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"Stability Indicating RP-HPLC Method Development and Validation for the Analysis of Desidustat in Tablet Dosage Form"

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ABSTRACT:

A simple, accurate, precise, rapid, specific, sensitive and selective Reverse Phase HPLC method was developed and validated for stability indicating RP-HPLC method development & validation for the analysis of Desidustat in Tablet Dosage Form. The approach was validated for Desidustat using ICH recommendation over a range of 3-7 ppm. An analytical column C18 (25cm × 0.46 cm) Hypersil BDS was used. At a flow rate of 1.0 ml/min, the mobile phase was Methanol: Acetonitrile (70:30 % v/v). The elution was examined using detector SPD-20A with detection wavelength of315 nm. The retention time for Desidustat is 9.520 Accuracy, Precision, Specificity, Sensitivity & Selectivity was ni accordance with ICH guideline Q1A (R2). The method can be successfully employed for the stability indicating RP-HPLC method development & validation for the analysis of Desidustat in Tablet Dosage Form.

KEYWORDS: Desidustat in RP-HPLC Method

INTRODUCTION

- High performance (or high pressure) liquid chromatography (HPLC) is a separation technique in which a sample mixture is introduced into a stream of solvent (the mobile phase) which then flows over a surface (the stationary phase) consisting of either spherical particles or a film coated onto such particles. Separation results from differences between sample components in their relative affinity for the stationary and mobile phases.

Basic Diagram of HPLC is Shown Below in Figure 1.1

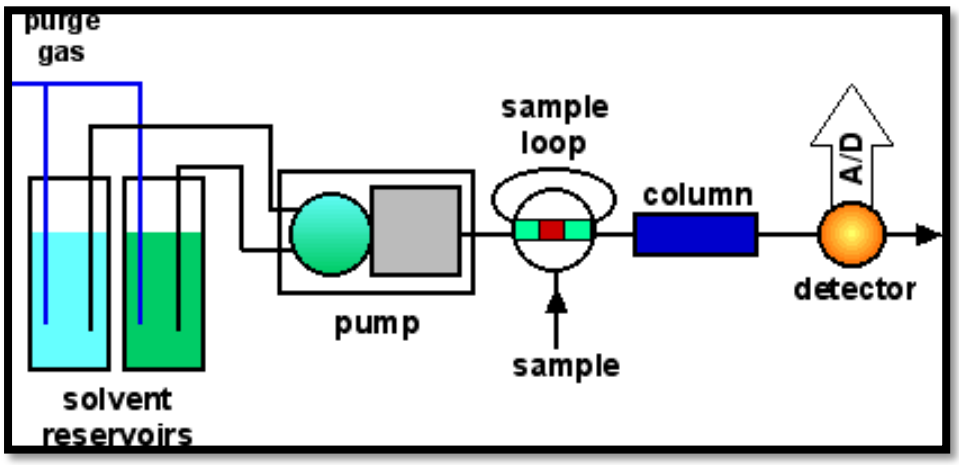
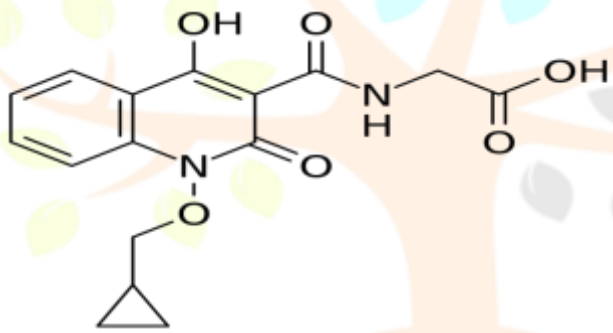


Fig. 1.1: Basic Diagram of HPLC

- There are no any RP-HPLC methods reported for estimation for the desidustat.
- Desidustat is use for treatment of anemia associated with chronic kidney disease of the hypoxia-inducible factor prolyl hydroxylase inhibitor class.



Structure of Desidustat,

Materials and Methods

- **Instrument specification**

Table 1 Instrument specification for High Performance LC

Make	Shimadzu
Model	LC-20AT
Type	Binary Gradient
Detector	SPD-20A
Software	Spinchrom
Column	C_{18} (25cm x 0.46 cm) Hypersil BDS
PH-meter	Systronic

Table 1 Instrument specification for weighing balance

Make	Mettler Toledo
Melting point apparatus name	Analab

Table 2 Instrument specification for melting point apparatus

Make	Shimadzu
Model	LC-20AT
Type	Binary Gradient
Detector	SPD-20A
Software	Spinchrom

HPLC METHOD DEVELOPMENT

❖ Selection of wavelength:

✓ The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. Drug solution of Desidustat (5 ppm) was prepared in Methanol. This drug solution was scanned in UV region of 200-400 nm and overlay spectrums were recorded. So, 315 nm was selected for simultaneous estimation of drug in RP-HPLC method.

Sample preparation

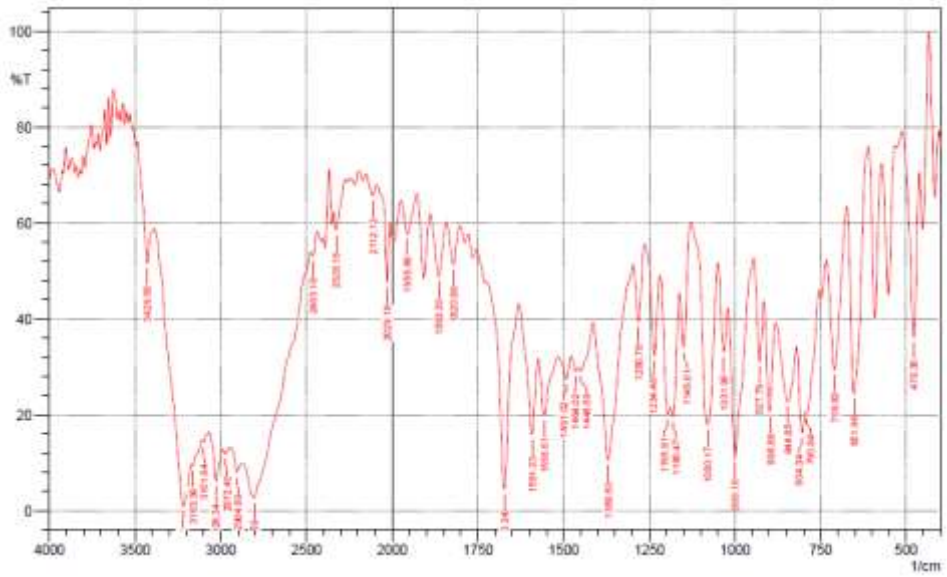
	Preparation of solution
Preparation of Hydrochloric acid solution (0.1 N)	0.86 ml of Concentrated HCl transferred in 100 ml volumetric flask and dilute with methanol upto 100 ml.
Sample Stock Solution (Desidustat 50 ppm):	Take tablet Powder equivalent to 50 mg of Desidustat was transferred into 100 ml volumetric flask, Add 50 ml of Methanol and Shake for 15 min and made up volume with Methanol. The solution was filtered through what man filter paper.

- **RESULTS AND DISCUSSION**

Table 2 Melting point data of **Desidustat**

Drug	**Reported Melting Point** [18]	**Observed Melting Point**
Desidustat	212-214°C	211-213°C

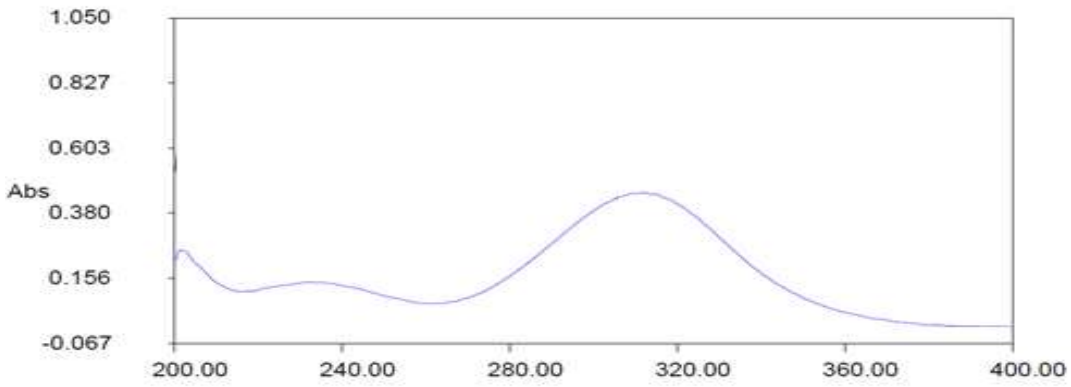
Identification by IR Spectra



Interpretation of FT-IR Spectra of Desidustat

Functional Group	Observed Frequency (cm^{-1})
N-H	1456
S=O	1131
C=C	1564
C-N	1030

❖ **Selection of Analytical/Detection wavelength**



UV Spectra of Desidustat (5 ppm) at 315 nm

❖ **Selection of Mobile Phase:**

Trial contains various mobile phase which are considered of Methanol, Water and Acetonitrile in different proportions and different volumes at different flow rate were tried. On the basis of various trial the mixture of Methanol : Acetonitrile 70:30 %v/v, at 1.0 ml/min flow rate, proved to be better than the other mobile phase in terms of peak shape, theoretical plate and asymmetry.

Sr. No	Mobile Phase	Remark
1	Water : Methanol (50:50 %v/v)	No Peak observed
2	Water : Methanol (40:60 %v/v)	Peak of Desidustat confirmed With Irregular Shape
3	Water : Methanol (30:70 %v/v)	Peak of Desidustat confirmed but not sharp
4	Methanol : Acetonitrile (50:50 %v/v)	No changes occurred in Peak shape by used Acetonitrile in place of Water
5	Methanol : Acetonitrile (70:30 %v/v)	Good Resolution & sharp peak of both drugs obtained. (Rt: Desidustat 9.520 min).

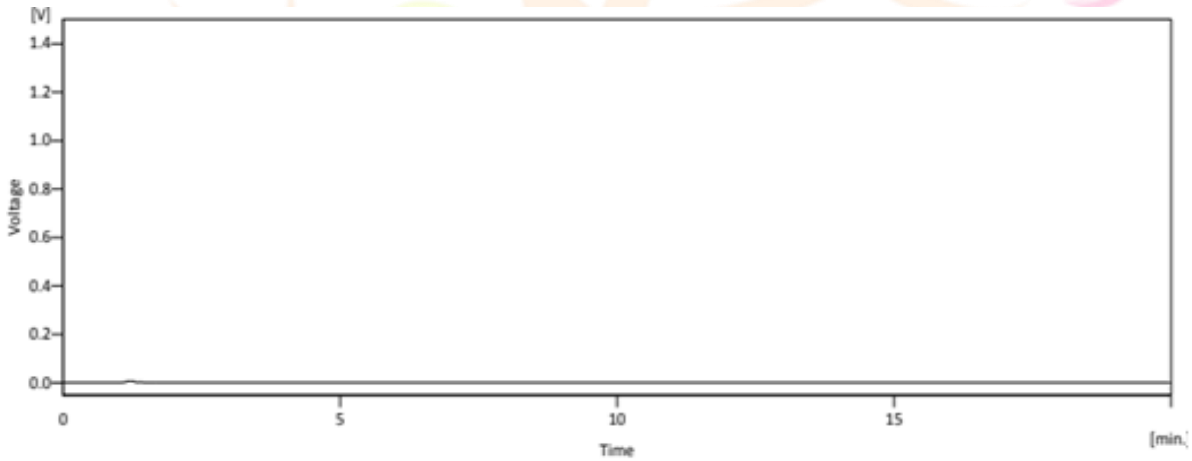


Figure: HPLC Chromatogram of Desidustat in Methanol : Water

(50:50)

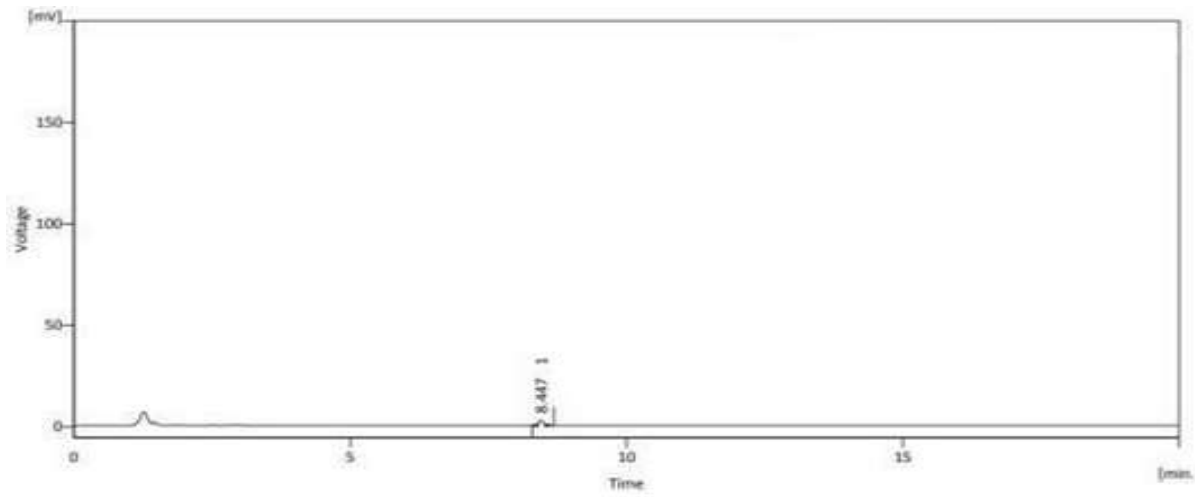


Figure: HPLC Chromatogram of Desidustat in Methanol : Water

(60:40)

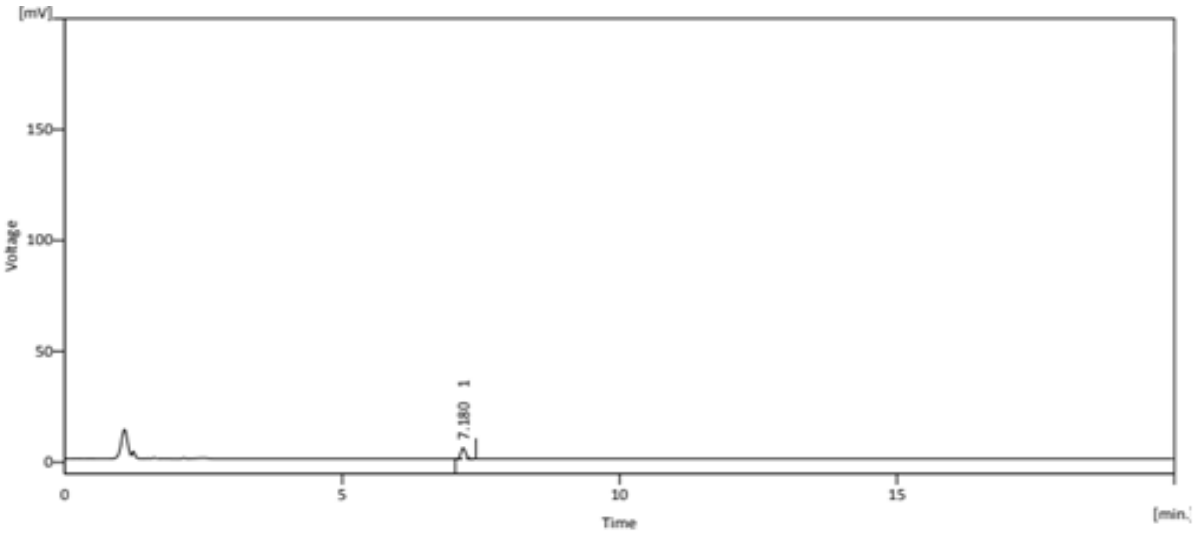


Figure: HPLC Chromatogram of Desidustat in Methanol : Water(70:30)

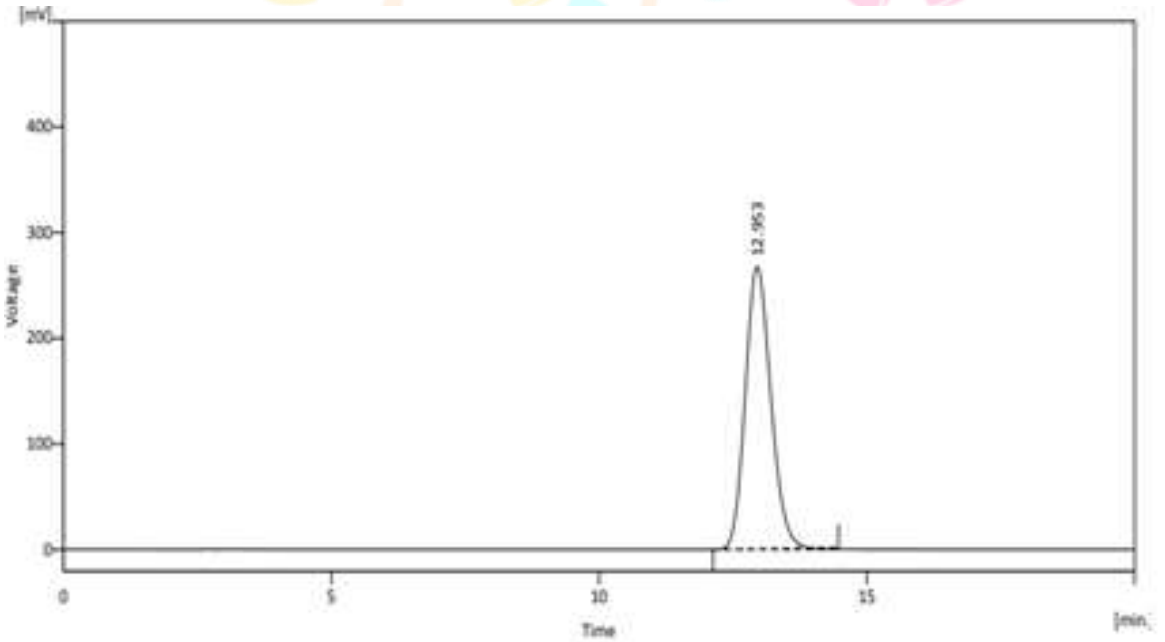


Figure: HPLC Chromatogram of Desidustat in Methanol : Acetonitrile

(50:50)

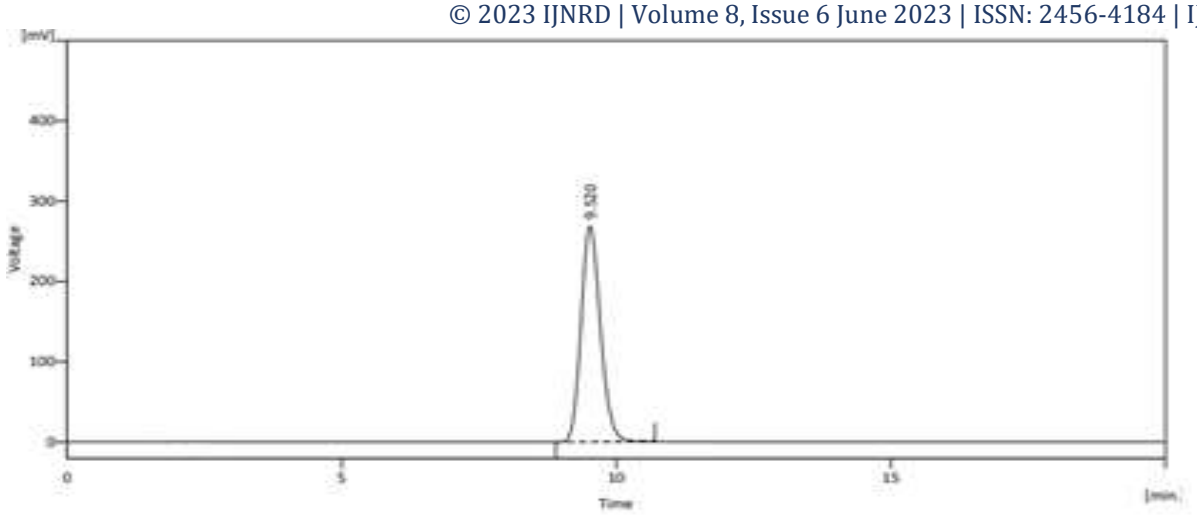


Figure: HPLC Chromatogram of Desidustat in Methanol : Acetonitrile (70:30)

1.1.1. Stability indicating RP-HPLC method for simultaneous estimation of Desidustat

- To calculate the standard area and sample response below confirmation runs were taken as a part of the analysis.

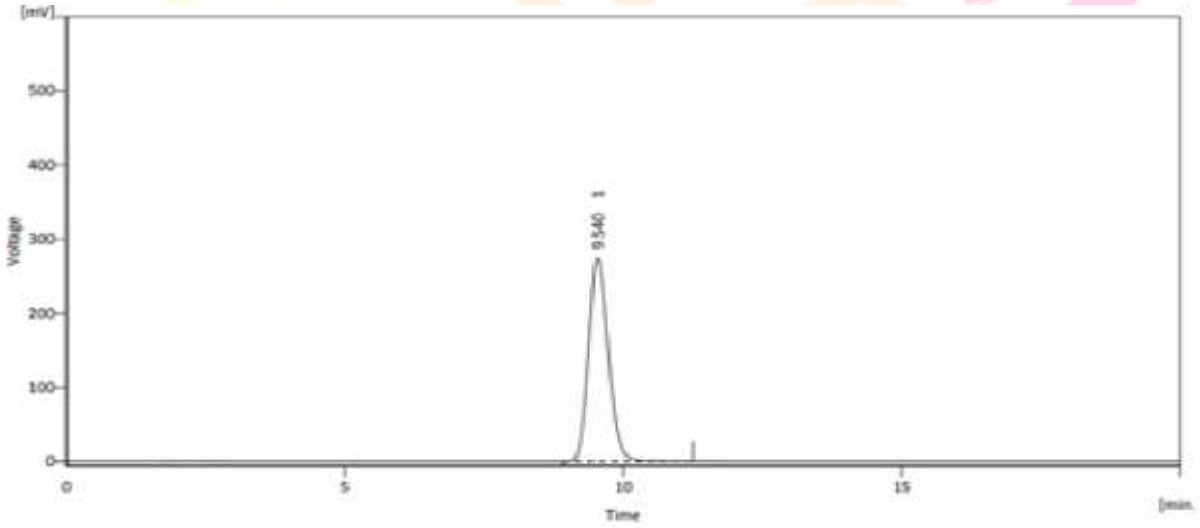


Figure: Desidustat standard stability

Desidustat	
Area	**6566.48**

1.1.2. Acid Degradation:

After degradation the drug solution with 0.1 N Hydrochloric acid for 4 hour, 1.687-17.635 % degradation was observed in Desidustat.

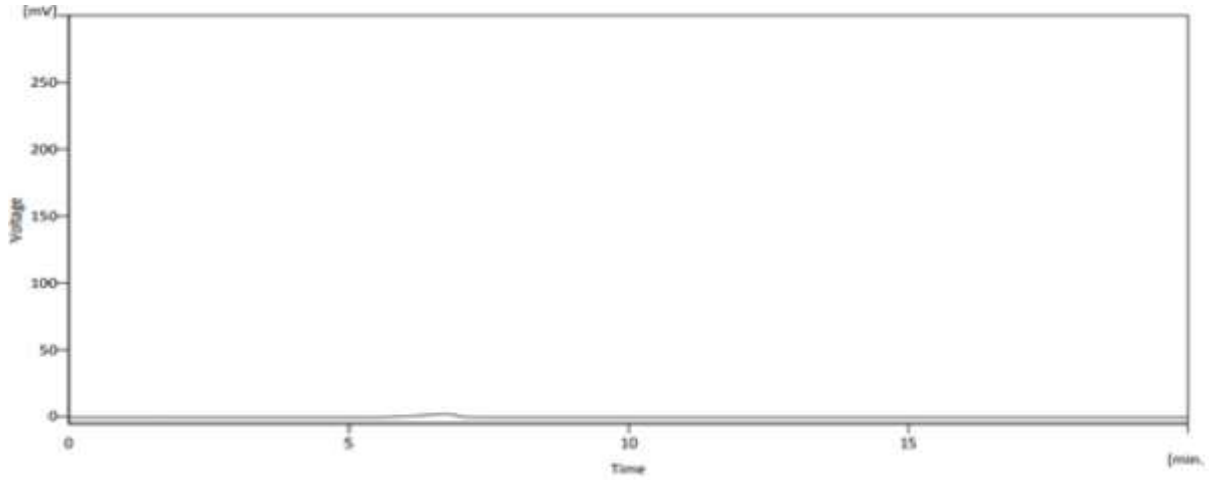


Figure: Acid Degradation Blank

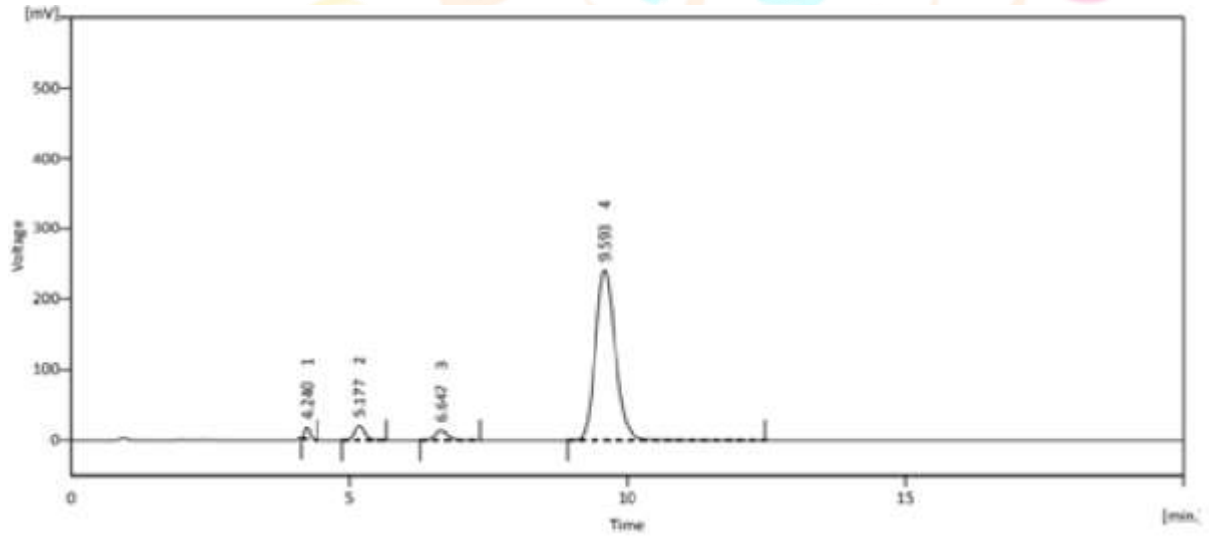


Figure: Chromatogram of Acid Degradation of Desidustat

Table: Results of Acid Degradation study of Desidustat

Parameter	Reagent for Degradation	Reagent for Neutralization	Time	Area of sample	% degradation of sample
Acid Desidustat	2ml 0.1N HCl	2ml 0.1N NaOH	1 hr	1371.452	1.687
			2hrs	1393.308	5.798
			3 hrs	1111.594	10.686
			4 hrs	1015.179	17.635

1.1.3. Base Degradation:

After degradation the drug solution with 0.1 N Sodium Hydroxide for 4 hour, 3.21-16.275% degradation was observed in Desidustat.

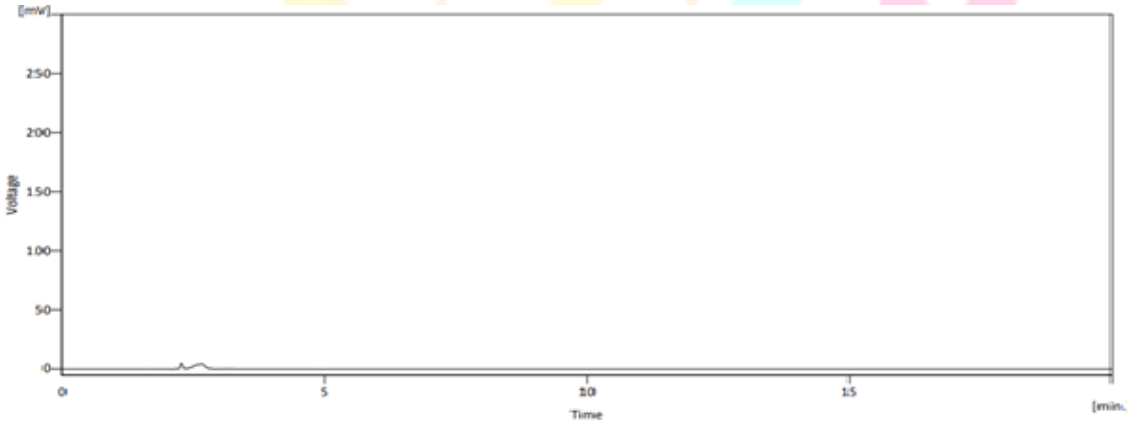


Figure: Chromatogram of Base Degradation Blank

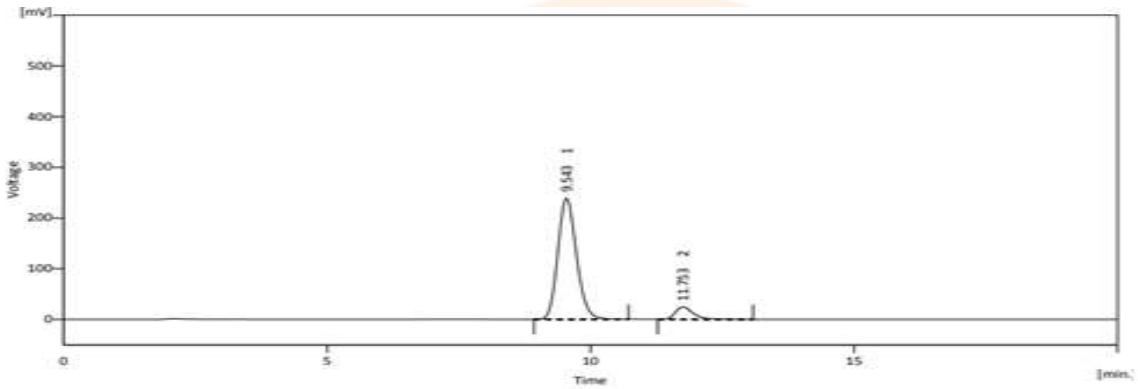


Figure: Chromatogram of Base Degradation of Desidustat

Table: Results of Base Degradation study of Desidustat

Parameter	Reagent for Degradation	Reagent for Neutralization	Time	Area of sample	% degradation of sample
Base Desidustat	2ml 0.1N NaOH	2ml 0.1N HCl	1 hr	1242.253	3.251
			2hrs	1159.190	8.257
			3 hrs	1100.226	11.506
			4 hrs	1075.683	16.275

1.1.4. Oxidation Degradation

After degrading the drug solution with 3% Hydrogen peroxide for 4 hours, 3.794-16.734% degradation observed in Desidustat.

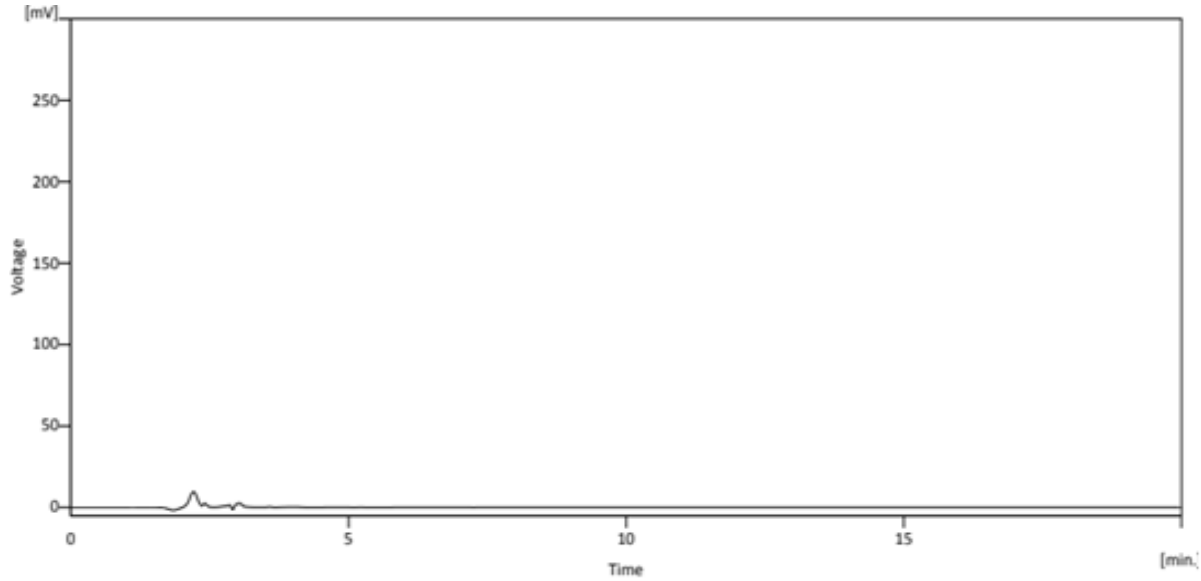


Figure: Chromatogram of Oxidation Degradation (Blank)

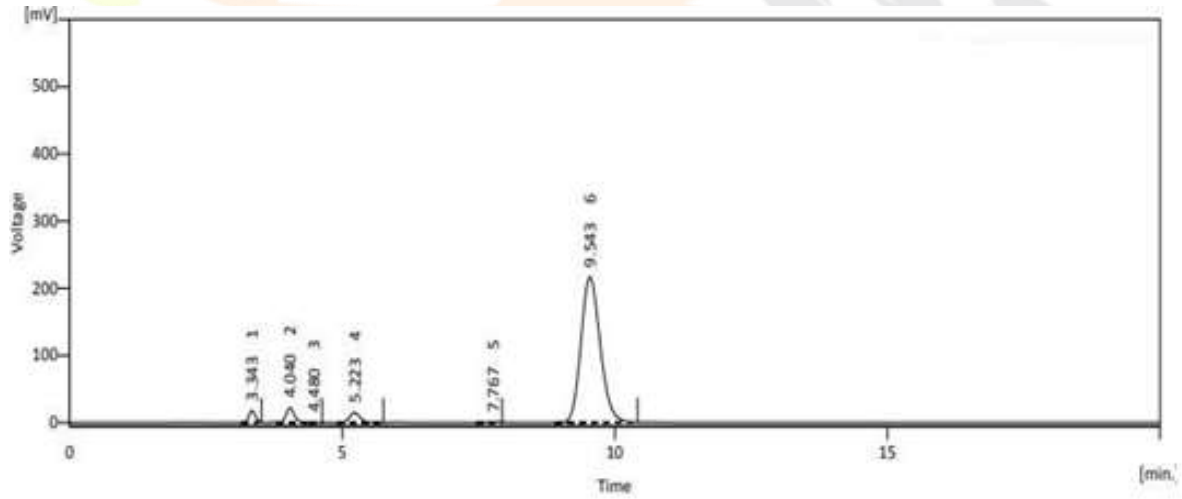


Figure: Chromatogram of Oxidation of Desidustat

Table: Results of Oxidation Degradation study of Desidustat

Parameter	Reagent for Degradation	Reagent for Neutralization	Time	Area of sample	% degradation of sample
Oxidation Desidustat	3 % H_2O_2	--	1 hr	1448.870	3.794
			2 hrs	1397.510	5.495
			3 hrs	1263.958	9.913
			4 hrs	1069.317	16.734

1.1.5. Photo Degradation

When drug solution was exposed to direct UV light for 8 hour and tested at 1 hr, 4hrs, and 8hrs, 2.568 to 15.368 % degradation was observed in Desidustat.

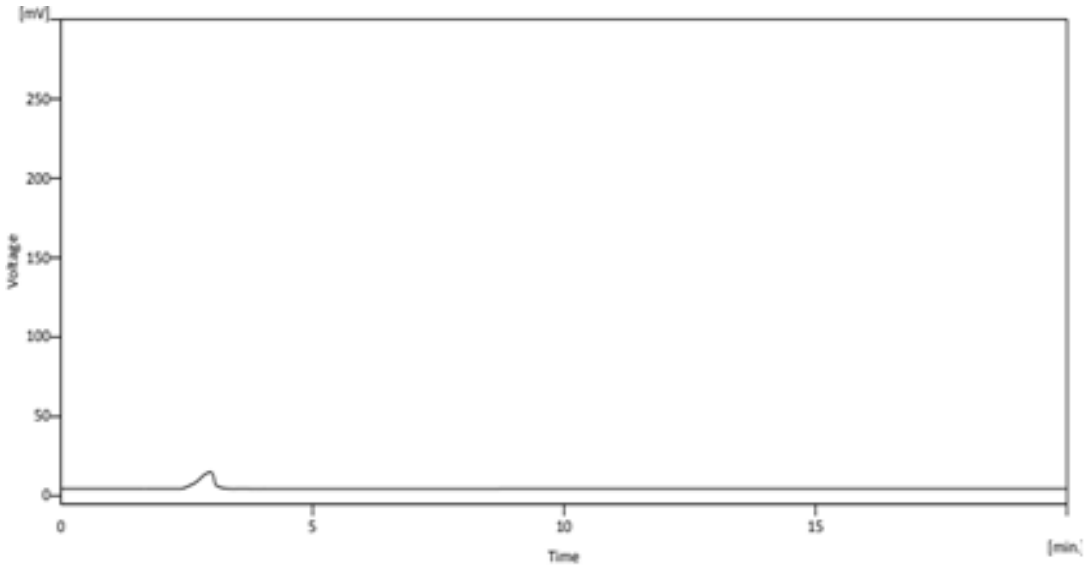


Figure: Chromatogram of Blank for Photo Degradation

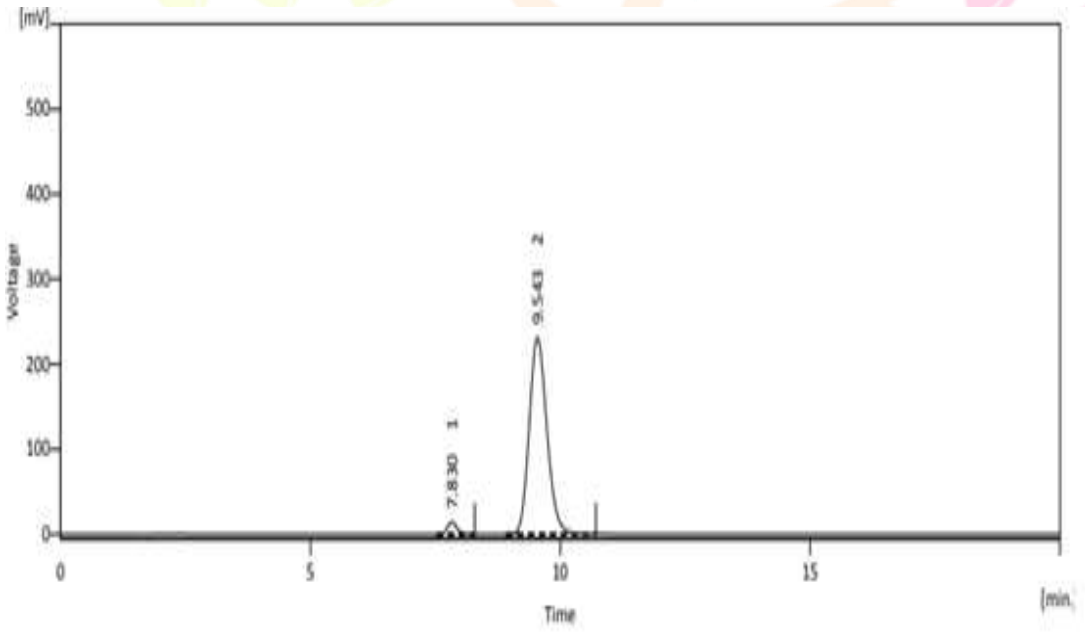


Figure: Chromatogram of Photo Degradation of Desidustat

Table: Results of Photo Degradation study of Desidustat

Parameter	Reagent for Degradation	Reagent for Neutralization	Time	Area of sample	% degradation of sample
Photo Desidustat	--	--	1 hr	1465.287	2.568
	--	--	4 hrs	1200.414	5.246
			8 hrs	1018.885	15.368

1.1.6. Thermal Degradation

After heating the drug solution at 80 °C for 6 and 8 hours, 5.499 and 13.167 %degradation was observed in Desidustat.

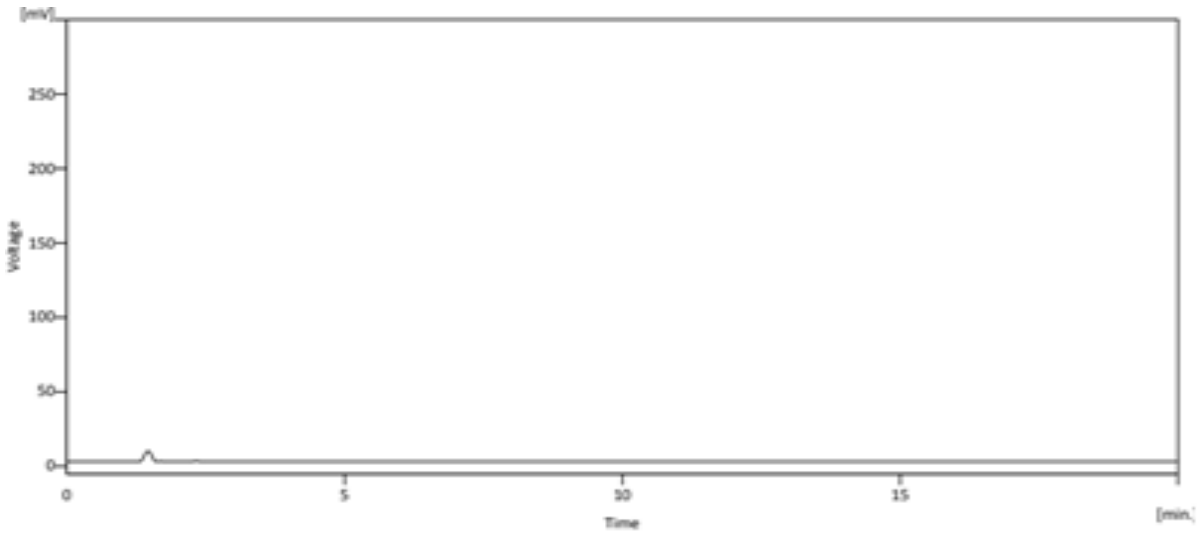


Figure: Chromatogram of Thermal Degradation (Blank)

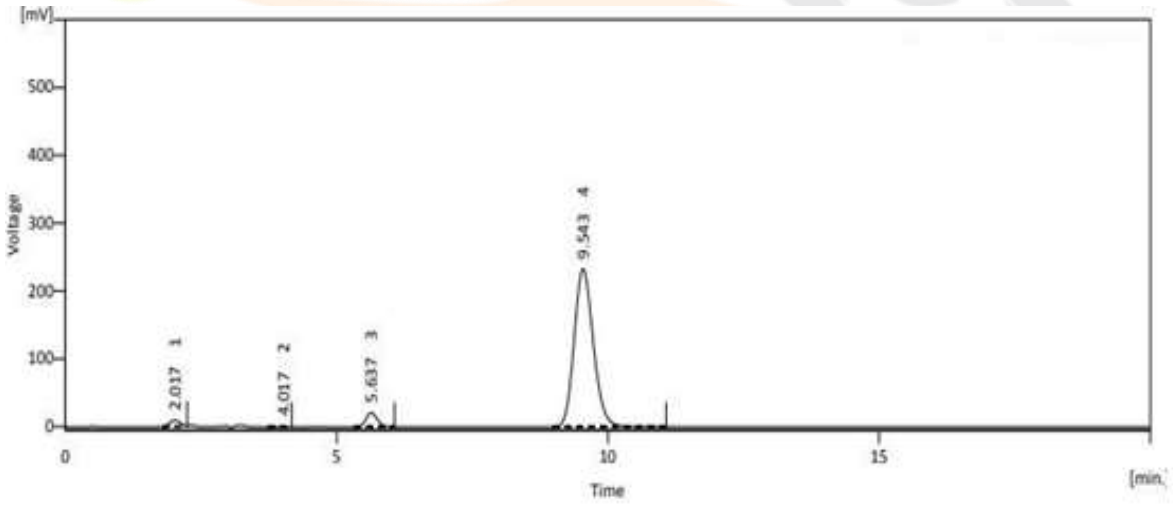


Figure: Chromatogram of Thermal Degradation Of Desidustat

Table: Results of Photo Degradation study of Desidustat

Parameter	Reagent for Degradation	Reagent for Neutralization	Time	Area of sample	% degradation of sample
Thermal Desidustat	--	--	6 hr	1257.455	5.499
	--	--	8 hrs	1391.063	13.167

1.1.7. Summary of Forced Degradation study by Stability Indicating RP-HPLC Mehtod.

- **Calculation for Stability:**

Table: Desidustat standard for stability

Drugs	Area
Desidustat	1487.644

Table: Desidustat % Degradation

Parameter	Reagent for Degradation	Reagent for Neutralization	Time	Area of Sample	% degradation of sample
Acid	2ml 0.1N HCl	2ml 0.1N NaOH	1hr	1371.452	1.687
			2hrs	1393.308	5.798
			3hrs	1111.594	10.686
			4hrs	1015.179	17.635
Base	2ml 0.1N NaOH	2ml 0.1N HCl	1hr	1242.253	3.251
			2hrs	1159.190	8.257
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Oxidation	3% H_2O_2	--	1hr	1448.870	3.794
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			3hrs	1263.958	9.913
			4hrs	1069.317	16.734
Photo	--	--	1hr	1465.287	2.568
			4hrs	1200.414	5.246
			8hrs	1018.885	15.368
Thermal	--	--	6hr	1257.455	5.499
			8hrs	1391.063	13.167

1. METHOD VALIDATION

8.1. Linearity and Range: (n=6)

✓ The linearity for Desidustat was assessed in range of 3-7 ppm. 3, 4, 5, 6, 7 mL solutions were pipetted out from the Stock solution of Desidustat and transferred into 100 ml volumetric flask and made up with mobile phase to obtain 3, 4, 5, 6 and 6ppm.

8.2. Precision

I. Intraday precision (n=3)

✓ Solution containing (3, 5, 7 ppm) of Desidustat was analyzed three times on the same day and % RSD was calculated.

II. Interday precision (n=3)

✓ Solution containing (3, 5, 7 ppm) of Desidustat was analyzed three times on the different day and % RSD was calculated.

III. Repeatability (n=6)

✓ The data for repeatability of peak area measurement for Desidustat (3 ppm) based on six measurements of same solution of Desidustat (3 ppm) and % RSD was calculated.

8.3. Accuracy (% Recovery) (n=3)

Desidustat

✓ 3 ppm drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10 mL. The area of each solution peak was measured at 315 nm. The amount of Desidustat was calculated at each level and % recoveries were calculated.

8.4. LOD and LOQ

✓ LOD and LOQ were calculated as follows:

✓ LOD = 3.3× SD/slope of calibration curve

✓ LOQ = 10 × SD/slope of calibration curve

✓ Where, SD = Standard deviation of intercepts

8.5. Robustness

✓ Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (± 0.2) 4.2 and 3.8.
3. Ratio of Mobile phase was changed (±2) Phosphate Buffer: Methanol (62:38 %v/v) and Phosphate Buffer: Methanol (58:42 %v/v).

❖ **Method Validation**

- **Linearity and Range**

Linearity data for Desidustat evaluated, the regression line equation for Desidustat was as following: for Desidustat y = 334.78x - 12.753 and Linear correlation was obtained between peak area and concentration of Desidustat in the range of 3-7 ppm. The linearity of the calibration curve was validated by the value of correlation coefficients of the regression (r). The overlain chromatogram of Desidustat presented in Figure 8.1. The linearity data were presented in Table 8.1

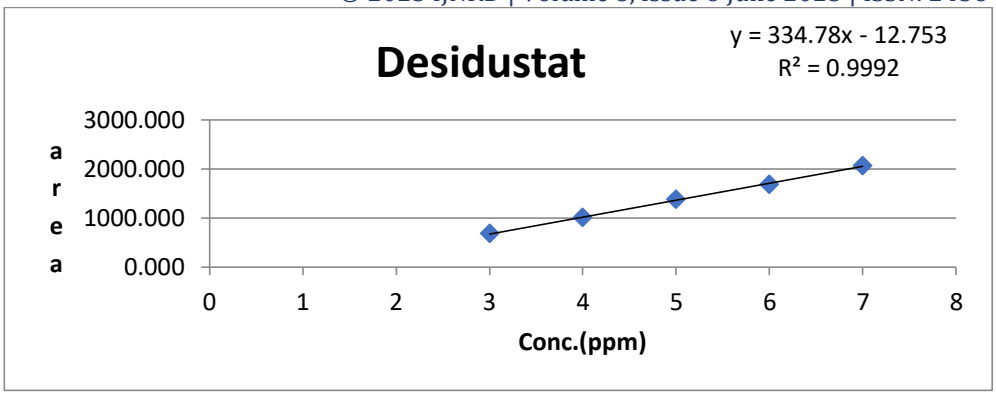

Fig. 8.1 Calibration Curve of Desidustat (3-7 ppm) at 315 nm

Table 8.1.: Linearity for Desidustat

Sr. No	Concentration(ppm)	Area ± SD (n=6)	% RSD
1	3	618.437±6.445	1.168
2	4	1060.194±7.622	0.947
3	5	1537.955±8.717	0.799
4	6	1846.379±11.083	0.685
5	7	2061.686±12.597	0.621

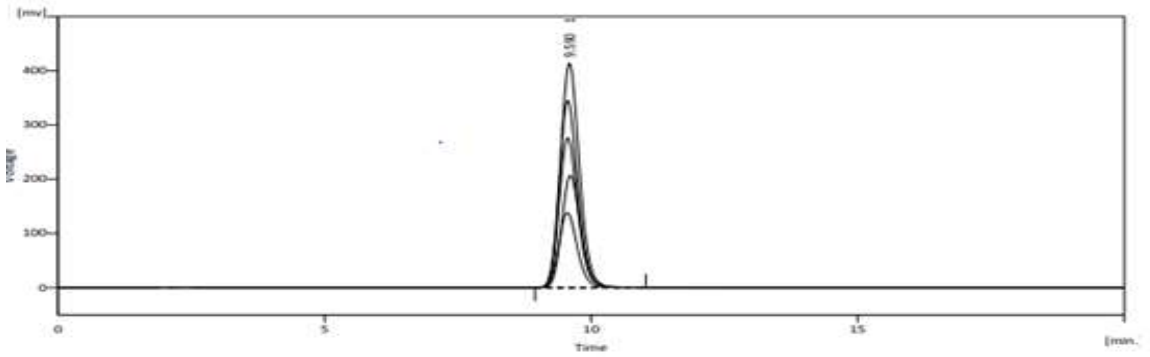

Fig. Overlay in chromatogram of linearity of Desidustat (3-7 ppm)

- **Precision**

I. Intraday Precision

- The data for intraday precision for Desidustat showed inTable 8.2.

Table 8.2: Intraday Precision data for estimation of Desidustat

Sr. No.	Desidustat		
	Conc. (ppm)	Area Mean ± S.D. (n=3)	% R.S.D
1	3	686.421 ± 8.897	1.747
2	5	1281.583± 11.354	0.980
3	7	2121.452± 16.136	0.793

II. Interday Precision

- The data for interday precision for Desidustat showed in Table 8.3

Table 8.3: Inter-day precision data for estimation of Desidustat

	Desidustat		
Sr. No.	**Conc. (ppm)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	3	683.252 ± 8.972	1.763
2	5	1379.594 ± 11.888	0.998
3	7	2123.459± 17.286	0.839

III. Repeatability

- The data for repeatability precision for Desidustat showed in Table 8.4.

Table 8.4.: Repeatability data for Desidustat

Desidustat				
Sr. No.	Conc. (ppm)	Area	Mean ± S.D (n=6)	% R.S.D
1.	5	1265.546 1255.610 1275.906 1278.668 1271.774 1274.553	1270.342±7.470	0.518

- **Accuracy**
  - Accuracy of the method was confirmed by recovery study from Desidustat at three level of standard addition. The results of Desidustat showed in Table 8.5. Recovery greater than 99 % with low SD justified theaccuracy of the method.

Table 8.5.: Recovery data for Desidustat

Sr.NO.	Conc. Level (%)	Sample amount (ppm)	Amount Added (ppm)	Amount recovered (ppm)	% Recovery	% Mean Recovery ± S.D
1	80 %	3	2.4	2.415	100.625	100.736 ± 0.724
2		3	2.4	2.408	100.333	
3		3	2.4	2.430	101.250	
4	100 %	3	3	3.004	100.133	99.599 ± 0.731
5		3	3	2.985	99.500	
6		3	3	2.975	99.166	

7	120 %	3	3.6	3.617	100.472	100.314 ± 0.266
8		3	3.6	3.604	100.111	
9		3	3.6	3.613	100.361	

- **LOD and LOQ**
  - The Limit of detection (LOD) and Limit of quantification (LOQ) of Desidustat found to be as per below table 8.6

Table 8.6.: LOD and LOQ

	Desidustat
LOD	0.165 ppm
LOQ	0.539 ppm

- **Specificity**
  - In the chromatogram sample and standard both peaks were observed at the same time which indicates that there is no interference.

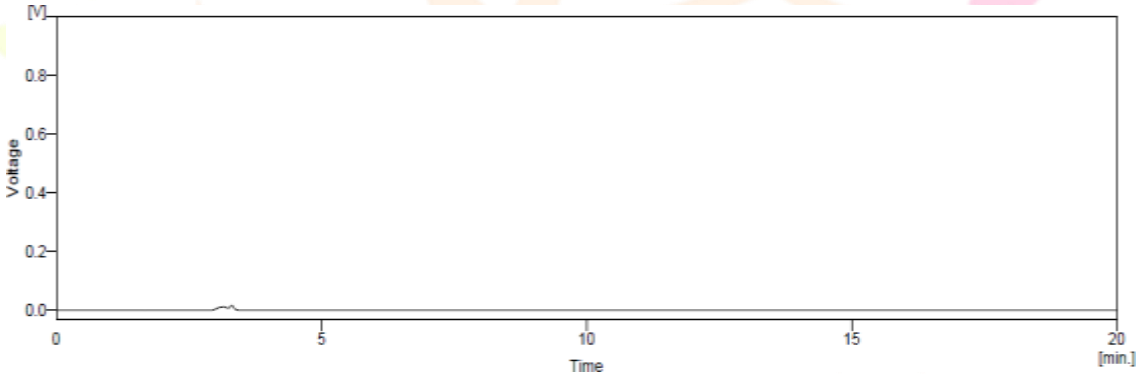

Fig.: HPLC Chromatogram of Blank

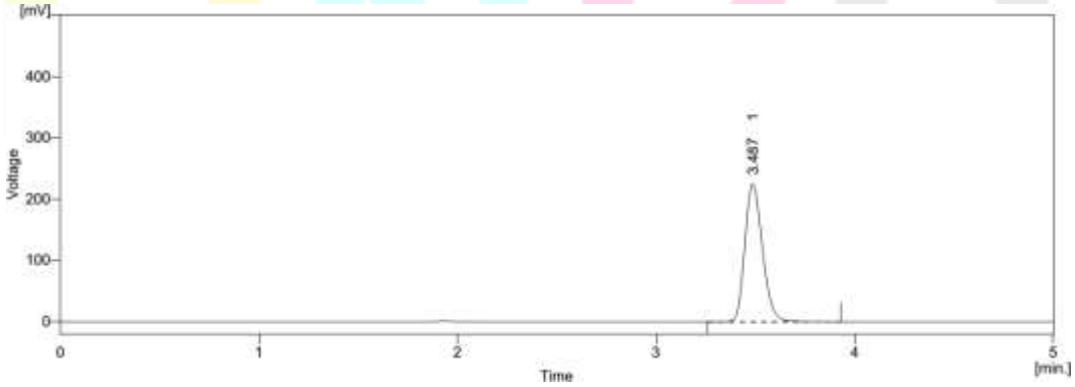

Fig.: RP-HPLC Chromatogram of Desidustat

  - The Chromatograms of Desidustat standards and sample were showed no interference with the Chromatogram of Desidustat Blank, so this method is Specific.

- **Robustness**
  - The data for Robustness for Desidustat showed in Table 8.7.

Table 8.7.: Robustness data for Desidustat

Sr. No.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	1283.284	1310.780	1372.748	1293.065	1369.052	1304.322
2	1316.891	1344.275	1413.130	1316.762	1408.879	1341.581
3	1342.351	1352.503	1421.411	1322.290	1417.146	1351.151
SD	20.156	22.100	26.036	15.525	25.715	24.741
% RSD	1.462	1.654	1.857	1.184	1.839	1.857

2. REFERENCES

1. "Introduction to Chronic kidney disease ", September-2018, http://www.healthline.com/health/vaginal-yeast-infection#Overview1
2. "Introduction to High performance Liquid Chromatography", Sept-2017, http://www.chem.utoronto.ca/coursenotes/analsci/chrom/hplc.html
3. "Introduction to High performance Liquid Chromatography", Sept-2017, http://www.hitachihightech.com/global/products/science/tech/ana/lc/basic/course1.ht ml
4. "Introduction to Analytical Parameters of HPLC", Sept-2017, http://chemistry.unt.edu/~tgolden/courses/Theory_Of_HPLC_Chromatographic_Para meters.pdf
5. "Introduction to Analytical Method Validation", Sept-2017, https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q 2_R1/Step4/Q2_R1_Guideline.pdf
6. ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva 1996.
7. Brummer H, "How To Approach A Forced Degradation Study", *Life sci. tech bul.,* *2011*
8. "Drug profile for Desidustat, May-2022 http://www.drugbank.ca/Salts/DBSALT001382
9. "Drug profile for Desidustat, Desidustat -2022 https://academic.oup.com/jat/article/45/2/184/5841139?login=false
10. Indian Pharmacopoeia 2022, The department of health, social services and public safety, London

11. Mandik SK, Siras SS, "RP-HPLC Method Development and Validation for the Estimation of Desidustat in Bulk and Tablet Dosage form" *Int. J. ChemtechRes.*, **2017,** 10(1), 573-580
12. Rongxin Q**. "**Determination of Desidustat by HPLC". *Chin. J. modern App. Pharm.*, **2022**
13. Albet C, Fernandez JM, Rozman E, Perez JA, Sacristan A, Ortiz JA "High-Performance Liquid Chromatography". *J Pharm Biomed Anal.* **1992**,10(2-3), 205-11.
14. Faizi M, Singh UK. "RP-HPLC and UV spectrophotometric methods" *J.of Pharmacology,* **2016**, 8(7), 740-745
15. Mahmoud A, Alamin A. "Purity assessment and determination of pharmaceutical formulations based on spectrophotometric and chromatographic approaches". *Euro. J. Chem.*, **2015,** 6(4), 381-386
16. Salama A, Mohamed A. "Stability indicating methods for the determination by miclle enhanced spectroflurimetr and TLC densitometry". *Bul. Chem. Soc. Etiop.*, **2009,** 23(2), 167-174
17. 35. Nemutlu E, Yardimci C, Ozaltın N. "Determination of pharmaceutical preparations by capillary zone electrophoresis". *Anal. Chem. Act*. **2005**,547(1,15), 83–88
18. Wang Y, Pang L, Wu M, Ou N, "A validated LC–MS/MS method for determination" *J. Chrom.*, **2009,** 877(31), 4047-4050
19. Indian Pharmacopoeia 2022, Government of India, Ministry of health and family welfare, ghaziyabad, pp1328-1329.
20. United state pharmacopoeia-2021, USP30-NF25, United state food and drug administration.
21. British Pharmacopoeia 2019, The department of health, social services and public safety, London.
22. De suza LR, Azevedo C, Madeira K, Moriea S "Validation of a stability-indicating HPLC method with diode array detection for the determination of aqueous suspension for nebulizer" *Asi. J. of Bio and pharm. sci.*, **2013,**3(19), 4-9.
23. Prajapati NB, "Development and validation of RP-HPLC method for simultaneous estimation." *Int. J of pharma Res*,**2013** 5(2), 41-50.