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Method Development and Validation of Desidustat in Solid Dosage Form by RP-HPLC Method

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ABSTRACT:
A reverse-phase high-performance liquid chromatography (RP-HPLC) method was validated for the quantitative estimation of Desidustat in pharmaceutical tablet dosage forms. The calibration curve for Desidustat was linear in the concentration range of 25 µg/mL to 150 µg/mL with an r^2 value of 0.9850, indicating good linearity. The percentage recovery of Desidustat ranged from 99.8% to 101.66%, demonstrating excellent accuracy. Reproducibility was confirmed by adding known amounts of pure drug to previously analyzed samples, which showed recovery values close to 100%. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.024 µg/mL and 0.07 µg/mL, respectively. Precision studies revealed a system precision of 0.45%, method precision of 1.56%, and intermediate precision of 1.517%, all within acceptable limits. The method was successfully applied to the analysis of Desidustat in bulk formulations and commercial tablet samples, demonstrating its rapid, precise, and accurate capability for routine analysis. This validated RP-HPLC method is suitable for the estimation of Desidustat in tablet dosage forms.
KEYWORDS: Desidustat, RP-HPLC, Validation, Accuracy, Precision.

I. INTRODUCTION:

High-Performance Liquid Chromatography (HPLC) is a widely used and powerful analytical technique for the separation, identification, and quantification of compounds in pharmaceutical, biological, environmental, and chemical samples. Among the different HPLC techniques, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) stands out due to its versatility, efficiency, and ability to analyze a broad range of compounds, including both polar and non-polar substances. This makes it a critical tool in the pharmaceutical industry for the analysis of Active Pharmaceutical Ingredients (APIs), excipients, impurities, and degradation products in drug formulations.

The validation of an RP-HPLC method is a critical step in ensuring that the developed analytical method is reliable, accurate, and suitable for its intended use. Method validation is a regulatory requirement as stipulated by global standards such as the International Council for Harmonisation (ICH) Q2(R1) guidelines and is essential for demonstrating that the method performs consistently and with the desired level of precision and accuracy. Validated RP-HPLC methods are crucial for ensuring product quality, regulatory compliance, and patient safety.

The validation process typically involves evaluating several key performance parameters, including:

1. **Accuracy**: The degree of closeness between the true value and the measured value. It ensures that the RP-HPLC method provides correct results under specified conditions.
2. **Precision**: The degree of consistency of repeated measurements under the same conditions. Precision is typically evaluated through intra-day and inter-day variability testing.
3. **Specificity**: The ability of the method to accurately measure the analyte in the presence of other components such as excipients, impurities, and degradation products.
4. **Linearity**: The method's ability to provide results that are directly proportional to the concentration of the analyte over a specified range. This is crucial for ensuring accurate quantification.
5. **Range**: The interval between the lower and upper concentration limits over which the method is accurate and precise.

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6. **Robustness**: The ability of the method to remain unaffected by small, deliberate changes in the method parameters, such as temperature, mobile phase composition, and flow rate.
7. **Limit of Detection (LOD) and Limit of Quantification (LOQ)**: These parameters define the smallest concentration of an analyte that can be reliably detected and quantified by the RP-HPLC method.

Method validation by RP-HPLC ensures that the developed analytical method is capable of producing accurate, reproducible, and reliable results. It plays a vital role in quality control, regulatory submission, and stability testing of pharmaceutical products. By meeting the stringent requirements for method validation, RP-HPLC serves as a trusted tool for ensuring the safety, efficacy, and quality of pharmaceutical products throughout their lifecycle.

Desidustat, a novel selective inhibitor of hypoxia-inducible factor prolyl hydroxylase domain (HIF-PHD), has gained attention for its role in treating anemia associated with chronic kidney disease (CKD). As the demand for precise and reliable formulations of pharmaceutical compounds increases, the need for robust analytical methods to ensure the quality and consistency of drug products becomes paramount.

High-Performance Liquid Chromatography (HPLC) is one of the most widely employed techniques for the analysis of pharmaceuticals due to its high resolution, sensitivity, and versatility. Among the various HPLC techniques, Reverse Phase (RP) HPLC is particularly advantageous for the analysis of solid dosage forms due to its efficiency in separating non-volatile compounds, including active pharmaceutical ingredients (APIs) like Desidustat, from other excipients present in the formulation.

The development and validation of an RP-HPLC method for Desidustat in solid dosage form is crucial for the pharmaceutical industry to ensure quality control, regulatory compliance, and safety of the final product. This involves optimizing parameters such as column selection, mobile phase composition, flow rate, and detection wavelength to achieve the desired separation and sensitivity. Furthermore, the method must be validated in terms of accuracy, precision, specificity, linearity, range, and robustness to ensure it meets regulatory guidelines such as ICH Q2(R1) for analytical method validation.

This study aims to establish a reliable RP-HPLC method for the quantification of Desidustat in solid dosage forms, focusing on method development and validation. Through this work, we seek to provide an analytical tool that can be utilized for routine quality control and stability testing of Desidustat formulations in accordance with international pharmacopeial standards.

II. DRUG PROFILE:

DRUG NAME: DESIDUSTAT

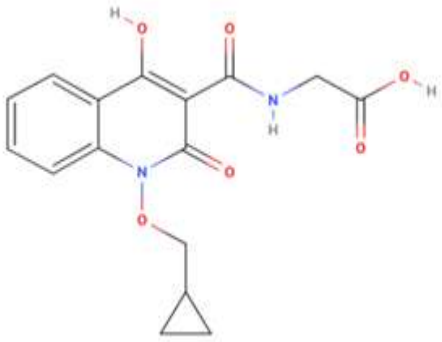

Figure 1 : Structure Of Desidustat

- **IUPAC Name : (**1,2 dihydroquinoline -3-carbonyl)glycineN-[1-(cyclopropylmethoxy)-4-hydroxy-2-oxoquinoline-3 carbonyl]amino acid.
- **Molecular Formula:** $C_{16}H_{16}N_2O_6$
- **Molecular Weight:** 332.31g/ml
- **Appearance :** clear, colorless and odorless
- **Solubility :** It is soluble in organic solvents such as ethanol, DMSO and Dimethyl Formamide (DMF) and sparingly soluble in aqueous buffer.
- **Polar Surface Area :** 16.17
- **Brand Name :**oxemia
- **Appearance :** clear, colorless and odorless
- **Route of administration:** Oral

Pharmacodynamics: Decreased hepcidin, increased EPO, serum iron, haematocrit and haemoglobin levels, and increased reticulocytes and RBCs in normal/nephrectomized rats and/or rodent model of chemotherapy-or inflammation-induced anaemia Increased haemoglobin and EPO levels in healthy volunteers Decreased hepcidin levels to a greater extent than darbepoetinalfa in patients with non-dialysis-dependent CKD Decreased hepcidin levels to a similar extent as epoetinalfa in patients with dialysis-dependent CKD.

Pharmacokinetics: Time to peak plasma concentration 2.5 h after single 50–150 mg dose in

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dialysis-dependent patients No accumulation after multiple dose administration Mean elimination half-life 6–15 h after single 50–150 mg dose in dialysis-dependent CKD patients and 6–14 h after multiple doses of 100–200 mg on alternate days for 6 weeks in pre-dialysis CKD patients.

III. MATERIALS AND METHODS:

METHOD OF ANALYSIS:

Chromatographic conditions:

Column : 250mm x 4.6mm,5µ
Detector : 245 nm
Flow : 1.0 ml/min
Injection volume : 20 microlitre
Temperature : Ambient

Diluent: Mix Ethanol: Water in the ratio of 300:700

Mobile phase: Filtered and degassed mixture of Ethanol:Water in the ratio of 350:650

Standard preparation: Weighed accurately about 0.100g of Desidustat working standard into a 100 ml volumetric flask, added 70 ml of diluent, shake and sonicate to dissolve the content, make up the volume with diluent . Pipette out 5 ml of resulting solution to 100 ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collected the filtrate after discarding the few ml of the filtrate.

Assay preparation: Weighed 20 tablets, triturate to a fine powder. Weigh accuraately about 1.33g powdered tablet (equivalent to 0.100g of Desidustat) into a 100 ml volumetric flask. Added 70 ml of diluent shake for 15 minutes and sonicate for 15 minutes, and made up the volume with diluent, pipette out 5 ml of filtrate to 100 ml with diluent. Filtered the solution through 0.45 micron membrane filter. Collect the filtrate after discarding the first few ml of the filtrate.

System Suitability Preparation: Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 1.33g of powdered tablet (equivalent to 0.100g of Desidustat)) into a 100 ml volumetric flask. Added 70 ml of diluent shake for 15 minutes and sonicate for 15 minutes, and made up the volume with diluent, pipette out 5 ml of filtrate to 100 ml with diluent. Filtered the solution through 0.45 micron membrane filter. Collect the filtrate after discarding the first few ml of the filtrate.

Procedure: Separately injected equal volumes (about 20µl) of the standard preparation and the assay preparation into the chromatograph, recorded the chromatograms, and measured the responses for the Desidustat peak.

System Suitability: Chromatograph the standard preparation and record the peak responses direct under procedure.

1. The column efficiency is not less than 2000 theoretical plates.
2. The tailing factor of the peak is not more than 2.0.
3. The relative standard deviation for the replicate injections is not more than 2.0%

PRECISION:

To establish the precision of the analytical method by using the following two methods:

1.System Precision: Establish the repeatability of the analytical method by estimating the assay for six different sample preparations of the same batch. Calculate the assay for all six sample preparations and report the %RSD for the same.

Preparation of Blank: Use diluent as blank.

Preparation of Standard Solution: Weighed accurately about 0.100g of Desidustat working standard into a 100ml volumetric flask, added 70ml of diluent, shake and sonicated to dissolve the content, made up the volume with diluent. Pipette out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collect the filtrate after discarding the few ml of the filtrate.

Preparation of Sample Solution: Weigh 20 tablets, triturate to a fine powder. Weigh accurately about 0.100g powdered tablet (equivalent to 1.33g of Desidustat) in to a 100ml volumetric flask. Added 70ml of diluent shake for 15 minutes and sonicated for 15 minutes, and made up the volume with diluent, pipette out 5ml of filtrate to 100ml with diluent. Filter the solution through 0.45 micron membrane filter. Collect the filtrate after discarding the first few ml of the filtrate.

Procedure: Injected separately 20µl of blank, standard and sample preparations into the chromatograph and measured the peak responses for the major peak. Calculate the content of Desidustat the individual solutions.

Acceptance Criteria: The relative standard deviation for the assay values of six sample preparations of same batch should not be more than 2.0%

2. Intermediate Precision (Ruggedness):

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A different analyst using a HPLC system with a different similar column on a different day should carry out this experiment.

Estimating the assay for six different sample preparations of the same batch. Calculate the assay for all six sample preparations and report the %RSD for the same.

Blank Preparation: Use diluent as blank.

Preparation of standard solution: Weighed accurately about 0.100g of Desidustat working standard into a 100ml volumetric flask, added 70ml of diluent, shake and sonicated to dissolve the content, made up the volume with diluent. Pipette out 5ml of resulting solution to 100ml volumetric flask made up with diluent. Filtered through 0.45 micron membrane filter. Collect the filtrate after discarding the few ml of the filtrate.

Preparation of Sample Solution: Weigh 20 tablets, triturate to a fine powder. Weigh accurately about 0.100g powdered tablet (equivalent to 1.33g of Desidustat) in to a 100ml volumetric flask. Added 70ml of diluent shake for 15 minutes and sonicated for 15 minutes, and made up the volume with diluent, pipette out 5ml of filtrate to 100ml with diluent. Filter the solution through 0.45 micron membrane filter. Collect the filtrate after discarding the first few ml of the filtrate.

Procedure: Injected separately 20µl of blank, standard and sample preparations into the chromatograph and measured the peak responses for the major peak. Calculate the content of Desidustat the individual solutions.

Acceptance Criteria:

a) The relative standard deviation for the assay values of six sample preparations of same batch should not be more than 2.0%

b) The difference in the assay of same batch of Desidustat Tablet 100mg between two analysts should not be more than 2.0%

LINEARITY AND RANGE:

Objective: To establish the linearity of the analytical method for assay using the following two methods.

Linearity and range for Desidustat working standard: Demonstrate the linearity of the analytical method for assay by injecting the various concentration of standard preparations prepared in the range of 25%to 150% into the chromatograph, covering six different concentrations. Draw a plot between the Concentration vs Peak response of Desidustat. Report the slope, intercept and regression coefficient from the plot obtained for Concentration vs Peak response of Desidustat in standard preparation.

Preparation of analytical solutions for linearity and range for Desidustat standard preparations:

a) Blank Preparation: Use diluent as blank.

b) Standard stock solution preparation: Transfer an accurately weighed quantity of about 100mg of Desidustat working standard into 100 ml volumetric flask, add 20 ml of diuent, sonicate for 10 minutes to dissolve and made to volume with mobile phase. From the stock solution 10 ml was pipette out into the 100 ml volumetric flask.

c) 25% Linearity standard solution preparation (12.5ppm): Pipette out 6.25 ml of standard stock solution into 50 ml volumetric flask and make up to volume with diluent.

d) 50% Linearity standard solution preparation (25.0ppm): Pipette out 12.5 ml of standard stock solution into 50 ml volumetric flask and make upto volume with diluent.

e) 75% Linearity standard solution preparation (37.5ppm): Pipette out 18.75 ml of standard solution into 50 ml volumetric flask and make up to volume with diluent.

f) 100% Linearity standard solution preparation (50ppm): Pipette out 25 ml of standard stock solution into 50 ml volumetric flask and make up to volume with diluent.

g) 125% Linearity standard solution preparation (62.5ppm): Pipette out 12.5 ml of standard stock solution into 50 ml volumetric flask and make up to volume with diluent.

h) 150% Linearity standard solution preparation (75.0ppm): Pipette out 15.0 ml of standard stock solution into 50 ml volumetric flask and make up to volume with diluent.

Calculations: Draw a plot between the concentration vs the average peak responses of canagliflozin peak for all the above studies. Calculate slope, intercept and regression coefficient from the plot obtained.

Acceptance criteria: The regression coefficient for all the various plots should not be less than 0.999.

ACCURACY / RECOVERY:

Objective: To establish the accuracy of the analytical method is the closeness of sample results obtained by method to the value by using recovery study.

Procedure: Perform the recovery studies by adding known quantity of Desidustat working standard to known quantity of placebo (Desidustat tablet 100mg excipient mixtures) in the range of

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50% to 150% of the sample concentration. Report the percentage recovery in relative standard deviation for all the values of % recovery.

a) Blank preparation: Use diluent as blank.
b) Standard Preparation: Weighed accurately about 0.100g of Desidustat working standard into a 100 ml volumetric flask, added 70 ml of diluent, shaked and sonicated to dissolve the content, made up the volume with the diluent. Pipette out 5 ml of resulting solution to 100 ml volumetric flask made up to the volume. Filtered through 0.45 micron membrane filter. Collect the filtrate after discarding the few ml of the filtrate.
c) 50% recovery solution preparation: Weighed 20 tablets, triturate to a fine powder. Weighed accurately about 1.33 g powdered tablet (equivalent to 0.100g 0f Desidustat) into a 100 ml volumetric flask containing 50 mg of Desidustat add 70 ml of diluent. Sonicate for 10 minutes to dissolve and make up to the volume with diluent and mix. Filter through 0.45µ membrane filter. Dilute the above solution as 10 ml to 50 ml with diluent. Repeat this procedure for another two sample preparations.
d) 100% recovery solution preparation: Weighed 20 tablets, triturate to a fine powder. Weigh accurately about 0.21g powdered tablets (equivalent to 0.100g of Desidustat) into a 100 ml volumetric flask containing 100 mg of Desidustat add 70 ml of diluent. Sonicate for 10 minutes to dissolve and make up to the volume with diluent and mix. Filter through 0,45 micron membrane filer. Diluted the above solution as 10 ml to 50 ml with diluent. Repeat this procedure for another two sample preparations.
e)150%recovery solution preparation: Weighed 20 tablets, triturate to a fine powder. Weigh accurately about 1.33g powdered tablets (equivalent to 0.100g ofDesidustat) into a 100 ml volumetric flask containing 150 mg of Desidustat add 70 ml of diluent. Sonicate for 10 minutes to dissolve and make up to the volume with diluent and mix. Filter through 0.45 micron membrane filter. Dilute the above solution as 10 ml to 50 ml with diluent, Repeat this procedure for another two sample preparations.
Procedure: Separately inject 20 µl of standard and sample preparations of recovery solutions into the chromatograph and measure the peak responses for the major peak. Calculate the % recovery solutions using the following expressions.

Recovery Calculation:

$$\textbf{Assay Percentage} = \frac{\text{peak area of spl x wt of std x 5 x 100 x 100 x 99.8 x 100 x avgwt}}{\text{std area x 100 x 100 x wt of sample x 5 x 100 x 100}}$$

$$\textbf{\% recovery} = \frac{\text{Mg of Desidustat working standard recovered}}{\text{Mg of Desidustat working standard added}} \times 100$$

Acceptance criteria for recovery study: The percentage of recovery should be in between 97.0% to 103.0%. The relative standard deviation (RSD) of all recovery values should not be more than 2.0%.

STABILITY OF ANALYTICALSOLUTIONS:
Objective: To establish the stability of analytical solutions by injecting the standard and sample solutions at periodic intervals up to 32 hrs.

Preparation of analytical solutions
a) Blank preparation: Use diluent as blank.
b) Standard solution preparation: Weighed accurately about 0.100g of Desidustat working standard into a 100 ml volumetric flask, added 70 ml of diluent, shaked and sonicated to dissolve the content, made up the volume with the diluent. Pipette out 5 ml of resulting solution to 100 ml volumetric flask made up to the volume. Filtered through 0.45 micron membrane filter. Collect the filtrate after discarding the few ml of the filtrate.
c) Sample solution preparation: Weighed 20 tablets, triturate to a fine powder. Weigh accurately about 0.21g powdered tablets (equivalent to 0.100g of Desidustat) into a 100 ml volumetric flask containing 100 mg of Desidustat add 70 ml of diluent. Sonicate for 10 minutes to dissolve and make up to the volume with diluent and mix. Filter through 0,45 micron membrane filer. Collect the

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filtrate after discarding the first few ml of the filtrate.

Procedure: Inject 20µl of blank, resolution solution, standard preparation and sample preparations into the chromatograph and record the chromatogram. Measure the peak responses for major peak for all solutions. Continue the chromatography with periodic injections in duplicate for standard and sample preparations in the interval of 4hrs or suitable interval depending on the instrument utilization and sequence of injections.

Calculation: Calculate the average peak response and %RSD for initial 5 replicate injections of standard preparations. Calculate the %RSD for average peak response of standard and sample preparations for periodic intervals.

Acceptance criteria: The % RSD of peak response for the major peak of both standard and sample solutions at periodic intervals should not be more than 2.0%.

IV. RESULTS AND DISCUSSION:

VALIDATION OF RP-HPLC
METHOD SPECIFICITY:

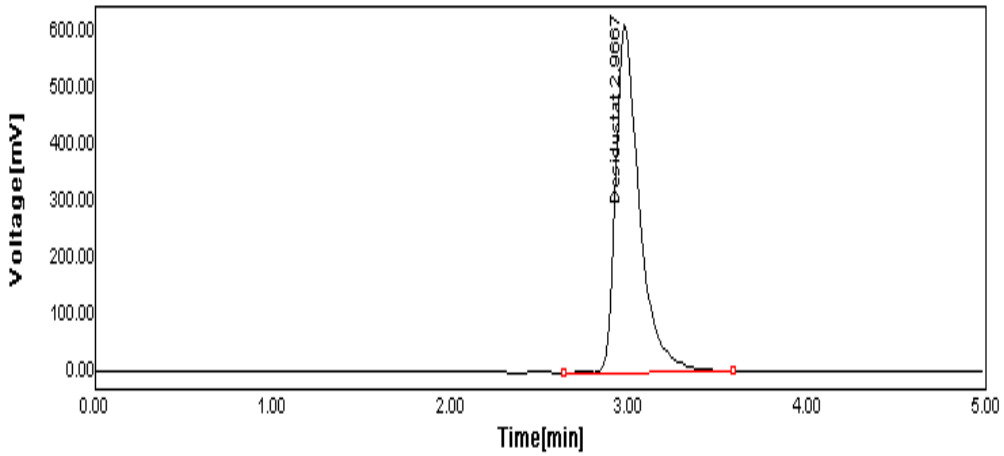

Figure 2:Method Specificity

Chromatogram of Desidustat sample for specificity

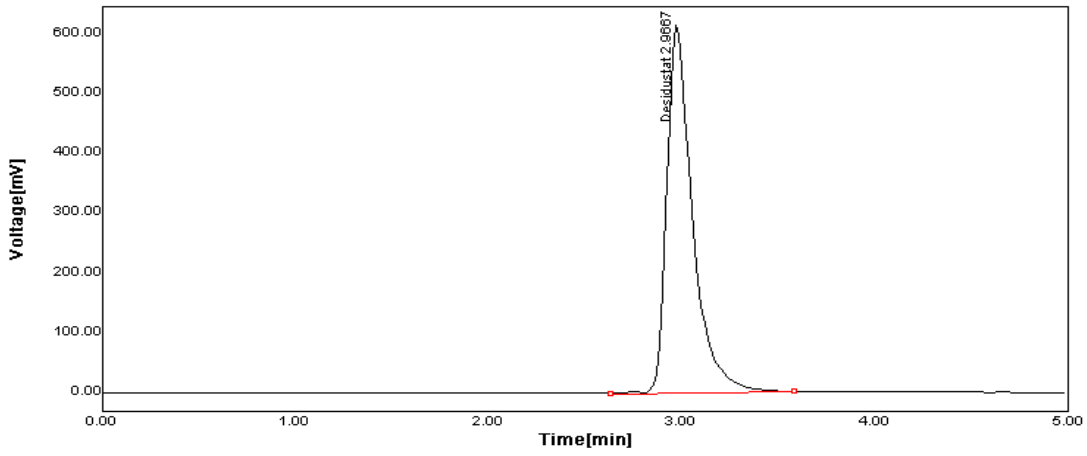

Figure 3:Chromatogram of Desidustat sample for specificity

Result:
The retention time of Desidustat peak in standard preparation: **2.9667**minutes

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The retention time of Desidustat peak in sample preparation: **2.9667**minutes

INTERFERENCE OF BLANK AND IMPURITY:

Table 1:Interference Of Blank And Impurity

NAME	INTERFERENCE	RETENTION TIME
BLANK	NIL	NA

SYSTEM SUITABILITY:

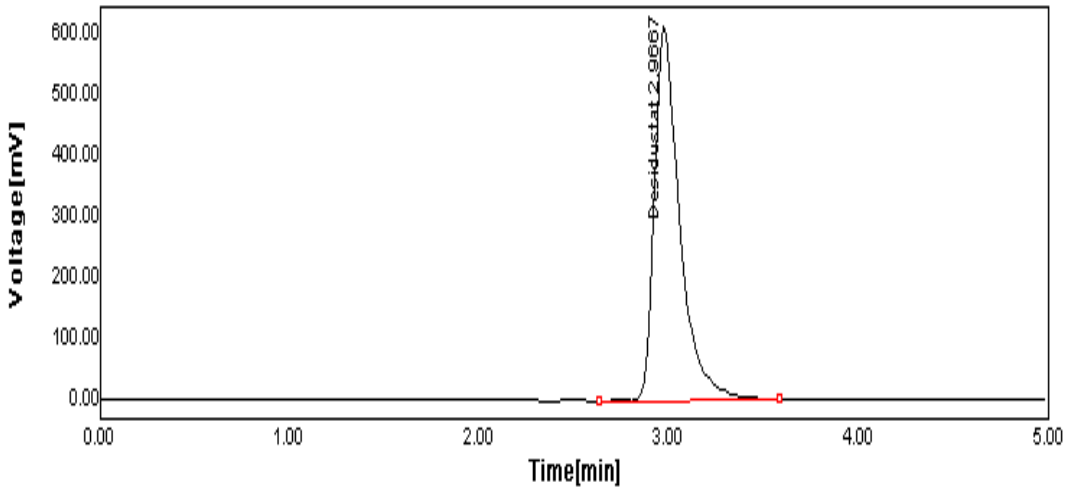

Figure 4:Chromatogram of Desidustat standard for system suitability

Result:

Table 2:Chromatogram of Desidustat standard for system suitability

No.	Name	RT[min]	Area[mV*s]	TP	Height%
1	Desidustat	2.9667	5716.2188	3053.5	100.00
Sum			5716.2188		

Table 3: Data for system suitability:

Injections	RT	Peak area	USP plate count	USP tailing
1	2.9667	5716.2188	3053.5	1.23
2	2.9667	5605.3749	3037.8	1.25
3	2.9667	5500.4745	3032	1.22
4	2.9667	5456.7474	3024.8	1.27
5	2.9667	5353.7473	2986.5	1.24
6	2.9667	5297.8923	2884.7	1.28
Mean	2.9667	9408.701	3003.217	1.248333

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Std deviation	**0**	**7819.972**	**2504.667**	**0.023166**
%RSD	**0**	**83.11**	**83.39**	**1.868**

Result: The % RSD of all the parameters like retention time, area, theoretical plates and tailing factor was within the limit. So the method passes

SYSTEM PRECISION:

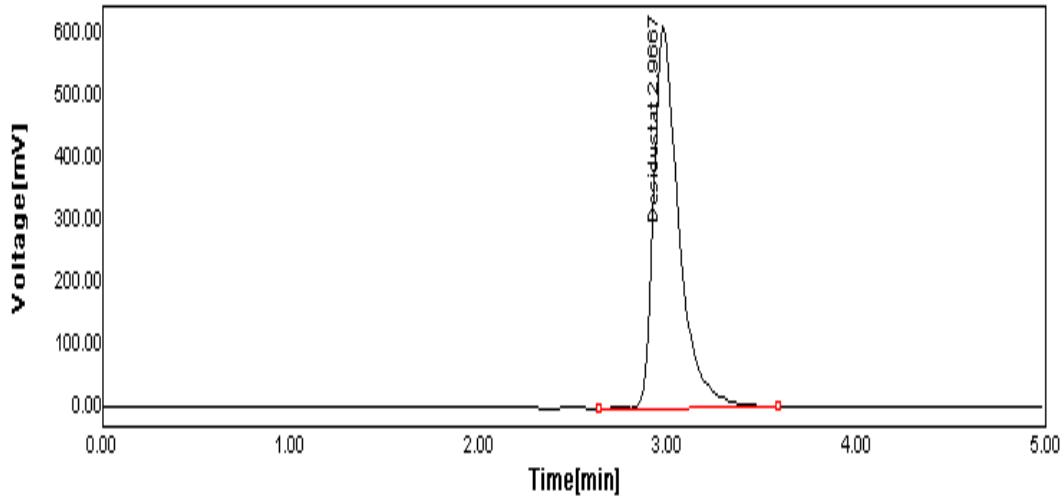

Figure 5:Chromatogram of standard Desidustat for system precision

Table 4: Data for system precision

Injections	**Retention Time**	**Peak Area**
1	**2.9667**	**5716.2188**
2	**2.9667**	**5605.3749**
3	**2.9667**	**5500.4745**
4	**2.9667**	**5456.7474**
5	**2.9667**	**5353.7473**
Mean	**2.9667**	**9408.701**
Std deviation	**0.00**	**7819.972**
%RSD	**0**	**83.11**

The %RSD of replicate of standard injections of standard solution is within the specified acceptance criteria.

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METHOD PRECISION :

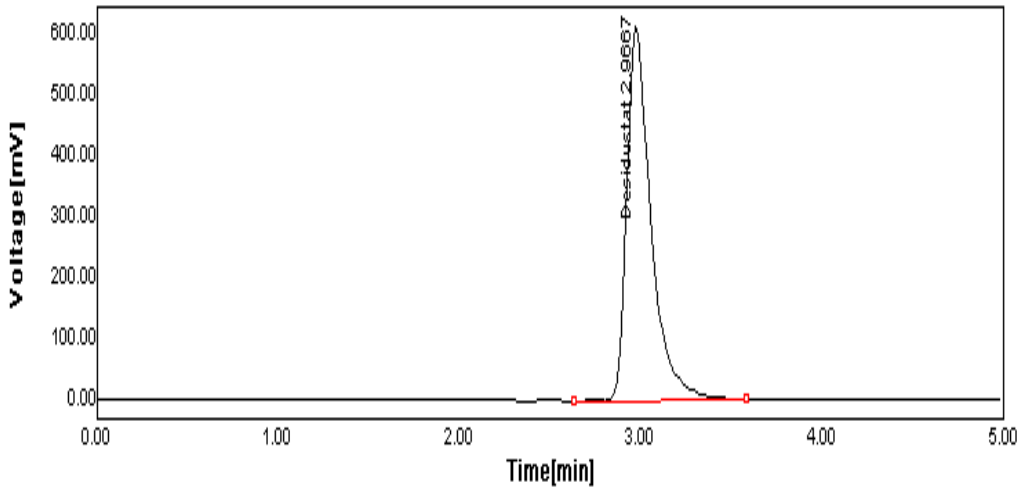

Figure 6:Chromatogram of standard Desidustat for Method precision

Table 5: Data for Method precision

Samples	Peak Area	Weight of sample	Assay	
			in mg	in %
1	5716.2188	1330	5.121	98.8
2	5605.3749	1330	5.123	98.55
3	5500.4745	1330	5.122	99.4
4	5456.7474	1330	5.120	100.53
5	5353.7473	1330	5.124	98.5
6	5297.8923	1330	5.119	99.3
Mean			5.121	99.18
Std deviation			0.699	0.6939
%RSD			0.145	0.698

INTERMEDIATE PRECISION:

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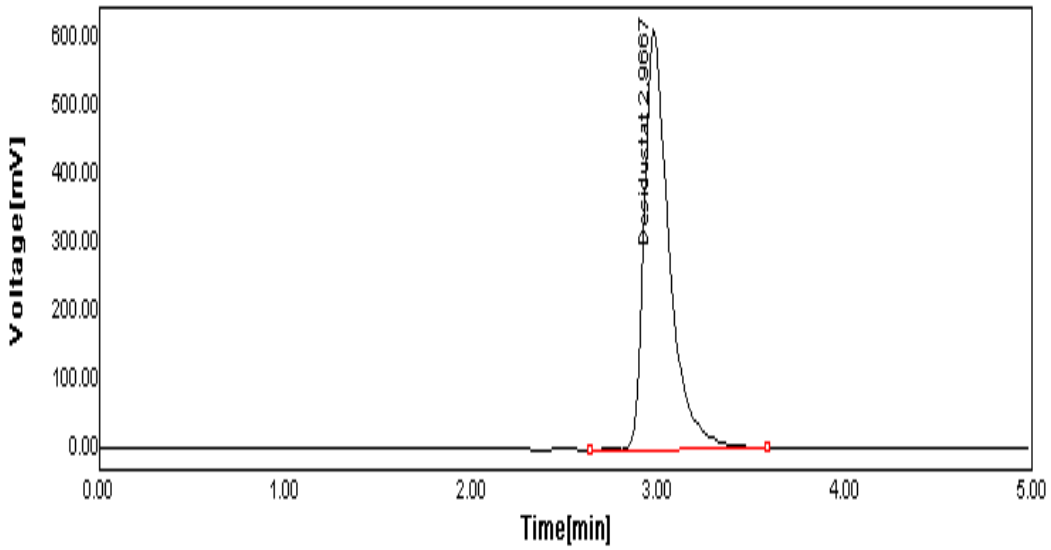

Figure 7:Chromatogram of standard Desidustat for intermediate precision

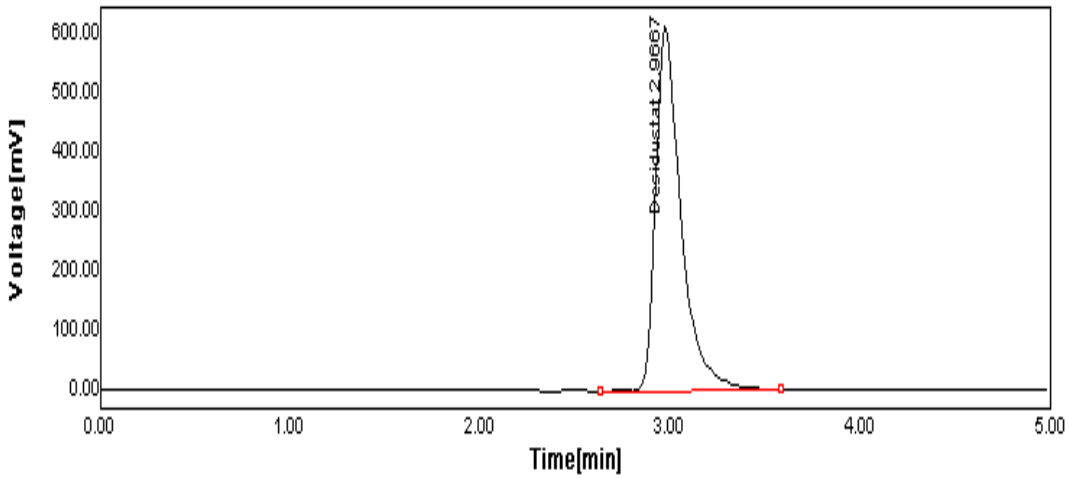

Figure 8:Chromatogram of standard Desidustat for intermediate precision

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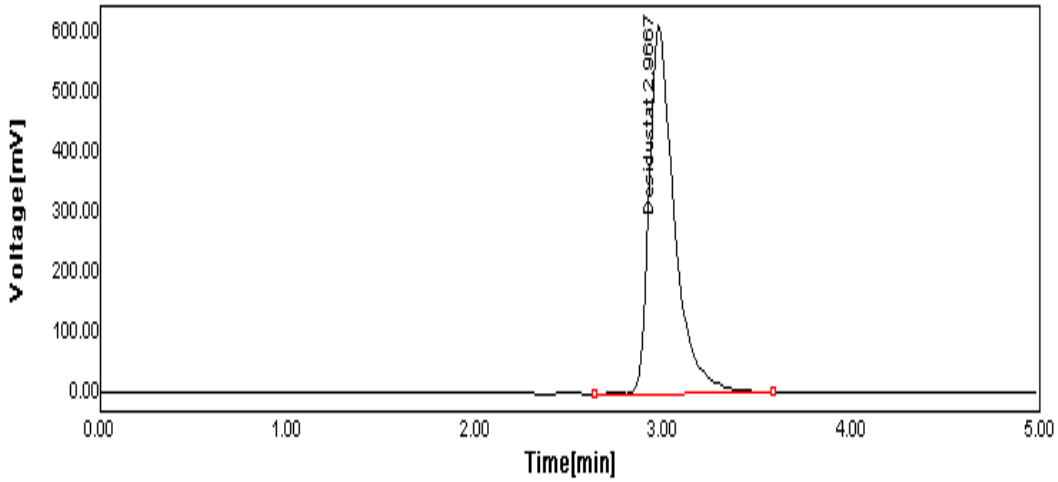

Figure 9:Chromatogram of standard Desidustat for intermediate precision

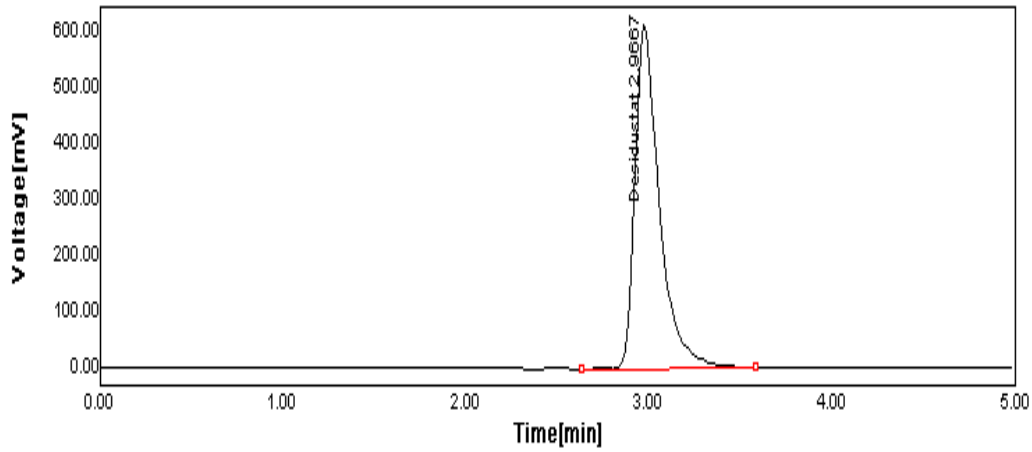

Figure 10:Chromatogram of standard Desidustat for intermediate precision

Table 6: Data for Intermediate Precision

Day	Sample Injection	Peak Area	Weight of Sample	Assay	
				In mg	In %
	1	5716.2188	1330	5.121	98.8
1	2	5605.3749	1330	5.123	98.55
	3	5500.4745	1330	5.122	99.4
2	4	5456.7474	1330	5.120	100.53

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	5	5353.7473	1330	5.124	98.5
3	6	5297.8923	1330	5.119	98.3
			Mean	5.121	99.18
			Std deviation	0.699	0.6939
			%RSD	0.145	0.698

LINEARITY AND RANGE:

Table 7: Linearity study for Desidustat

Sample No	% Level	Concentration (µg/ml)	Area
1	25	10	5297.8923
2	50	20	5353.7473
3	75	30	5456.7474
4	100	40	5500.4745
5	125	50	5605.3749
6	150	60	5716.2188
		Slope	18600
		Standard deviation	135.516
		Correlation coefficient	1

Linearity Curve:
Concentration vs Curve

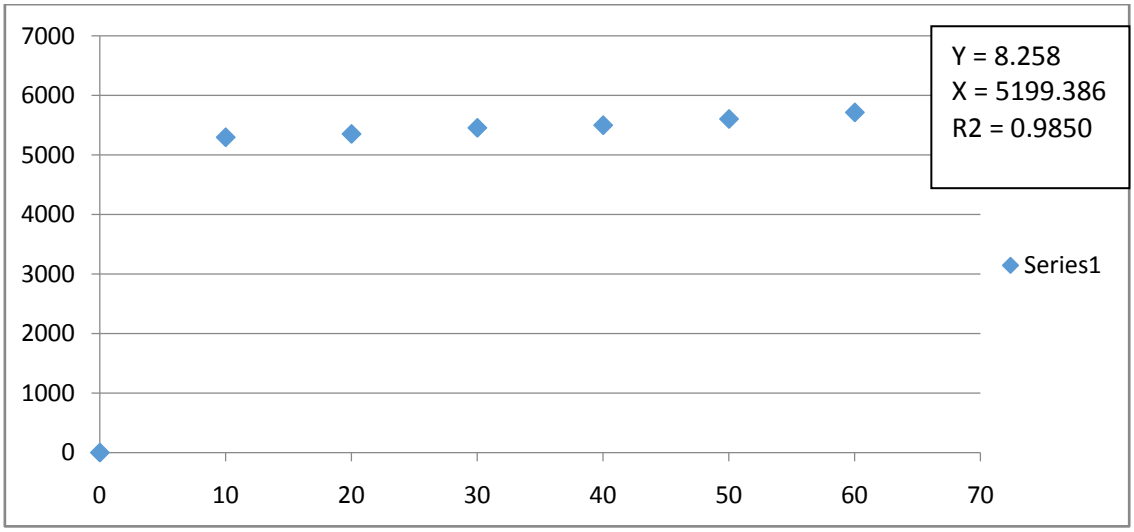

Figure 11:Linearity curve of Desidustat

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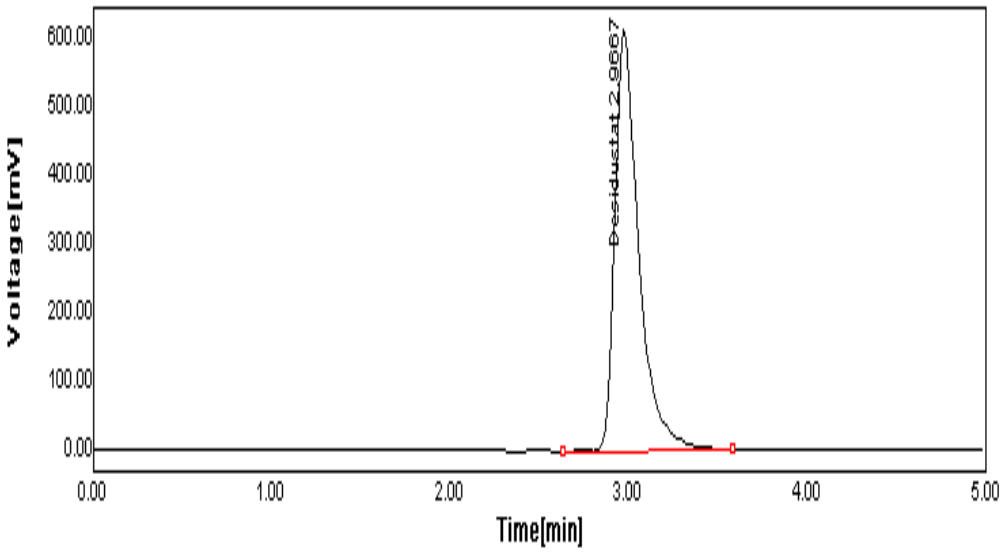

Figure 12:Chromatogram of standard Desidustat for linearity (25%)

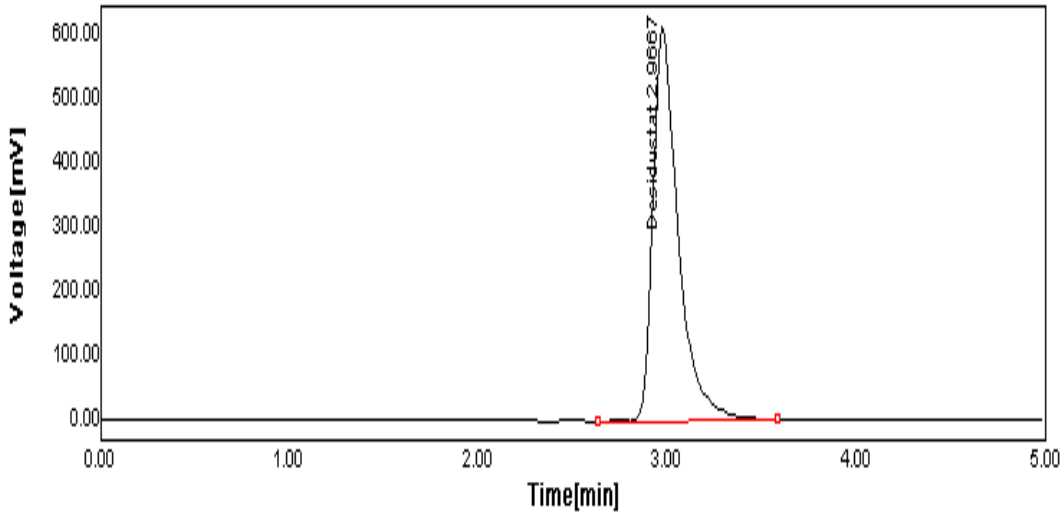

Figure 13:Chromatogram of standard Desidustat for linearity (50%)

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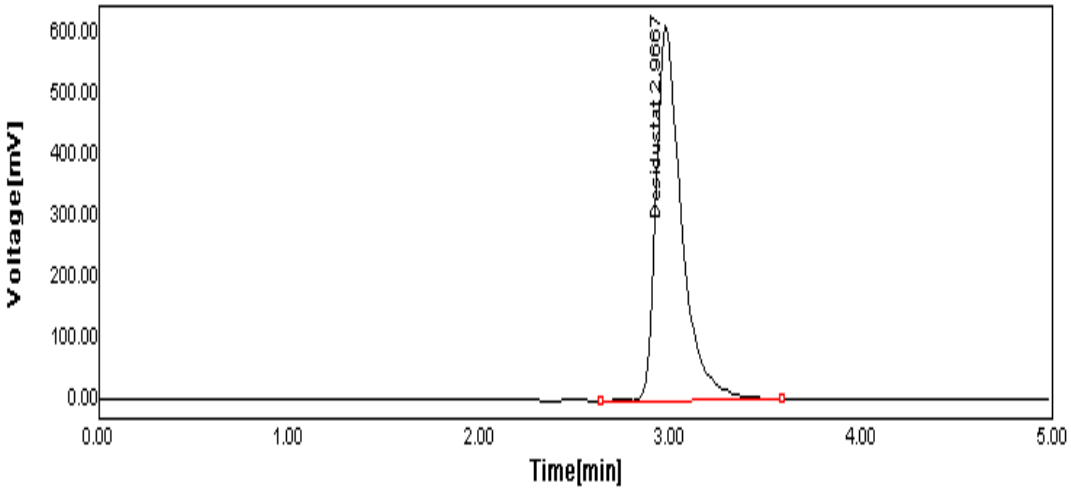

Figure 14:Chromatogram of standard Desidustat for linearity (75%)

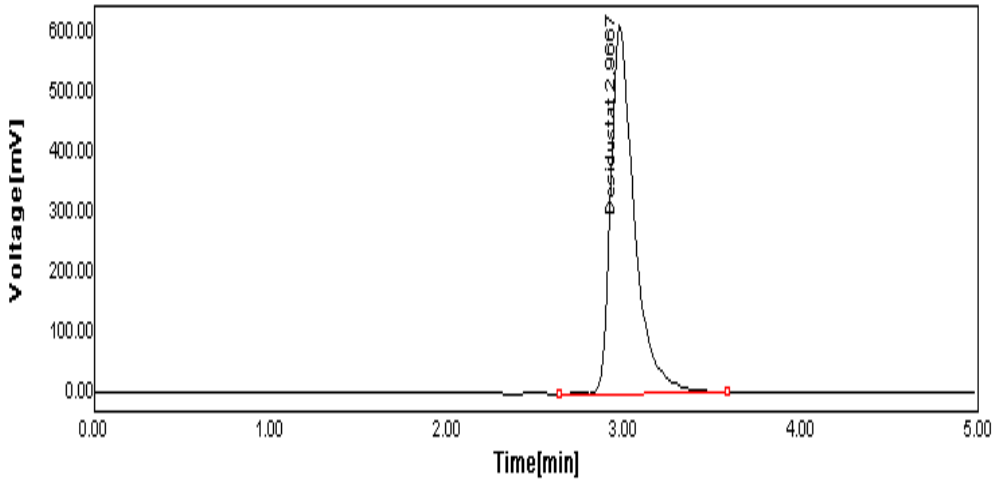

Figure 15:Chromatogram of standard Desidustat for linearity (100%)

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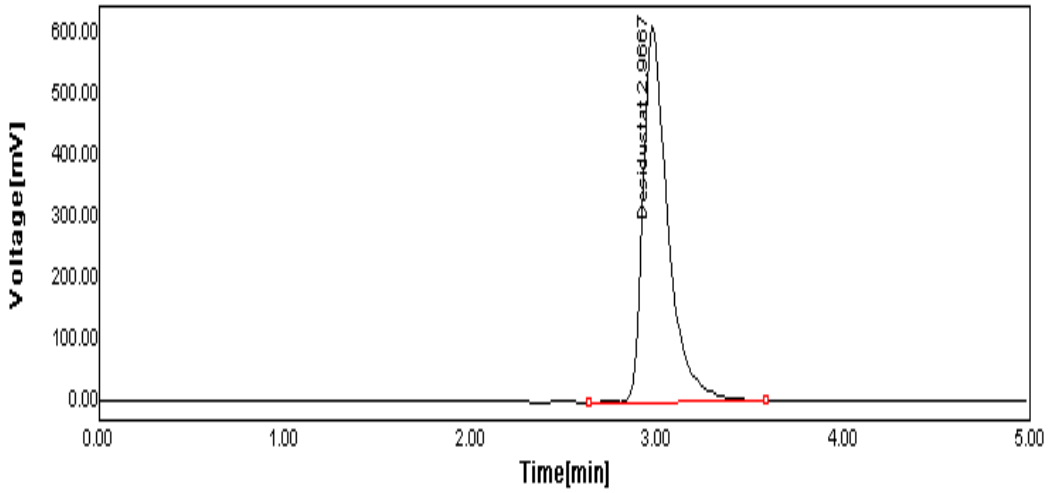

Figure 16:Chromatogram of standard Desidustat for linearity (125%)

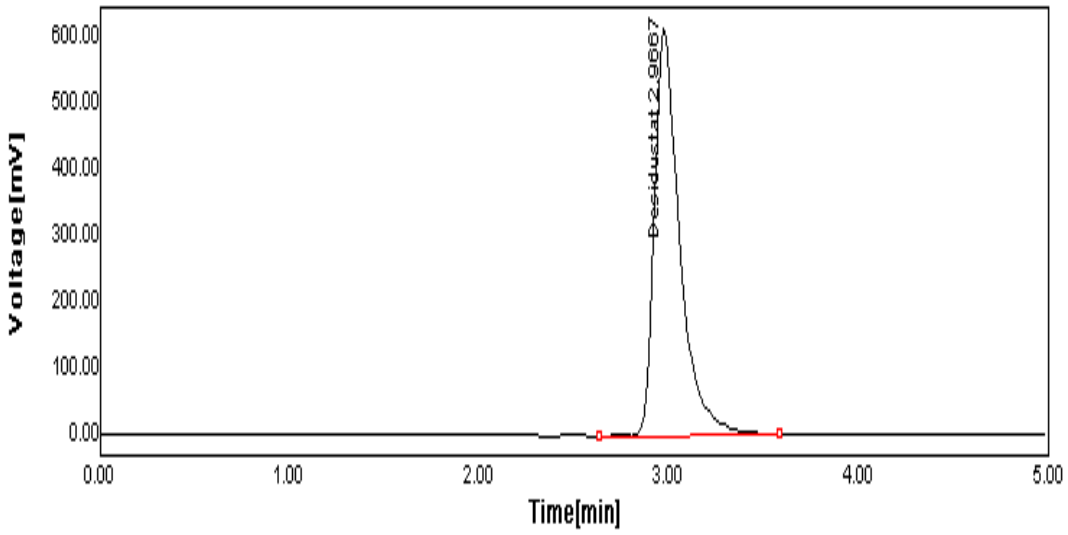

Figure 17:Chromatogram of standard Desidustat for linearity (150%)

METHOD ACCURACY:
DETERMINATION:

A known amount of Desidustat was spiked on Desidustat tablets 25mg tablet powder (equivalent to 100 mg of Desidustat) in order to produce recovery levels at 100% and 150% of the Desidustat working concentration of 50 micron/ml. Spiked assay samples were prepared in triplicate, injected in duplicate and the percentage recovery was calculated.

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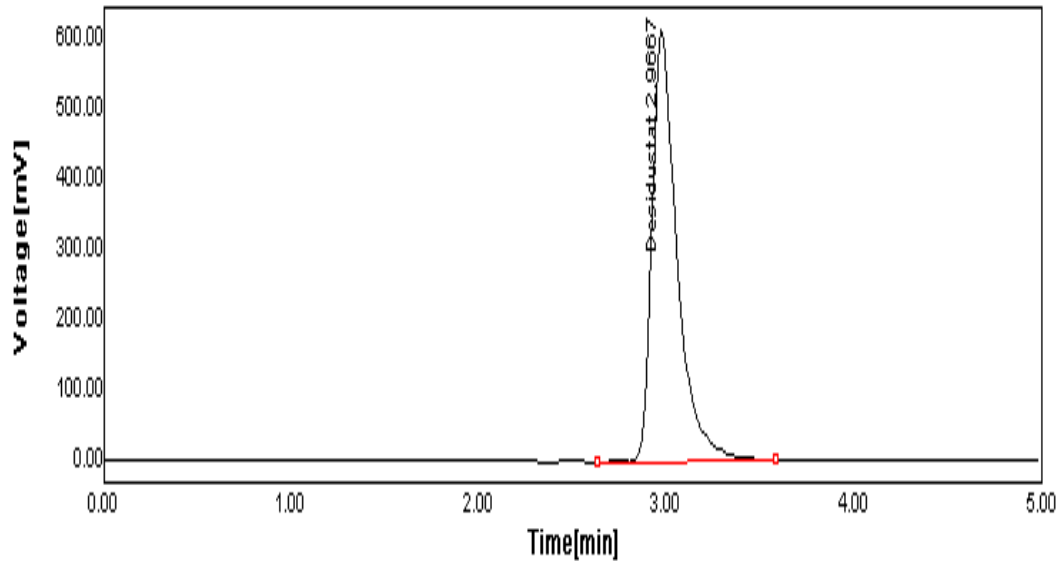

Figure 18:Chromatogram of standard Desidustat for method accuracy (50%)

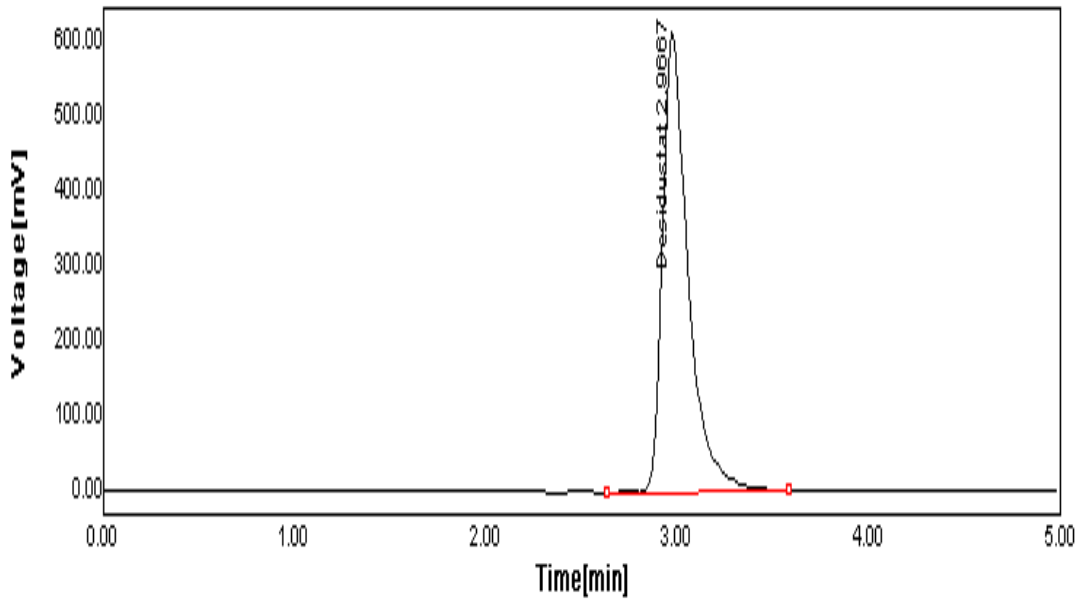

Figure 19:Chromatogram of standard Desidustat for method accuracy (100%)

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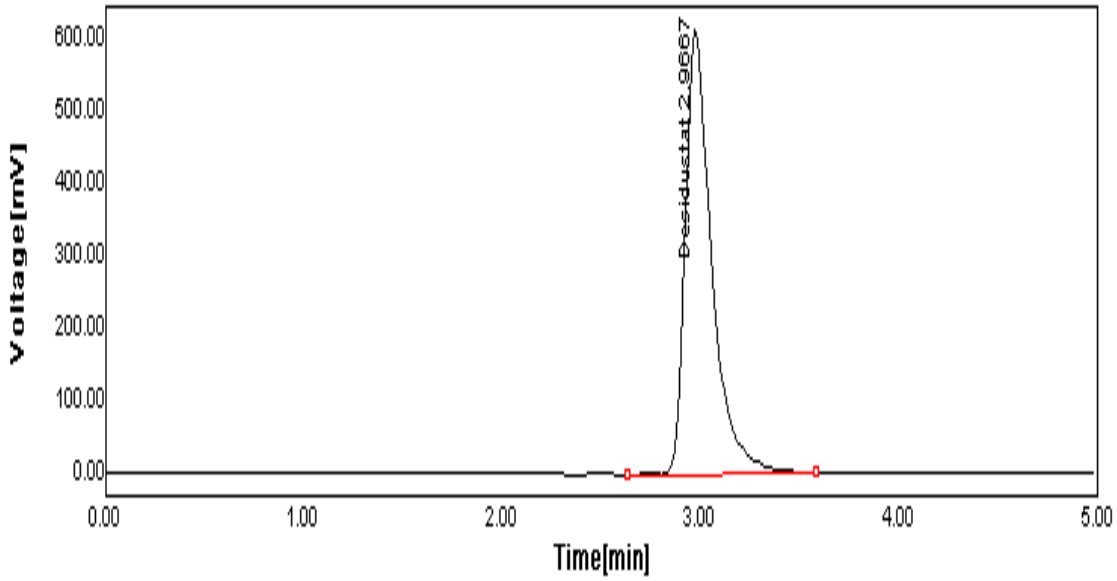

Figure 20:Chromatogram of standard Desidustat for method accuracy (150%)

Table 8: Method accuracy study of Desidustat

Sample No	Theoretical %	Mean Peak Area	Recovery		Mean (%) Recovery	Std deviation	%RSD
			In (mg)	In (%)			
1	50	5716.2188	100	100.48	99.66	62.84	0.773
2	50	5605.3749	100	99.50			
3	50	5500.4745	100	100.55			
1	100	5456.7474	100	99.93	99.71	23.27	0.2655
2	100	5353.7473	100	99.63			
3	100	5297.8923	100	99.57			
1	150	5287.2642	100	99.10	99.31	1.40	0.0110
2	150	5276.3749	100	99.54			
3	150	5256.6745	100	99.30			

The values for the range of recovery levels from 50% - 150% of the Desidustat working concentration (25 micron/ml) confirm to the acceptance criteria. The % Desidustat recovered at each of the levels falls between 99.6% -101.6% and the % RSD of all determinations at each level was not more than 2.0% therefore the method is considered accurate.

LIMIT OF DETECTION (LOD) and LIMIT OF QUANTIFICATION (LOQ):

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Table 9: Data for LOD and LOQ

Sample No	% Level	Concentration (µg/ml)	Area
1	25	25	5716.2188
2	50	50	5605.3749
3	75	75	5500.4745
4	100	100	5456.7474
5	125	125	5353.7473
6	150	150	5297.8923
		Slope	18600
		Standard deviation	135.516
		Correlation coefficient	1
		LOD	0.024µg/ml
		LOQ	0.07µg/ml

The LOD and LOQ Desidustat was calculated from the following formula,

LOD = 3.3σ/S

Where the σ = the standard deviation of the response,S = the slope of the calibration curve

LOQ = 10 σ/S

Where the σ = the standard deviation of the response, S = the slope of the calibration curve

SOLUTION STABILITY:

Chromatogram:

Figure 21:Chromatogram of standard Desidustat for Solution Stability

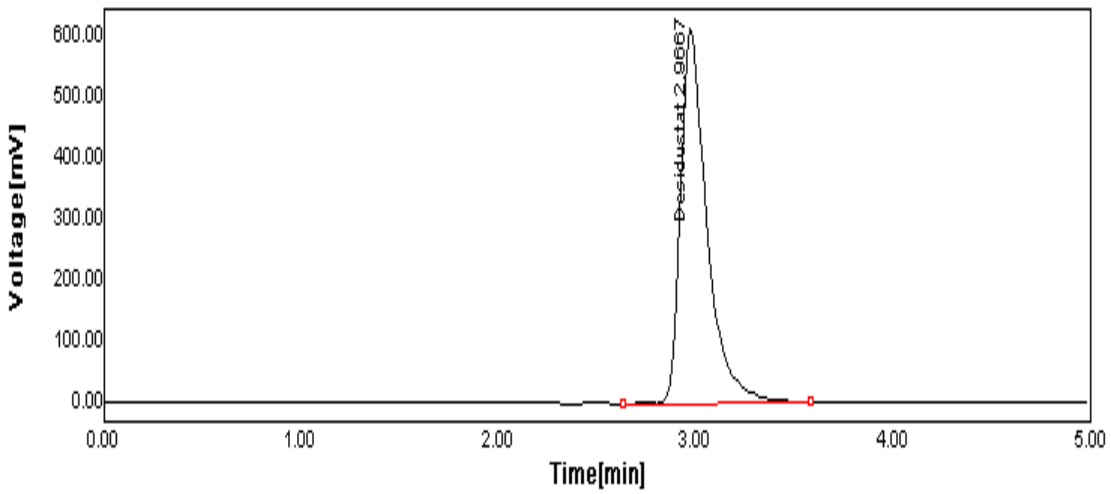

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Table 10: Data for standard solubility stability

S.NO	Time In Hours	RT	Peak Area
1	0	2.96	5716.2188
2	4	2.96	5605.3749
3	8	2.96	5500.4745
4	12	2.96	5456.7474
5	16	2.96	5353.7473
6	Mean	2.96	9408.701
7	Std deviation	0.00	7819.972
8	%RSD	0	83.11

Result:
The RSD of obtained standard area is not more than 2.0%. Therefore, the solutions is considered stable.

V. CONCLUSION:

The validated method was to quantitatively estimate the amount of Desidustat in pharmaceutical tablet dosage form using RP-HPLC method. The calibration curve for Desidustat was found to be linear in the range of 25µg/ml too 150µg/ml r2=0.9850 indicating a good linearity.The percentage recovery of sample was found to 99.8 to 101.66%w/w for Desidustat indicating the good accuracy of the method.To evaluate the validity and reproducibility of the method known, amount of pure drug was added to previously analysed samples and these samples were reanalyzed by proposed method, the percentage recovery was found to be close to 100% for all the methods.The limit of detection and limit of quantification was done by using linearity data, slope and standard deviation of the linearity samples were found to 0.024 µg/ml and 0.07µg/ml respectively.The % relative standard deviation (%RSD) values for system precision was 0.45% method precision was 1.56% and intermediate precision was 1.517%. The system precision and method precision were found to be less than 2% and so the method is said to be precise.The proposed method was applied for analysis of Desidustat in bulk formulation.The content and the percentage label claim of drugs in market sample indicate that the proposed method is rapid, precise and accurate for the routine estimation of Desidustat in tablet dosage form.

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