A Validated Rp-HPLC Method for the Determination of Desidustat in Pharmaceutical Dosage Forms

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Abstract- An accurate, sensitive, precise, fast isocratic reverse phase HPLC (RP-HPLC) method has been developed and validated for the quantification of Desidustat in bulk and pharmaceutical tablet dosage forms. With acetonitrile as the organic solvent, the best separation was achieved on a 250 mmx 4.6 mm i.d., 5µ-particle size Inertsil®-Octadecyl-silyl-3V-Reverse-Phase-C_{18}-column with 0.03M Potassium Dihydrogen Orthophasphate in water pH: 3.2 with Orthophosphoric acid: Acetonitrile (55:45) as the mobile phase solvent in the isocratic mode of elution at a speed of 0.8 ml.min^{-1}. UV detection was at 246 nm. Retention time of Desidustat was 4.67 minutes. With a correlation coefficient of about 0.999, peak-response was obtained as a function of concentration over the range of 40 to 120 µg/ ml for Desidustat. Desidustat was shown to have a percentage assay of 98.6%. Desidustat had a limit of detection of 0.05 mcg/ ml and a limit of quantification (LOQ) of 0.15 mcg/ ml. The presence of excipients in the formulation had no effect on the assay method. The procedure is appropriate for use in QC- laboratories since it is economical and precise.

Keyword: Acetonitrile, Anemia, Desidustat, Oxemia, RP-HPLC, Water.

INTRODUCTION

Desidustat (INN, also known as ZYAN1) [1] is a drug for the treatment of anemia of chronic kidney disease. This drug with the brand name Oxemia is discovered and developed by Zydus Life Sciences[2] for the treatment of anaemia associated with chronic kidney disease (CKD), COVID-2019 infections and chemotherapy induced anaemia [3]. Desidustat reduces the requirement of recombinant erythropoietin requirement in anemia, and decreases EPO-resistance, by reducing IL-6, IL-1β, and anti-EPO antibodies [4]. IUPAC name of Desidustat is N-[[1-(cyclopropylmethoxy)-1, 2-dihydro-4-hydroxy-2-oxo-3-quinolinyl] carbonyl]-glycine. Desidustat is an inhibitor of hypoxia-inducible factor prolyl hydroxylase (HIF-PH) [5] and stimulates erythropoiesis. Desidustat is under investigation in clinical trial NCT04012957 in the treatment of Anemia in CKD. It is currently being investigated against anemia of inflammation and COVID-19. It has a molecular weight of 332.31g/mol with a molecular formula $C_{16}H_{16}N_2O_6$. It reduces levels of hypoxia-inducible factor-1α (HIF-1α), a transcription factor regulated by HIF-PH enzymes, in rat liver and kidney. Desidustat increases the expression of the red blood cell- and iron transport-related genes *Epo, Fpn1,*and *Hamp* in rat liver in a model of anemia induced by peptidoglycan-polysaccharide (PGPS) [6]. It increases plasma levels of erythropoietin in rats by 10.3- to 40-fold when administered at doses of 15 and 30 mg/ kg respectively. Desidustat (15 and 30 mg/kg) also increases plasma levels of erythropoietin and hemoglobin,as well as the number of circulating red blood cells, in nephrectomized rats in a model of chronic kidney disease-induced anemia. It increases hemoglobin levels and the number of circulating red blood cells in a mouse model of anemia induced by the DNA-crosslinking agent cisplatin.

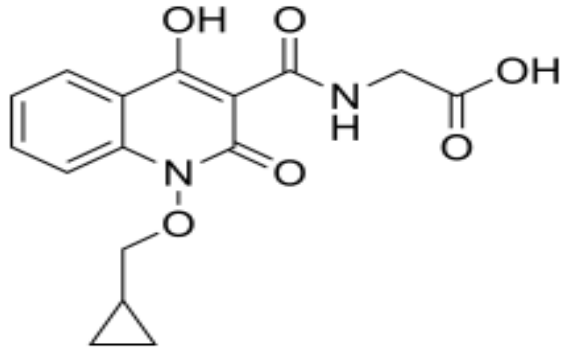

Fig 1: Structure of Desidustat

It is metabolically stable, has minimal potential to cause clinical drug-drug interactions (DDIs), and demonstrates discriminable pharmacokinetic properties for the treatment of anemia [7]. Hardly any techniques for the determination of Desidustat in oral fixed dosage form have been published. Furthermore,

no official or preliminary monograph on this analyte has been published in any of the compendial pharmacopoeias. The goal of this study was to develop an accurate and efficient RP-HPLC method to estimate Desidustat in unit dosage forms for oral administration. The validation of the devised approach is also addressed in this study, as per ICH guidelines [8].

Experimental:

Chemicals and Reagents:

- 99%, Desidustat pure was acquired from Srikem labs Pvt Ltd, Mumbai, India.
- Rankem-Fine-Chemicals of HPLC- Grade-Acetonitrile
- *ortho*-Phosphoric acid, 85% (v/v), Qualigen-Fine chemicals.
- Potassium Di hydrogen Orthophosphate, Qualigen-Fine chemicals.
- HPLC Grade water, SD-Fine chemicals.

Chromatographic-Instrument:

Quantitative RP- HPLC was carried out on a Waters 2996 high-performance liquid chromatograph with a PDA detector module, which included an automated injector with a 20 microliters injection volume and a quadra-pump. The column utilized was a Reverse Phase Inertsil Octa Decyl-S-3V-C_{18} column (250mmx 4.6 mm internal diameter with particle size 5µm). EMPOWER Software was installed on the HPLC equipment. The column temperature was adjusted to 25^0C and eluted over 20.0 minutes at a mobile solvent speed of 0.8 ml. min^{-1} under isocratic conditions. The mobile phase used was 0.03M Potassium Dihydrogen Orthophosphate in water pH:3.2 with Orthophosphoric acid: Acetonitrile (55:45v/v). It was degassed and filtered via 0.45mm Nylon membrane filters before use. For the analyte, UV detection at 246 nm was used as wavelength of detection with a PDA detector. H_20: CH_3CN in a ratio of 50:50 (v/v) was used as the diluent to make the standard dilutions. Desidustat was eluted at 4.7 minutes.

Preparation of the Primary Standard Drug solutions: To make the primary standard stock solution, 100.3 mg of Desidustat was dissolved in a volumetric flask (100ml) and diluted with the diluent (50:50 v/v H_2O: CH_3CN), sonicated for 15 minutes and diluted up to 100ml with the diluent to get the primary standard stock solution containing 1000µg-$ml^{-1}$of Desidustat.

Preparation of Working Standard Drug Solution: After adding 5 ml of the primary working standard solution to the 50 ml volumetric flask, the flask was filled with 50 ml of the diluent. This resultant mixture, which includes 100 ug/ ml of Desidustat, was suitable for use as a working standard solution. The stock solutions were kept in a cool, dark place that was controlled to be four degrees Celsius.

Sample Preparation: After measuring the weight of each individual tablet, the average weight of twenty Oxemia® pills was calculated. After crushing the tablets into a powder form a sample containing 130.4 mg of Desidustat tablet powder was obtained, which was then weighed, shifted to a 100ml pre-calibrated-measuring flask, and dissolved in a blend of acetonitrile and aqueous media with a volumetric ratio of 50:50 (v/v). After being sonicated in diluent and strained via Whattman 41 filter paper, the resultant primary working sample solution has 1000µg-$ml^{-1}$of Desidustat. After quantitatively transferring 5ml of the filtrate to a 50 ml pre-calibrated-measuring flask, the diluents were added to bring the volume of the solution to 50 ml. This served as a working testing solution having 100µg-$ml^{-1}$of Desidustat. The stock solution was kept in a dark place at 4º C.

Discussion and Results:

The purpose of this research was to create a chromatographic technique for the quantifiable determination of fixed-dose of Desidustat.

Optimized Chromatographic Conditions:

Elution solvents: 0.03M Potassium Dihydrogen Orthophosphate in water pH: 3.2 with Orthophosphoric acid: Acetonitrile (55:45 v/v)
Elution mode: Isocratic
Column: Inertsil ODS C-18-3V (250 x 4.6mm, 5µm particle size)
Flow rate: 0.08.0 ml/ min
Injection volume: 20 µl
Detector: Photo diode array (PDA)
Wavelength (λ_{max}): 246 nm
Column temperature: Ambient
Diluent: HPLC Grade Water: Acetonitrile (50:50 v/v)
Run time: 20 minutes
Retention time: 4.7 mins

Linearity: Aliquots of Desidustat working stock solutions was placed in various 10ml volumetric flasks and the volume was made up to the 10ml with the mobile phase, yielding in final strengths of 40-120 $\mu g.ml^{-1}$(Table 2). The peak areas and retention times of each of these drug solutions (loaded at 20µL) were measured thrice in the column. Using a PDA-detector set at 246 nm, a linearity-graph was generated by plotting peak areas-vs- Desidustat concentrations in $\mu g\text{-}ml^{-1}$.

Accuracy: The accuracy of the method was found by evaluating the drug recovery using the standard-spiking method. To assess if the analyte contained in the formulation caused positive or negative interventions, known amounts of the drug equivalent to 12 percent standard drug solution was added to 80 percent, 100 percent and 120 percent of the target test concentrations of the formulation. Each set-of-addition was replicated thrice at each dilution level. The results were compared to a competent reference standard after extraction of sample preparation. The percentage of analyte recovered by the assay was used to assess the accuracy. Table 3 shows the results of accuracy investigations on standard solution and process-related impurity; recovery measurements suggest that the procedure was accurate.

Precision: Quality-control samples in 100 % (w/v) dilution were used to assess intraday and inter-day precision. On the same day, six replicates of the target concentrations were examined for intra-day variation, and six replicates were examined for inter-day variation on three different days. The method's repeatability was indicated by the low RSD value (1%). (Table-4)

Limits of Detection and Quantification: The method's LOD was set at the lowest concentrations of active pharmaceutical component with a signal-to-noise (S/N) ratio of around 3 (LOD). The lowest active therapeutic medication concentration that can be assessed with acceptable precision and accuracy while maintaining a signal-to-noise (S/N) ratio of roughly 10 (LOQ) was also determined.

Method Applicability: The newly created method was evaluated by applying it to pharmaceutical tablets Oxemia for the estimation of Desidustat.

Results and Discussion:

Optimization of Chromatographic Conditions:

An isocratic RP- HPLC procedure for assaying the active ingredients was developed due to lack of an easy, economical, reproducible, and quick-to-use method for the determination of Desidustat concentrations in formulary matrices. The effect of various HPLC technique variables was examined on the result of the study to optimize the chromatographic parameters, various proportions of CH_3CN: *O*-H_3PO_3, CH_3CN-H_2O and CH_3CN-KH_2PO_4 buffer were tested. After several early investigatory tests, 0.03M Potassium Dihydrogen Orthophosphate in water pH: 3.2 with Orthophosphoric acid: Acetonitrile binary system at the proportion of 55:45 (v/ v) was chosen over other mobile phases because it resulted in improved resolution of active component. This procedure gave the good detection of analyte after multiple exploratory & investigatory trail runs. The active pharmaceutical analyte had excellent UV sensitivity and was interference-free at 246 nm. The analyte peak was highly defined and showed no incidence of tailing under these conditions. The set of conditions previously noted in this article were chosen for additional validation after considering the entire body of data acquired from this extensive study.

Method Validation Tests [9,10]:

Method precision (RSD percent), method accuracy (recovery percent & % RSD,), linear range (r^2) and LOD & LOQ were explored as recommended method validation characteristics.

Linearity: With a correlation coefficient of 0.9999, the graph of chromatographic-peak areas of the analyte versus respective concentration was shown to be linear in the band of 40-120 $\mu g.\ ml^{-1}$ for Desidustat (Table 2). The least square fit data of linear regression analysis derived from the measurements is given in Table 1. Desidustat is y = 06 x. Table 1 presents the regression parameters for this technique that include USP Tailing, resolution and % RSD. These findings suggest that there was a significant correlation.

Accuracy: Individual recovery of analyte at 80 %-dilution level on w/v basis, 100 %-dilution level on w/v basis and 120 %-dilution level on w/v basis of prescribed concentrations was 111.58 percent to 96.41 percent for Desidustat demonstrating the method's

accuracy. The % RSD was usually less than 1% in these data, demonstrating that the technique seems to be very accurate and generates consistent results (Table 3)

Precision: Table 4 summarizes the intraday and interday fluctuation in precision analysis. The method's repeatability is indicated by the low RSD value (less than-1%). These results show that the approach has a high level of precision and repeatability, both within a single analytical run and across multiple runs.

Limit-of-Detection & Limit-of-Quantifications: Desidustat has a limit of detection of 0.1 µg /ml and a limit of quantification (LOQ) of 0.3 µg/ ml. These numbers illustrate the method's high sensitivity, which is essential in most investigations, as well as the fact that it can be used to detect and quantify the analyte over a wide concentration range.

Specificity: The Retention time for Desidustat was determined to be 5.7 minutes, according to the representative chromatogram given in Figure 1. When the pharmaceutical tablet matrices were evaluated, no indication of excipient interference signal was observed in the respective retention time of the chromatogram. It indicates that the analyte was not disturbed of probable merging peaks. As a result, this technique can be employed with certainity.

Table 1: Regression analysis & Operating-System Suitability Results:

Study-Parameter	Desidustat
Retention Time (min)	4.7
Peak areas	10785318
Percentage of peak areas	99.74
USP-Tailing	1.7
Theoretical Plates	3307.18
Resolution	3.89
Linear range in (µg/ml)	40-120
Limit-of-Detection in $\mu g.ml^{-1}$	0.05
Limit-of-Quantification in $\mu g.ml^{-1}$	0.15
Correlation-Coefficient (r^2)	0.999
Assay-in-Percentage (%)	98.6

Table 2: Summary of the standard calibration Curve for Linearity experiment

Calibration Standard Dilution Level	Concentration of Desidustat (µg/ml)	Peak Area
40 %	40	4544621
60 %	60	6660386
80%	80	8774974
100 %	100	10998263
120 %	120	13057751

Table 3: Accuracy evaluation by Spike-analysis method

Accuracy study at 80% target level	Injection Number	Desidustat	
		Standard Soln.	Spiked Soln.
Oxemia-® tablet dosage form solution at 80% level was spiked with 12% of standard solution of API	1	8744228	10106220
	2	8809551	10074005
	3	8753545	10093097
	Mean area	8766895	10092127
	Std. Dev	18017.0	19067
	% RSD	0.2	0.19
	% Recovery		111.58
80% of the target concentration is equivalent to 80 µg/ ml in acetonitrile: water 60:40 v/v as diluent.			
Accuracy study at 100% target level	Injection Number	Desidustat	
		Standard Soln.	Spiked Soln.
Oxemia-® tablet dosage form solution at 100% level was spiked with 12% of mixed standard solution of API's	1	10882221	12058358
	2	10869631	12077756
	3	10871979	12064992
	Mean area	10874826	12068257
	Std. Dev	4471.00	9649
	% RSD	0.1	0.11
	%Recovery		104.83
100% of the target concentration is equivalent to 100 µg/ ml in acetonitrile: water 60:40 v/v as diluent.			
Accuracy study at 120% target level	Injection Number	Desidustat	
		Standard Soln.	Spiked Soln.
Oxemia-® tablet dosage form solution at 120% level was spiked with 12% of mixed standard solution of API's	1	13068755	14087731
	2	13115820	14201561
	3	13085480	14128890
	Mean area	13092289	14144670
	Std. Dev	51885	59000
	% RSD	0.4	0.39
	%Recovery		96.41
120% of the target concentration is equivalent to 120 µg/ ml in acetonitrile: water 60:40 v/v as diluent.			

Table-4: Evaluation of precision with-in-day and day-to-day analysis

Intra-Day Precision study of 100% standard dilution containing 100 µg/ ml of Desidustat			Inter-Day Precision study of 100% standard dilution containing 100 µg/ ml of Desidustat	
S. No	Desidustat		Desidustat	
	Ret. time	Peak area	Ret. time	Peak area
1	4.751	10595478	4.713	10782533
2	4.746	10893761	4.721	10777350
3	4.756	10718520	4.731	10923971
4	4.757	10629343	4.750	11022996
5	4.758	10821513	4.737	10917448
6	4.762	10559182	4.748	10764037
Average	4.755	10720584	4.733	10893305
Std. Dev	0.006	66885	0.014	25589
% RSD	0.12	0.66	0.30	0.21

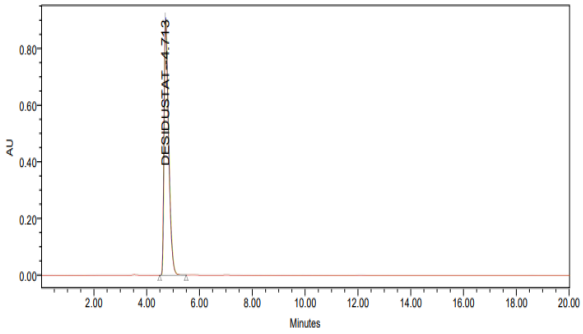

Figure 1: Chromatogram of Desidustat 100µg/ml analyzed by optimized Isocratic RP-HPLC method.

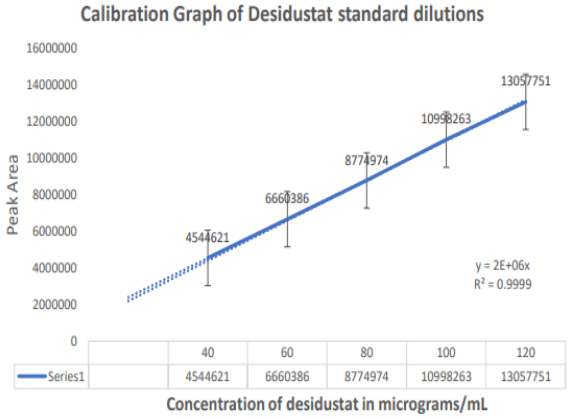

Figure-2: Linearity graph of Desidustat standard solution

CONCLUSION

In this study, an economical, efficient and commonly available HPLC method for the analysis of Desidustat in pharmaceutical matrices was devised. This method's key advantages are its significantly reduced cost, ease of use, ease of operation and reduced run time. All these features are critical in operation, especially when analyzing a large number of samples. The validation experiments demonstrated that the procedural approach has a large calibration concentration range, adequate precision & accuracy, and practically reliable sensitivity. The method can be used for regular analysis in formulation QC-studies and allows for a straightforward, selective, sensitive, and specific assessment of Desidustat.

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