1]. Body liquids, electrolytes, and waste can develop as constant kidney illness advances. Chronic kidney disease (CKD), which affects 8 to 16% of the global population, is frequently misdiagnosed by patients and healthcare professionals. 1-4 Albuminuria is defined as a glomerular filtration rate (GFR) of less than 60 ml/min/1.73 m2. In patients with CKD and renal insufficiency, hematuria or disorders like polycystic or dysplastic kidneys occur less frequently than once every 24 hours and last for more than 90 days.

Desidustat is a low-dose oral drug from Zydus Cadila designed to treat chronic kidney disease (CKD), coronavirus, and anemia. Prolyl hydroxylase (HIF-PH) is a hypoxia-inducible nuclear factor. Decidastat inhibits the catalytic domain of prolyl hydroxylase and stabilizes the production of erythropoiesis-activating erythropoietin and hypoxia-stimulated transforming growth factor [4]. In Walk 2022, Desidustat is the first drug to be approved in time for dialysis treatment in elderly CKD patients instead of dialysis. Desidustat was developed for the treatment of frailty in CKD patients in China, for the management of COVID-19 infection in Mexico, and the treatment of chemotherapy-related frailty in the United States [4].

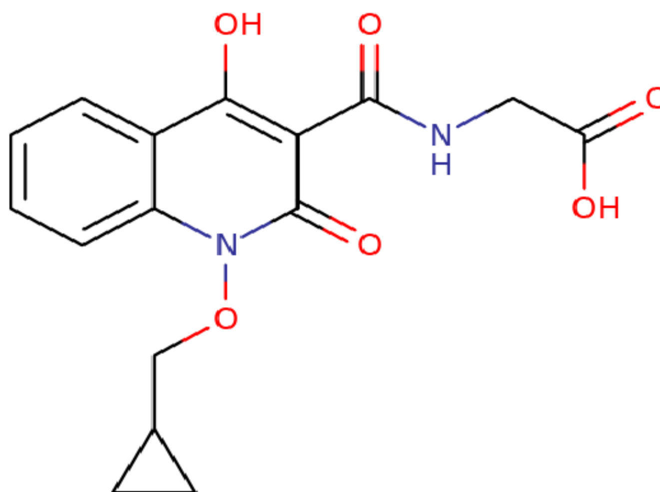

Figure 1: Chemical structure of Desidustat

Weakness is a difficulty of chronic kidney disease (CKD) portrayed by diminished erythropoietin (EPO) and diminished iron digestion. Anemia is linked to lower quality of life,

more cardiovascular events, more dialysis needs, and more death. Traditional medicines incorporate iron enhancements and erythropoietin stimulators (ESAs) [5].

Testing for formulation stability, testing for dispersion, and analyzing raw materials and synthetic products are all quality control measures. HPLC is a novel method for analyzing various samples, and pharmaceutical companies typically need to measure a large number of quality control samples [6, 7]. The point of this study was to create an exact, dependable, fast, and particular HPLC strategy for the assurance of desidustat in tablet measurement structure. Forced degradation techniques like exposure to high temperatures, acidic conditions, and ultraviolet light were used to evaluate the stability of the isolates. The created strategy was completely approved against the ICH rules [8,9] and the desidustat estimations were constrained by the presence of required debasement items. As a result, it can be concluded that this strategy could be used to control desidustat quality in the pharmaceutical sector [10].

The separation method known as high-performance liquid chromatography (or high-performance liquid chromatography) involves introducing the mixture into a stream of dissolved (solid phase) or spherical (stationary phase) particles. These particles cover the film completely. The sample components' differing relative affinities for stationary and mobile phases cause isolation [11-13].

Forced dispersion studies use materials that can be tested with atomic force to make stronger dispersions of drugs and pharmaceuticals under rapid conditions. According to the ICH guidelines, pressure testing is used to test strategies for determining particle stability, determining degradation pathways, identifying potential contamination, and demonstrating robustness. These rules, on the other hand, are limited and do not provide any practical instructions for carrying out stress tests. Although formal corruption investigations are considered unnecessary for formal security programs [14,15,16].

They are an authoritative and calculated necessity for drug recuperation. In this study, a 5 m analytical column was utilized for the separation of desidustat Phenomenex C18 (250 mm X 4.6 mm). Samples were taken in a methanol and water solution mobile phase (70:30% v/v) at a flow rate of 1.0 ml/min. The vehicle model and column temperature are set to ambient conditions. The indicator was set to a frequency of 232 nm and had an infusion volume of 20 µl.

Rajendra D. Dighe / Afr.J.Bio.Sc. 6(11)(2024).326-344

2. MATERIAL AND METHOD:

2.1. Chemicals And Reagents:

Vidisha analytical presented the Pure desidustat medication as a gift. Potassium dihydrogen orthophosphate analytical reagent was purchased from Merck and Siddhi Lab. Acetonitrile, methanol, and water from (qualigens) were of HPLC grade.

2.2. Instrumentation:

An HPLC-1260 infinity II (Agilent) was used for the method development and validation HPLC binary gradient system, Detector (DEAX02386), Double beam UV visible spectroscopy (Jasco), Weighing Balance (CY224C) from Aczet for sample weighing, Bio-technic Ultra Sonicator (13.5L) and pH meter from Lab Man used for sample preparation.

2.3. Selection Of Solvents:

Methanol was selected as the solvent for dissolving desidustat.

2.4. Preparation Of Standard Stock Solutions:

Transfer 10 mg of desidustat to a 10 mL volumetric flask, add 7 mL of methanol until the standard is completely dissolved, and then dissolve in methanol (1000 ppm dilution) to prepare the stock solution. Then, at that point, 0.4 ml was blended in with 20 ml of methanol (20 ppm) and 0.1 ml with 20 ml of methanol (5 ppm).

2.5. Selection Of Analytical Wavelength:

Methanol blank and Desidustat standard solutions (20 and 5ppm) were scanned from 400nm to 200nm. The maximum absorption of the drug is determined. The results show that desidustat has maximum absorption at 232 nm.

3. METHOD DEVELOPMENT BY RP – HPLC:

3.1. Preparation Of Standard Stock Solution For Chromatographic Development:

To make a standard stock solution of desidustat, dissolve 10 mg of the drug in a dry, clean volumetric flask that is 10 mL in volume, add about 7 mL of methanol until the drug is

Rajendra D. Dighe / Afr.J.Bio.Sc. 6(11)(2024).326-344

completely dissolved, and then make up the volume with methanol (prepared to 1000 ppm). An additional 1 ml of the stock solution with the mobile phase of each experiment was diluted to 10 ml and injected into each experiment (100 ppm).

3.2. Selection Of Analytical Wavelength For HPLC Method Development:

The spectrophotometric maximum absorption wavelength was used as the analytical wavelength for the experiment, which was 232 nm.

3.3. Chromatographic Condition:

Desidustat was separated using a thermo fisher C18 analytical column with a particle size of 5 m (250 mm x 4.6 mm). The samples were extracted using a 0.1 ml/min mobile phase solution of methanol and water (30:70 vol/vol). The vehicle model and column temperature are set to ambient conditions. The injection volume was 20 microliters, the detector wavelength was 232 nm, and the temperature was 25 ± 3 °C.

3.4. Preparation Of System Suitability Test (Desidustat Standard Solution):

After weighing a volumetric flask of 20 milliliters and adding 15 milliliters of methanol, which was then sonicated to dissolve, the volume was filled to the brim with methanol. Pipette one milliliter of the standard stock arrangement into a volumetric cup that holds ten milliliters and mark it with the portable stage (100 g/ml = working focus). The chromatogram was then taken.

A pharmaceutical requirement called "system compatibility" is used to make sure that a chromatographic system is good for analysis. The test was performed by gathering information from five infusions of a standard medication arrangement and recording the outcomes.

3.5. Method Validation:

Chromatographic conditions are optimized for specificity, linearity, accuracy, precision, range, the limit of quantification (LOQ), the limit of detection (LOD), robustness, and system consistency parameters according to ICH Q2 (R1) guidelines [17].

4. RESULTS AND DISCUSSION:

Table 1: Method Development Initial Study

Column Used	Mobile Phase Ratio	Detection Wavelength	Flow Rate	Injection Volume	Run Time	Result	Conclusion
Phenomenex C17 (250 mm X 4.6mm ID, 5 µm)	Methanol : Water (70:30)	232 nm	1.0 ml/min	20µl	12 min	The resolution was not satisfactory	Method unaccepted
Phenomenex C17 (250 mm X 4.6mm ID, 5 µm)	Acetonitrile : Water (70:30)	232 nm	1.0 ml/min	20µl	12 min	Desidustat not eluted until 20 minutes	Method unaccepted
Phenomenex C17 (250 mm X 4.6mm ID, 5 µm)	Methanol : 0.05% OPA in Water (70:30)	232 nm	1.0 ml/min	20µl	12 min	Desidustat eluted at 7.5 minutes with unacceptable chromatography	Method unaccepted
Phenomenex C17 (250 mm X 4.6mm ID, 5 µm)	Acetonitrile : 0.05% OPA in Water (70:30)	232 nm	1.0 ml/min	20µl	12 min	Desidustat eluted at 4.11 with acceptable chromatography	Method Accepted

4.1. Optimization of RP-HPLC Method Chromatogram:

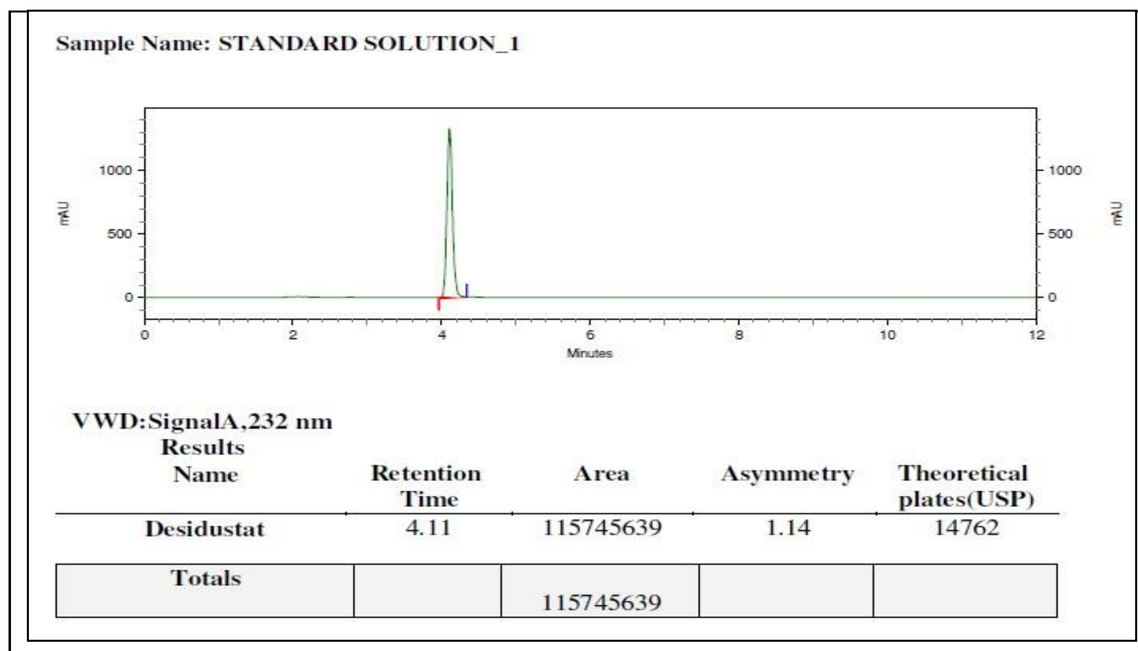

Fig. No. 2. Typical chromatogram Optimization of RP-HPLC Method Chromatogram

4.2. Filtration study:

An unfiltered centrifuged sample was used in the filter study. The example was then gone through a 0.45 PVDF channel and a 0.45 Nylon channel, with 5 mL of solution being discarded. (Sample of the mixture of tablets used in the filter study)

Table 2: Observations of the filtration study

Sample	Area	% Absolute difference
Unfiltered	114185417	NA
0.45 µ PVDF filter	114056381	0.11
0.45 µ Nylon filter	113856962	0.29

PVDF and nylon filters both meet the requirements for filter study, so they can be used.

4.3. Stability of analytical solution:

Rajendra D. Dighe / Afr.J.Bio.Sc. 6(11)(2024).326-344

Standards and test sample solutions were subjected to stability tests. Standard laboratory conditions were used for stability testing. For 12 to 24 hours, the solution is kept in typical laboratory lighting and analyzed. Dependability investigations of standard and test arrangements are completed by ascertaining the contrast between the test aftereffects of the arrangements at every strength point.

Table 3: Stability of analytical sample solution and standard solution

Sample solution			Standard Solution		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	114025634	NA	**Initial**	115685230	NA
12 Hours	113654178	0.33	**12 Hours**	115449857	0.20
24 Hours	112956967	0.94	**24 Hours**	114759615	0.80

*If the user wishes to inject the solution after 24 hours, they can check its stability.

Because both the standard solution and the sample solution were found to be stable for 24 hours, the prepared solution can be used for up to 24 hours.

4.4. Specificity:

The capacity to effortlessly access analytics using the anticipated component is one feature. Injecting a tablet sample, a placebo, a standard solution, and a blank to ensure maximum purity. The control was invalid and fake treatment didn't slow down R.T. Desidustat.

A standard solution that uses the same model solution R.T. As a result, the new chromatographic technique exceeds the specificity requirements.

4.5. Linearity:

Preparation of linearity solution

Five levels, ranging in concentration from 80% to 120%, have been prepared. Each level was injected three times. Conce plotted a linear graph. versus Mean Area. Determined capture, slant, and relapse coefficient.

Table 4: Linearity stock preparation

Standard weight	Diluted to (ml)	Volume taken (ml)	Diluted to (ml)	Conc. (µg/ml)
50	50	1	1	1000.00

Table 5: Linearity levels preparation assay of Desidustat

<table>
<tr><th>Level</th><th>Conc. (µg/ml)</th><th>ml of Stock</th><th>Diluted to</th><th>Area</th><th>Means</th><th>%RSD</th></tr>
<tr><td rowspan="3">80%</td><td rowspan="3">80.00</td><td rowspan="3">1.6</td><td rowspan="3">20</td><td>92651406</td><td rowspan="3">92663366</td><td rowspan="3">0.091</td></tr>
<tr><td>92753221</td></tr>
<tr><td>92585472</td></tr>
<tr><td rowspan="3">90%</td><td rowspan="3">90.00</td><td rowspan="3">1.8</td><td rowspan="3">20</td><td>104520217</td><td rowspan="3">104365621</td><td rowspan="3">0.132</td></tr>
<tr><td>104321659</td></tr>
<tr><td>104254986</td></tr>
<tr><td rowspan="3">100%</td><td rowspan="3">100.00</td><td rowspan="3">2.0</td><td rowspan="3">20</td><td>115748898</td><td rowspan="3">115724149</td><td rowspan="3">0.081</td></tr>
<tr><td>11562029</td></tr>
<tr><td>115803219</td></tr>
<tr><td rowspan="3">110%</td><td rowspan="3">110.00</td><td rowspan="3">2.2</td><td rowspan="3">20</td><td>126854957</td><td rowspan="3">126802830</td><td rowspan="3">0.040</td></tr>
<tr><td>126754983</td></tr>
<tr><td>126798549</td></tr>
<tr><td rowspan="2">120%</td><td rowspan="2">120.0</td><td rowspan="2">2.4</td><td rowspan="2">20</td><td>139152329</td><td rowspan="2">139361679</td><td rowspan="2">0.156</td></tr>
<tr><td>139345216</td></tr>
</table>

				139587492		

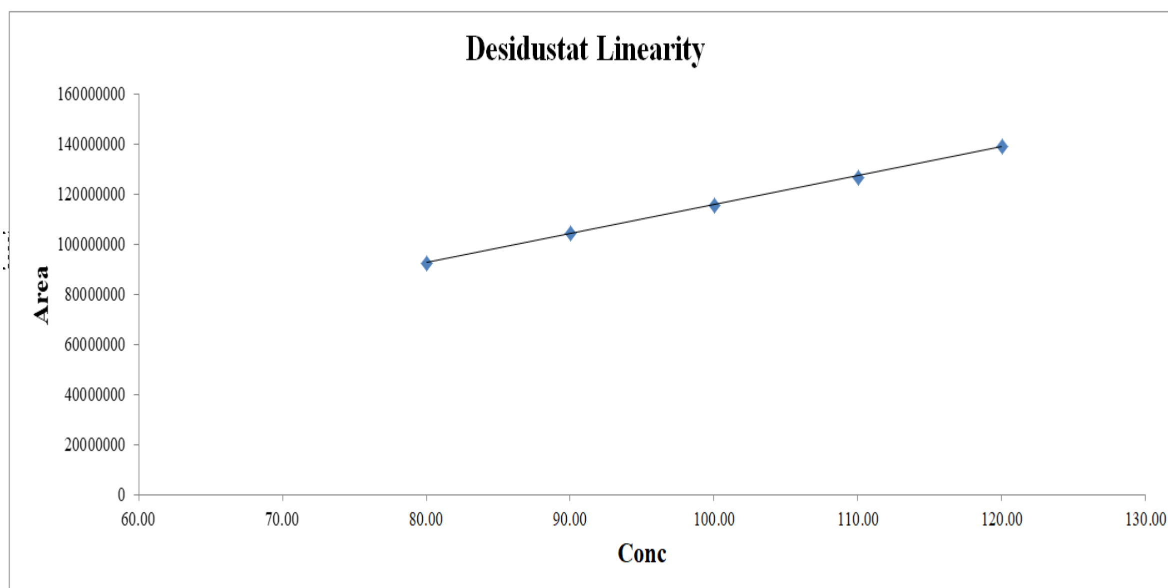

Figure No. 6: Calibration plot obtained for assay of Desidustat

The regression coefficient was found to be well within the accepted range.

4.6. Accuracy (% Recovery):

The degree to which the found value and the true value of the standard or accepted reference value are in close agreement is referred to as the analytical procedure's accuracy.

The accuracy will be between 80 and 120 percent of the functioning fixation. For each level of precision, three copies of the solutions were made. The mean recovery, the total recovery for each level, the percent RSD for each level, and the percent RSD for the total recovery were all determined.

Table 7: Accuracy Data for Desidustat

Level (80%)	Area	Recovered Conc.	Added Conc.	% Recovery	Mean Recovery	%RSD	Overall Recovery	% RSD overall
	92541235	80.04	80.40	99.55				

80	92612524	80.11	80.60	99.39	99.77	0.5212	99.73	0.684
	93986528	81.29	81.00	100.36				
100	115849561	100.20	100.60	99.60	99.82	0.5831		
	114663514	99.18	99.80	99.38				
	116396840	100.68	100.20	100.48				
120	139485634	120.65	119.60	100.88	99.62	1.1079		
	137985418	119.35	120.40	99.13				
	138045975	119.40	120.80	98.84				

% Recovery was found well within the acceptance range at all three levels.

4.7. Precision:

i. Precision Repeatability:

The precision of an analytical procedure is the degree of agreement between measurements taken from several identical test samples under specific conditions. Stretch exactness and repeatability are the two types of precision. A test model is where the tablet runs.

Precision was achieved through the preparation of six test samples (Tablet 6).

Table 8: Precision (repeatability) data

Sample	Area	% Assay
Sample 1	114052631	98.49
Sample 2	114585794	99.03
Sample 3	113251041	97.64

Sample 4	113659873	98.78
Sample 5	113956428	98.49
Sample 6	114163195	99.06
Mean		98.58
STD DEV		0.5240
% RSD		0.532

Precision passes the models, and six distinct tests show that there is no variation. Replicating the results is simple.

ii. Intermediate precision:

To verify the reproducibility of the results, is carried out by analyzing on a different day. Samples that were prepared in the same manner as the Repeatability parameter.

Table 9: Intermediate precision data

Sample	Area	% Assay
Sample1	113232516	97.47
Sample 2	113526312	98.35
Sample 3	113648573	98.54
Sample 4	114053164	99.13
Sample 5	114256896	98.59
Sample 6	113885219	98.43
Mean		98.42
STD DEV		0.5394
% RSD		0.548

Precision Plus Intermediate precision	Mean	98.500
	STD DEV	0.5141
	% RSD	0.522

4.8. Limit of detection (LOD) and limit of quantification (LOQ):

According to ICH rules, the limits of detection (LOD) and quantification (LOQ) were set at 3.3 SD/S and 10 SD/S, respectively. SD is the standard deviation of the reaction (Y-capture) and S is the slant of the alignment bend. The least analyte focus that produces a quantifiable response is the LOD, which has a motion toward a clamor proportion of 3. The LOQ is the most minimal analyte focus that gives a distinct and quantifiable reaction (motion toward commotion proportion of 10). The determined LOD and LOQ values are displayed in Table 6.

$$LOD = 3.3 \times S / SD$$

and

$$LOQ = 10 \times S / SD$$

Table 10: Limit of detection (LOD) and limit of quantification (LOQ) data for desidustat

Sr. No.	Drug	SD	LOD	LOQ
1	Desidustat	360730.196	1.03 pmm	3.11 ppm

4.9. Robustness:

The strength of a legitimate method is the extent of its capacity to remain unaffected by little, yet deliberate assortments in system limits and offers a hint of its immovable quality during customary use.

Determination: Standard arrangements were infused under various chromatographic circumstances as displayed in Table 9.

Rajendra D. Dighe / Afr.J.Bio.Sc. 6(11)(2024).326-344

Table 11: Method robustness for desidustat

Sr. No.	Conditions	Standard Solution	R.T.	Area	Asymmetry	Theoretical plate
1	Change in wavelength: +3 nm	Standard	4.11	94163279	1.16	15180
2	Change in wavelength: -3 nm	Standard	4.11	85164683	1.13	15149
3	Change in flow rate + 10%	Standard	3.73	81888911	1.11	14084
4	Change in flow rate - 10%	Standard	4.56	100360937	1.17	16009
5	Change in column over temperature: +2ºC	Standard	4.09	115965428	1.13	14846
6	Change in column over temperature: -2ºC	Standard	4.12	114853274	1.15	14564

Chromatography was not compromised by changes in ± 3 nm

5. FORCED DEGRADATION STUDY:

Force degradation is the more severe breakdown of drugs and pharmaceutical products under accelerated conditions, providing degraded materials that can be tested for atomic forces. The objective of force degradation studies was to validate the effective separation of desidustat from its degradation product. According to ICH regulations, pressure testing is used to find degradation pathways, identify potential adverse events, analyze the internal stability of particles, and evaluate robustness-demonstration procedures. The outcomes of forced degradation studies are shown in a table representable form in Table No. 10.

Table 12: Forced Degradation Study

Sr. No.	Degradation Parameter	% Degradation of Desidustat
1.	Acid Degradation (5 N HCl)	7.95 %
2.	Base Degradation (1 N NaOH)	9.49 %
3.	Peroxide Degradation (30% H_2O_2)	Nil
4.	Thermal Degradation (105°C for 47 Hours)	Nil
5.	Photolytic Degradation (Direct sunlight for 72 hours)	Nil

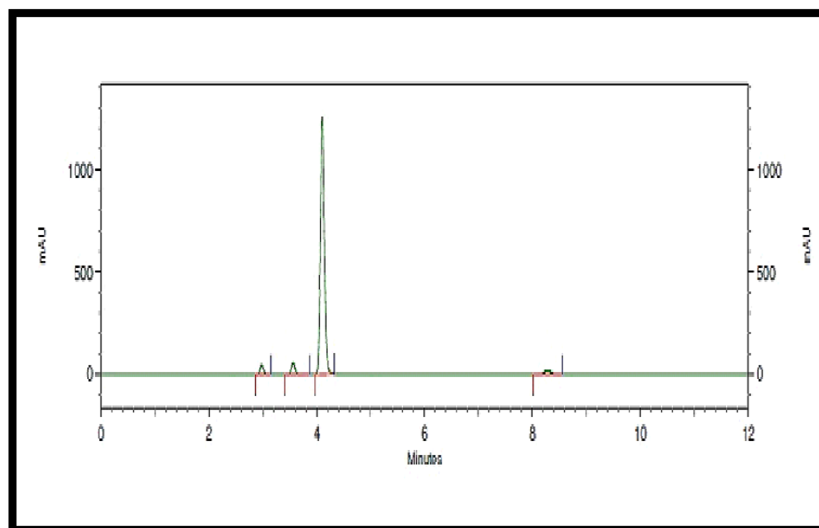

Figure No. 4: RP-HPLC Acid Degradation Chromatogram

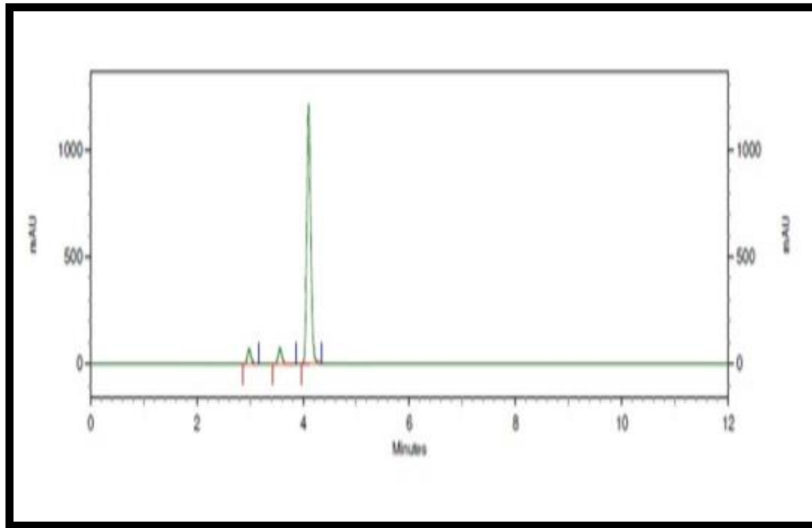

Figure No. 5: RP-HPLC Base Degradation Chromatogram

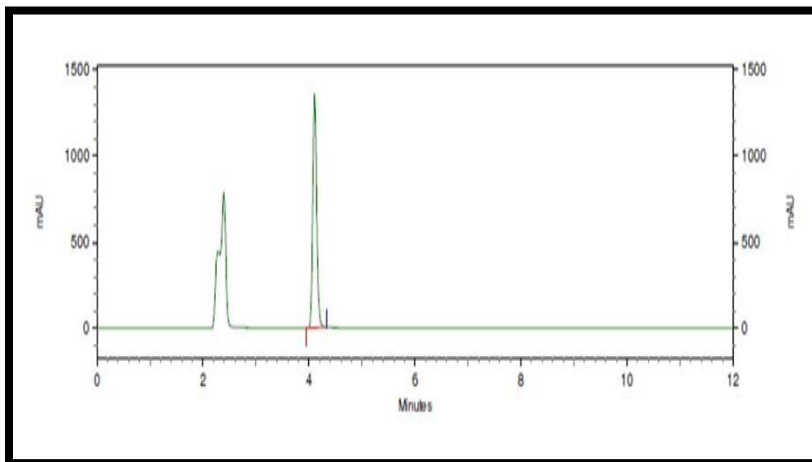

Figure No. 5: RP-HPLC Peroxide Degradation Chromatogram

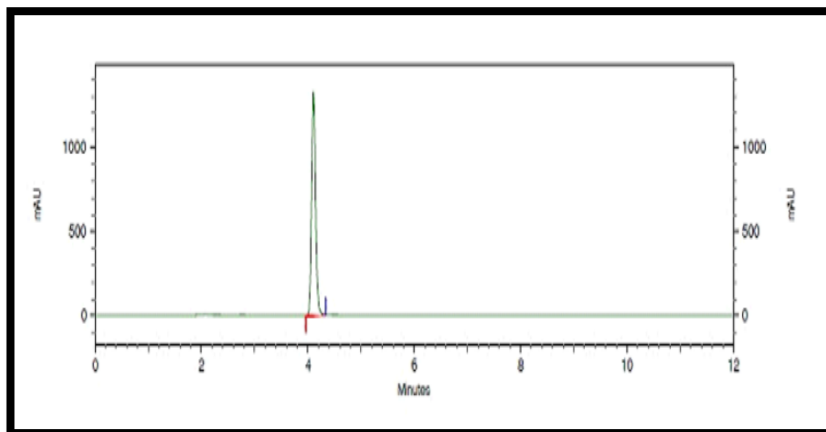

Figure No. 5: RP-HPLC Thermal Degradation Chromatogram

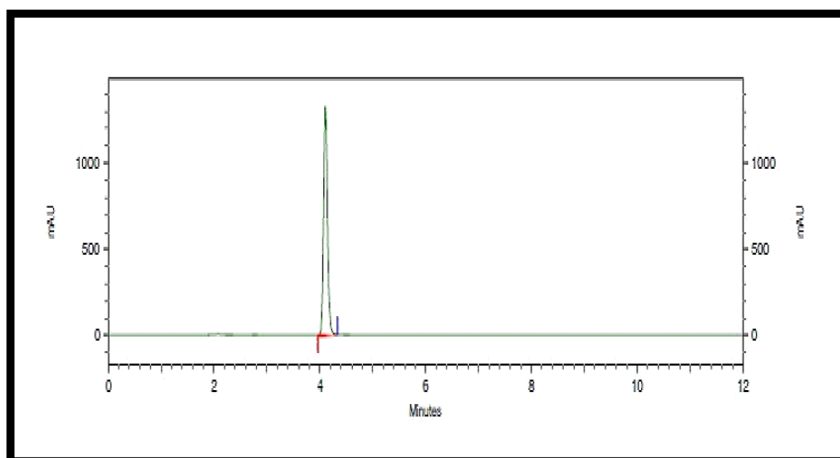

Figure No. 6: RP-HPLC Photolytic Degradation Chromatogram

CONCLUSION:

A fast and accurate isocratic RP-HPLC strategy was created and approved for assurance of a desidustat measurement structure as per ICH rules. For desidustat, the linearity of the arrangement was 5 degrees of working centralizations of 80, 90, 100, 110, and 120%. The RSD rate is under 2%, which demonstrates the precision of the created strategy. The upsides of this technique are precise capacity time and high awareness. To acquire the ideal chromatographic circumstances, the impact of the natural synthesis on the portable stage stream rate and framework similarity boundaries was contemplated. The results obtained show that the developed method is effective in terms of precision, accuracy, linearity, LOD, LOQ and

robustness. Therefore, this method can be used for systematic screening of desidastat drugs in pharmaceuticals. Therefore, this method was successfully used for routine analysis of desidustat.

REFERENCES:

1) Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. JAMA. 2007;298(17):2038–2047.

2) Hsu CY, Vittinghoff E, Lin F, Shlipak MG. The incidence of end-stage renal disease is increasing faster than the prevalence of chronic renal insufficiency. Ann Intern Med. 2004;141(2):95–101.

3) Plantinga LC, Boulware LE, Coresh J, et al. Patient awareness of chronic kidney disease: trends and predictors. Arch Intern Med. 2008;168(20): 2268–2275.

4) Dhillon S. Desidustat: First Approval. Drugs. 2022;82(11):1207-1212.

5) Patel R, Yadav P. Stability indicating RP-HPLC method development and validation for estimation of desidustat in tablet dosage form. 2023;12(7):1290-1304.

6) Dong MW. Handbook of pharmaceutical analysis by HPLC. Elsevier: United Kingdom, 2005;6:2-3.

7) International Conference on Harmonization (ICH). Text on validation of analytical procedure: methodology: Q2(R1), 2005. Available at: Accessed on: 19 May 2012.

8) ICH Harmonized Tripartite Guideline. Pharmaceutical quality systems Q10. International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use 2008.

9) ICH Harmonized Tripartite Guideline. Pharmaceutical development Q8(R2) International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use 2009.

10) Jaivik PM. Stability Indicating RP-HPLC Method Development and Validation for the Analysis of Desidustat in Tablet Dosage Form. IJNRD. 2023;8(6):b55-b73.

11) Bavand Savadkouhi M, Vahidi H, Ayatollahi AM, Hooshfar S, Kobarfard F. RP-HPLC Method Development and Validation for Determination of Eptifibatide Acetate in Bulk Drug Substance and Pharmaceutical Dosage Forms. Iran J Pharm Res. 2017;16(2):490-497.

12) Bhatt DA, Rane SI. QbD approach to analytical RP-HPLC method development and its validation. Int J Pharm Pharm Sci. 2011;3(1):179–187.

13) Bhawar HS, Thete S, Shinde GS. Development and validation of stability indicating RP-HPLC method for estimation of brexpiprazole from bulk and tablet form. J Drug Deliv Therapeut. 2019;9(4):141–145.

14) Singh S., Junwal M., Modhe G., Tiwari H., Kurmi M. Forced degradation studies to assess the stability of drugs and products. Trends. Anal. Chem. 2013;49:71-88.

15) Singh R., Rehman Z.U. Current trends in forced degradation study for pharmaceutical product development. J. Pharm. Educ. Res. 2012;3(1):54-63.

16) ICH guidelines, Q1A (R2): Stability Testing of New Drug Substances and Products (revision 2), International Conference on Harmonization. 2003.

17) Maithani M, Swivedi D, Gupta V, Bansal P. Analytical Method Development and Validation of Alectinib by RP-HPLC Technique. 2023;28(3):406-415.