Analytical method development and validation for simultaneous estimation of Dapagliflozin and Desidustat by RP-HPLC Method in prepared tablets

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***Abstract* —A simple, fast and economic reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous and quantitative analysis of Dapagliflozin and Desidustat in laboratory prepared tablets. The method was developed using the mobile phase comprising of methanol, water and trifluoroacetic acid in the ratio of 73:27:0.05 (v/v) over C-18 hypersil column (250 x 4.6 mm, 5 um) at ambient temperature. The flow rate was at 1.0 ml/min and the eluent was monitored by UV detection at 228 nm. The retention times for the nephroprotective drugs-Dapagliflozin and Desidustat were found to be 3.9and 4.3 minutes, respectively, with good resolution and symmetric peaks. The recoveries were found to be >99% for both Dapagliflozin and Desidustat, demonstrative of accuracy of the protocol. Inter-day and intra-day precision of the new method were less than the maximum allowable limit (RSD%>2.0) according to ICH, USP and FDA guidelines. The method showed linear response with correlation coefficient (r^2) values of 0.99988 for Dapagliflozin and 0.99995 for Desidustat. The LOD & LOQ of Dapagliflozin and Desidustat were found to be 0.16 ug/ml, 0.50 ug/ml & 0.55 ug/ml, 1.66 ug/ml respectively. Therefore, the method was found to be accurate, reproducible, sensitive and less time consuming and can be successfully applied for the assay of Dapagliflozin and Desidustat in combined formulations.**

***IndexTerms*— HPLC, Dapagliflozin, Desidustat, analysis, tablets**

1.Introduction

Chronic kidney disease (CKD) continues to be a global health concern, particularly among individuals with diabetes mellitus, where kidney function progressively declines due to metabolic, hemodynamic, and inflammatory factors. Effective therapeutic strategies increasingly involve the use of drug combinations that target multiple pathophysiological pathways to slow disease progression and improve patient outcomes.[1] Among such emerging nephroprotective agents, Dapagliflozin and Desidustat have shown considerable promise for use in CKD patients, both individually and potentially in combination therapies.[2]

Dapagliflozin is a selective sodium-glucose cotransporter-2 (SGLT2) inhibitor that was originally developed to manage type 2 diabetes mellitus. By inhibiting glucose reabsorption in the proximal tubules of the kidneys, it promotes glycosuria, leading to improved glycemic control independent of insulin.[3] Beyond its antidiabetic role, Dapagliflozin has demonstrated significant renal and cardiovascular protective effects, including reduction of albuminuria, decreased intraglomerular pressure, and slowed progression to end-stage renal disease.[4] These benefits have been well-documented in large-scale clinical trials such as DAPA-CKD, where Dapagliflozin was found effective even in non-diabetic CKD populations.[5]

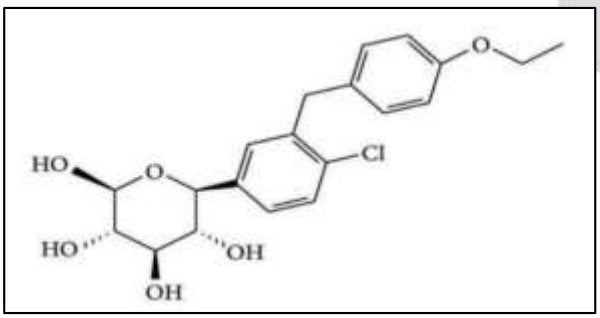

Fig no 1.1 Structure of Dapagliflozin

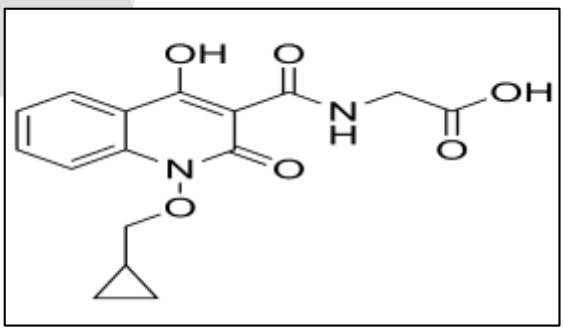

Fig no 1.2 Structure of Desidustat

Desidustat, on the other hand, is a novel oral hypoxia-inducible factor prolyl hydroxylase (HIF-PH) inhibitor developed for the treatment of anemia in CKD patients. It mimics hypoxic conditions by stabilizing HIF, thereby stimulating the transcription of erythropoietin and enhancing iron metabolism.[6] This mechanism makes Desidustat a compelling alternative to injectable erythropoiesis-stimulating agents (ESAs), offering better patient compliance and a favorable safety profile.[7] It not only corrects anemia but may also exert anti-inflammatory and cytoprotective effects beneficial in CKDGiven their complementary mechanisms—Dapagliflozin improving metabolic and renal outcomes, and Desidustat managing anemia—there is growing interest

in co-formulating these drugs into a single tablet dosage form.[8] However, from an analytical perspective, the simultaneous quantification of these compounds presents a unique challenge due to their differing chemical structures, polarities, and chromatographic behaviors. Despite the therapeutic relevance, there is currently a lack of validated methods for their combined estimation in pharmaceutical preparations.[9][10]

High-Performance Liquid Chromatography (HPLC) is a well-established and widely accepted analytical technique for quantifying pharmaceutical compounds due to its high sensitivity, accuracy, and reproducibility. The present study is aimed at developing and validating a simple, precise, and robust reverse-phase HPLC (RP-HPLC) method for the simultaneous estimation of Dapagliflozin and Desidustat in a combined tablet dosage form.[11] The method is optimized for effective separation and resolution of both drugs, and validated in accordance with the International Conference on Harmonisation (ICH) Q2(R1) guidelines. This method will serve as a critical tool for quality control, formulation development, and regulatory evaluation of nephroprotective combination therapies.[12]

I. Materials and Methods

1. Chemicals and Reagents

Desidustat and Dapagliflozin reference standards were purchased from Bulat pharmaceuticals pvt ltd, Haryana. HPLC-grade methanol, water and trifluoroacetic acid were procured from Merck (India). Potassium dihydrogen phosphate and acetonitrile (analytical grade) were procured from S. D. Fine Chem Pvt. Ltd. All solvents and reagents used were of analytical or HPLC grade

2. Instrumentation and Chromatographic Conditions

Chromatographic analysis was performed on a [YOUNG LIN ACME9000] HPLC system equipped with a UV-visible detector (730D) and auto-injector. Data acquisition and processing were carried out using Lab Solutions software.

- **Column**: Hypersil BDS C18 column (250 mm × 4.6 mm, 5 µm)
- **Mobile phase**: Methanol, water and trifluoroacetic acid (73:27:0.05v/v)
- **Flow rate**: 1.0 ml/min
- **Detection wavelength**: 228 nm
- **Injection volume**: 20 µL
- **Column temperature**: Ambient (25 ± 2°C)
- **Run time**: 07 minutes

3. Determination of the λmax

The standard solutions of Desi and Dapa having strengths 10 µg/ml were prepared in methanol. These solutions were scanned individually and in combination using a UV-visible spectrophotometer (range 200–400 nm) to determine the wavelength of maximum absorbance (λmax). Both drugs showed satisfactory absorbance at 228 nm, which was selected as the detection wavelength for RP-HPLC analysis due to good response and minimal baseline noise.

4. Preparation of Laboratory Tablets

Tablets were prepared using the direct compression method. Each tablet contained 50 mg of Desidustat and 10 mg of Dapagliflozin. The APIs were blended with suitable excipients, and the mixture was compressed using a single-punch tablet machine. These tablets were used for analytical purposes only.

5. Preparation of Standard Stock Solutions

Accurately weighed 50 mg of Desidustat & 10 mg of Dapagliflozin were transferred into a 100 mL volumetric flask, dissolved in the diluent, and the volume was made up to the mark to obtain a stock solution with a concentration of 500 & 100 µg/mL. From this stock solution, 2 mL was pipetted into a separate 20 mL volumetric flask and diluted to volume with the same diluent to achieve a final concentration of 50 & 10 µg/mL. The solution was shaken well and filtered through a 0.2 µm nylon syringe filter before injecting into the HPLC system (50µg/ml & 10 µg/ml).

6. Preparation of Sample Solution

10 tablets were weighed individually and then crushed into a fine powder using a mortar and pestle. An accurately weighed quantity of the powdered tablet, equivalent to the required amount of active pharmaceutical ingredients (APIs), was transferred to a 100 mL volumetric flask. The contents were dissolved in the diluent with vigorous shaking and sonicated for 2 minutes to ensure complete dissolution. From this solution, 2 mL was pipetted into a 20 mL volumetric flask, diluted to volume with the same diluent, shaken, and sonicated. The final solution was filtered through a 0.2 µm membrane filter prior to HPLC analysis.

7. Method Validation

The method was validated as per ICH Q2(R1) guidelines for the following parameters:

7.1 System Suitability

System suitability was assessed by injecting five replicates of a standard solution before sample analysis. Parameters evaluated included: Retention time (Rt), Theoretical plates (N), Tailing factor (T), Resolution (Rs), %RSD of peak areas. All values were within acceptable limits, confirming suitability of the system.

7.2 Specificity

Specificity was confirmed by analyzing placebo and sample solutions to check for interference at the retention times of the analytes.

7.3 Accuracy (Recovery Studies)

Accuracy was assessed by recovery studies at 80%, 100%, and 120% levels. Known quantities of standard drugs were added to pre-analyzed samples, and percentage recovery was calculated.

7.4 Precision

Precision was evaluated in terms of:

- **Intra-day precision**: Four replicates of sample solutions were analyzed on the same day.
- **Inter-day precision**: The same procedure was followed on two different days.
  Results were expressed as %RSD (Relative Standard Deviation).

7.5 Linearity

Linearity was evaluated by preparing standard solutions of Dapagliflozin (5– 15µg/mL) and Desidustat (25.0–75 µg/mL). Calibration curves were plotted between peak area and concentration, and correlation coefficients (R^2) were determined.

7.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated using the formula:

- **LOD** = $3.3 \times (\sigma/S)$
- **LOQ** = $10 \times (\sigma/S)$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

7.7 Robustness

Robustness was evaluated by making small, deliberate changes in method parameters:

- Flow rate (±0.5 mL/min)
- Mobile phase composition (±5%)

The effect on retention time, peak area, and resolution was observed.

7.8 Analysis of formulated tablet

The formulated tablet test preparation was prepared as described earlier, with 2 injections made from the tablet solution. The active pharmaceutical ingredient (API) content was determined by comparing the observed amount (in mg) of Dapagliflozin and Desidustat to the label claim. The assay was calculated based on the peak area from the chromatograms, and the percentage assay of the formulated tablet was determined, ensuring the method's accuracy and suitability for routine analysis.

II. Results and Discussions

1. Method Development

A reverse-phase HPLC method was successfully developed for the simultaneous estimation of Dapagliflozin and Desidustat in prepared tablet formulations. After evaluating various solvent systems and detection wavelengths, a mobile phase of methanol,

water and trifluoroacetic acid in the ratio of 73:27:0.05 (v/v) and detection at 228nm provided well-resolved, sharp, and symmetrical peaks for both analytes.

- **Retention time**: Dapagliflozin ~ 3.9 min; Desidustat ~ 4.3 min
- The method showed good peak symmetry and baseline separation.

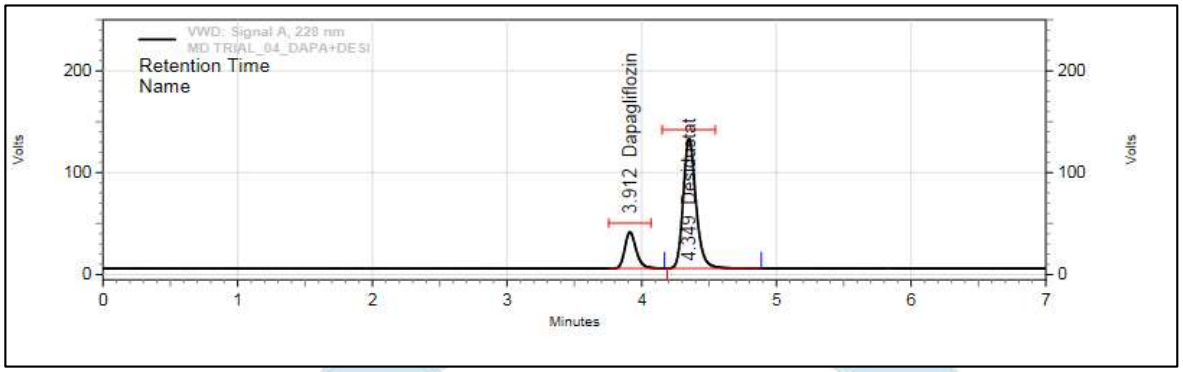

Fig no 3: Chromatogram showing resolved peaks of DESI & DAPA

2. System Suitability

System suitability parameters were assessed before validation. The results are summarized below:

Table no 1: System Suitability studies for Dapagliflozin

Dapagliflozin					
Name	**Area**	**RT (min)**	**TP (NLT 2000)**	**TF (NMT 2)**	**Resolution(NLT 2)**
Standard _Inj_01	3503020	3.892	10649	1.36	2.66
Standard_Inj_02	3481932	3.892	10690	1.34	2.67
Standard_Inj_03	3487112	3.892	10655	1.28	2.64
Standard_Inj_04	3484394	3.887	10734	1.32	2.65
Standard_Inj_05	3482641	3.882	10720	1.36	2.65
Mean	**3487820**	3.892			
SD	8728.8219	0.0045			
%RSD (NMT 2)	**0.25**	**0.11**			

Table no 2: System Suitability studies for Desidustat

Desidustat					
Name	**Area**	**RT (min)**	**TP (NLT 2000)**	**TF (NMT 2)**	**Resolution (NLT 2)**
Standard _Inj_01	13923413	4.315	10067	1.31	2.66
Standard_Inj_02	13911349	4.315	10799	1.23	2.67
Standard_Inj_03	13865464	4.310	10808	1.21	2.64
Standard_Inj_04	13825393	4.305	10848	1.34	2.65
Standard_Inj_05	13825585	4.300	10864	1.29	2.65
Mean	**13870241**	4.309			
SD	46220.8027	0.0065			
%RSD (NMT 2)	**0.33**	0.15			

Remark: Theoretical plates, resolution and Tailing factor observed within acceptance criteria, also %RSD of replicate injections for area and retention time observed within acceptance criteria, hence system is suitable for analysis of both Dapagliflozin and Desidustat. Hence System Suitability is justified.

3. Specificity

There was no interference from the blank or excipients. Well-resolved and pure peaks confirmed the method's specificity.

4. Accuracy (Recovery Studies)

Accuracy was evaluated by spiking known quantities of drugs into the matrix. The recovery was within acceptable limits

Table no 3: Accuracy studies of Dapagliflozin and Desidustat

Level (%)	Dapagliflozin			Desidustat		
	Mean % recovery	SD	%RSD (NMT 2)	Mean % recovery	SD	%RSD (NMT 2)
80%	99.99	0.8216	**0.82**	100.86	0.9567	**0.95**
100%	99.30	0.3016	**0.30**	99.59	0.4591	**0.46**
120%	99.73	0.7086	**0.71**	99.42	0.3037	**0.31**

5. Assay

%Assay of Dapagliflozin and Desidustat in test solutions 1 & 2 was found to be 99.07%, 99.92% & 99.72%, 99.60% respectively.

Table no 4: %Assay of Dapagliflozin

Name	Area	RT (min)	% Assay
Test solutions-1	3490071	3.882	**99.07**
Test solutions-2	3484797	3.878	**99.92**

Table no 5: %Assay of Desidustat

Name	Area	RT (min)	% Assay
Test solutions-1	13748117	4.295	**99.72**
Test solutions-2	13976515	4.286	**99.60**

6. Precision

Precision was evaluated by intra- and inter-day repeatability studies. The %RSD values were below 2% for both drugs, confirming the method's reproducibility under normal laboratory conditions.

Table no 6: Intraday precision data of Dapagliflozin & Desidustat

Name	Preparations	% Assay	
		Dapagliflozin	Desidustat
Set-1	prep-1	99.07	98.72
	prep-2	98.92	98.62
Set-2	prep-1	99.03	100.45
	prep-2	99.91	100.3
Mean		99.23	99.52
SD		0.4561	0.9533
% RSD (NMT 2)		**0.46**	**1.00**

Table no 7: Interday precision data of Dapagliflozin & Empagliflozin

Name	Preparations	% Assay	
		Dapagliflozin	Desidustat
Day-1	prep-1	99.07	98.72
	prep-2	98.92	98.60
Day-2	prep-1	99.73	99.24
	prep-2	98.27	99.84
Mean		99.25	99.10
SD		0.3522	0.5660
% RSD (NMT 2)		**0.35**	**0.57**

7. Linearity

Linearity was evaluated by analyzing five different concentrations within the expected working range. A strong correlation between concentration and peak area was observed, with correlation coefficients (R^2) greater than 0.999 for both drugs. This confirms the method's reliability across the tested concentration range.

8. LOD & LOQ

LOD and LOQ were determined based on. standard deviation and slope of the calibration curve. These values indicate the method's high sensitivity, capable of detecting and quantifying trace levels of both drugs

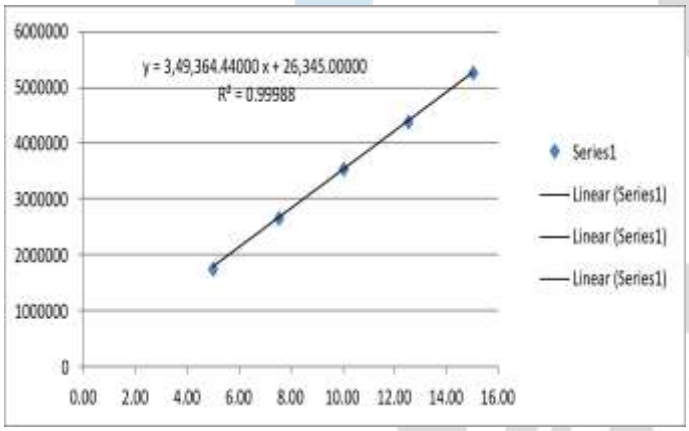

Fig no 4: Linearity curve for Dapagliflozin

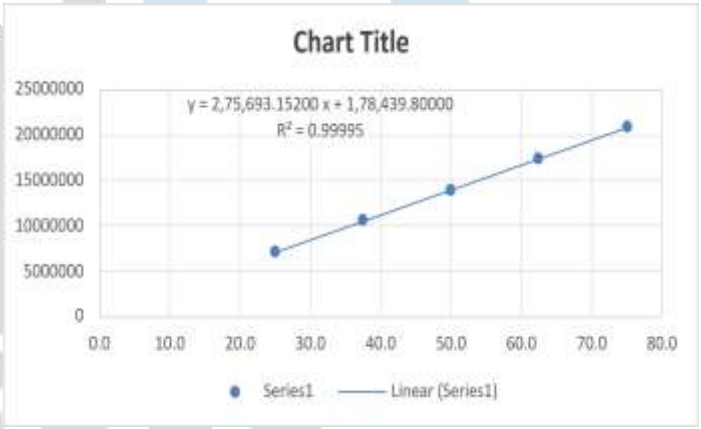

Fig no 5: Linearity curve for Desidustat

Table no 8: Linearity standards peak area along with LOD & LOQ for Dapagliflozin & Desidustat

Con. (ppm or ug/ml)	Area	Con. (ppm or ug/ml)	Area
5.00	1758467	25.00	7038884
7.50	2651026	37.50	10529441
10.00	3542930	50.00	13984506
12.50	4392977	62.50	17456459
15.00	5254547	75.00	20806197
Correlation coefficient (NLT 0.995)	**0.99988**	**Correlation coefficient (NLT 0.995)**	**0.99995**
Intercept	**26345**	**Intercept**	**178440**
SLOPE	**349364**	**SLOPE**	**275693**
STEYX	17443	**STEYX**	**45762**
LOD (ug/ml)	**0.16**	**LOD (ug/ml)**	**0.55**
LOQ (ug/ml)	**0.50**	**LOQ (ug/ml)**	**1.66**

9. Robustness

The robustness of the method was confirmed by deliberately altering flow rate & mobile phase. No significant variations were observed in retention time or peak area, proving that the method is reliable under slight changes in conditions.

Table no 9: Robustness changes in method parameters of Dapagliflozin & Desidustat

Name	Preparations	%Assay	
		Dapagliflozin	Desidustat
Original method parameters	Test prep-1	99.07	98.72
Original method parameters	Test prep-2	98.92	98.60
Pump, Flow 0.95 ml/min	Test prep	98.59	98.61
Pump, Flow 1.05 ml/min	Test prep	98.56	99.19
MeOH:Water:TFA, 70:30:0.05	Test prep	99.93	99.12
MeOH:Water:TFA, 76:24:0.05	Test prep	101.07	100.20
Mean		99.36	99.07
SD		0.9757	0.6081
%RSD (NMT 2)		**0.98**	**0.61**

11. Analysis of formulated tablet

The developed and validated RP-HPLC method was applied for the quantitative estimation of Dapagliflozin & Desidustat in the prepared tablet formulation. The sample was analyzed in triplicate, and the assay results were calculated based on the peak areas from the standard calibration curve. The percentage of the labeled amount found in the tablet was within the acceptable range of 98% to 102%, confirming that the method is suitable for routine analysis of these drugs in formulation. These results indicate that the prepared tablets complied with assay specifications and the method can reliably quantify both APIs without interference.

Table no 11: Formulated tablet test analysis (Dapagliflozin & Desidustat)

Name	Dapagliflozin		Desidustat	
	Test solution-1	Test solution-2	Test solution-1	Test solution-2
Area	3490071	3484797	13748117	13976515
RT (in min)	3.882	3.878	4.295	4.286
API obs in mg	9.9	9.89	49.36	49.3
Label claim	10	10	50	50
% Assay	**99.07**	**98.92**	**98.72**	**98.60**

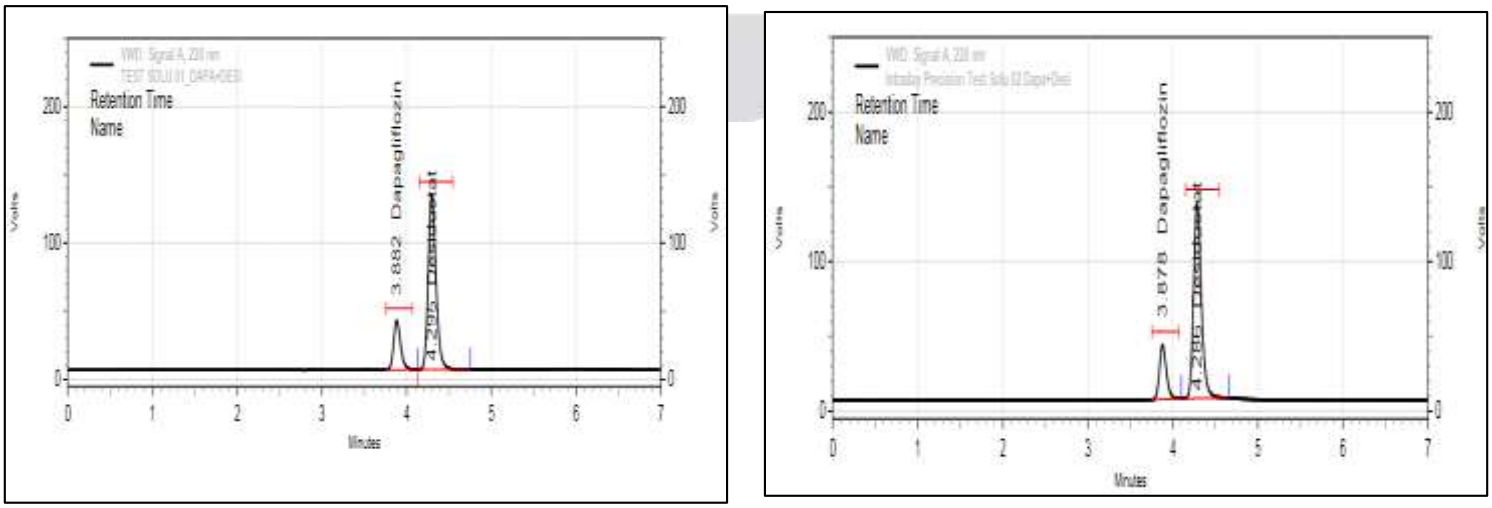

Fig no 6: Chromatogram of formulated tablet test solution-1 & test solution-2

III. Conclusion

A simple, precise, accurate, and robust reverse-phase HPLC method was successfully developed and validated for the simultaneous estimation of Dapagliflozin & Desidustat in prepared tablet formulations. The method demonstrated excellent linearity, precision, accuracy, specificity, and system suitability as per ICH guidelines. Low values of LOD and LOQ indicate the method's high sensitivity. The developed method was effectively applied to the analysis of formulated tablets, and the assay results were within acceptable limits. Overall, the method is reliable and suitable for routine quality control analysis of Dapagliflozin & Desidustat in pharmaceutical dosage forms.

IV. References

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