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R E S E A E C H A R T I C L E

Stability Indicating RP-HPLC Method for Simultaneous Estimation of Saxagliptin and Dapagliflozin in Pharmaceutical Dosage Form

Prathap.B[1*], Haribaskar.V[2], Kumar.B[3], Ramesh Dhani[4], Bhavya.CH[5], Kavitha.M[6], Harika.S[7]

Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Nellore – 524346, Andhra Pradesh, India.

A B S T R A C T

A simple, sensitive, accurate, precise and stability indicating RP-HPLC method has been developed for the simultaneous estimation of saxagliptin and dapagliflozin in combined pharmaceutical dosage form. The analysis was carried out at 225nm and the chromatographic separation was achieved with spursil C18 [250 X 4.6 X 5µ] column under 25°C temperature and using mobile phase 0.1%OPA (buffer): methanol: acetonitrile in a ration of 30:60:10v/v/v buffer pH adjusted to 3.8. The retention timef of saxagliptin and dapagliflozin were found to be 2.340 min and 5.081min respectively. The proposed method was validated according to ICH guidelines. The linearity study of saxagliptin and dapagliflozin was found in concentration range of 10 – 50 µg/ml and 20 - 100µg/ml and coefficient (r2) was found to be 0.9998 and 0.9991. The percentage recovery was obtained as 99.86%v/v and 100.64%v/v for saxagliptin and dapagliflozin. The percentage RSD was found to be less than 2.0%. The studies were carried out by conducting deliberate degradation of the sample with exposure to stress conditions like acidic, alkaline, thermal, oxidizing agent and light. This method was validated and met the regulatory requirements for specificity, linearity, LOD, LOQ, precision, accuracy, robustness and stability for the determination of saxagliptin and dapagliflozin in pharmaceutical dosage form by RP-HPLC.

Keywords: Saxagliptin, Dapagliflozin, RP-HPLC method

A R T I C L E I N F O

Corresponding Author
Prathap.B
Department of Pharmaceutical Analysis,
Ratnam Institute of Pharmacy,
Nellore – 524346, Andhra Pradesh, India.
MS-ID: IJCPS3722

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1. Introduction

Saxagliptin is chemically (1S,3S,5S)-2-{(2S)-2-amino-2-(3-hydroxyl adamantyl) acetyl}-2-azabicyclo(3.0.1)hexane-3-carbonitril figure 1 . It is a dipeptidyl peptidase-4 (DPP-4) inhibitor used for the treatment of type 2 diabetes [1,2]. It is a novel DPP-4 inhibitor with a high selectivity for DPP-4 compared with other dipeptidyl peptidase. DPP-4 inhibitors enhance endogenous concentrations of the insulin hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide.

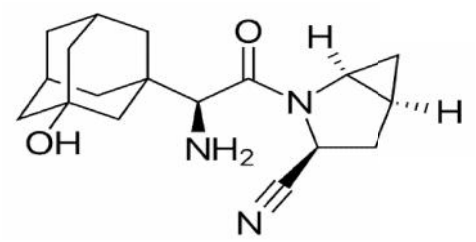

Fig 1: Structure of Saxagliptin

Dapagliflozin is chemically [(2S, 3R, 4R, 5S, 6R)-2-{4-Chloro3[(4-ethoxyphenyl)methyl]phenyl}-6-(hydroxyl methyl) oxane-3, 4, 5-triol figure 2.It inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2), which is responsible for at least 90% of the glucose reabsorption in the kidney [3,4]. Use of Dapagliflozin leads to blood glucose to be eliminated through the urine.

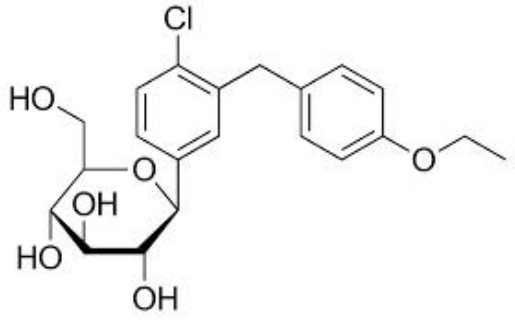

Fig 2: Structure of Dapagliflozin

The extensive literature survey revealed that there were UV spectroscopy, HPLC, LC-MS and HPTLC and stability indicating RP-HPLC methods [7 - 18] were available for the determination of saxagliptin and Dapagliflozin individually or in combination with other drugs. But no method was reported for simultaneous estimation of saxagliptin and dapagliflozin in combined dosage form using RP-HPLC method. The study was thus performed with an aim to develop a simple, economic, sensitive, rapid and accurate and precise method for the determination of saxagliptin and dapagliflozin in combined tablet dosage form.

2. Materials and Methods

Chemicals and reagents:

Saxagliptin and Dapagliflozin (API) were purchased from Pharma Train Labs, Hyderabad. Ortho phosphoric acid (OPA) purchased from SD fine chemicals (Hyderabad), methanol, acetonitrile and HPLC grade water obtained from Rankem. All the solvents used in the work were HPLC grade. The tablets containing 5mg of saxagliptin and 10m of dapagliflozin (QTERN) were purchased from local markets.

Instrumentation:

Waters (2695) HPLC using the software Empower 2, UV detector and Rheodyne injector with 20µl loop volume. All the glass wares used were 'A' grade.

Preparation of standard solution:

Accurately weighed and transferred 10mg of saxagliptin and 20mg of dapagliflozin into a 100ml volumetric flask and add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 3.0ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of sample solution:

Weigh accurately 20 tablets and average weight were found out and weight equivalent to 5 mg of saxagliptin and 10mg of dapagliflozin were taken into 100ml volumetric flask and dissolved in 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). The solution was filtered through 0.45µ Millipore Nylon filter. From the filtrate pipette out 3.0ml of solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of mobile phase:

Mixed 300ml (30%) of 0.1% OPA buffer pH 3.8, 600ml (60%) of Methanol and 100ml (10%) of Acetonitrile (HPLC) grade. Filter through 0.45µ Millipore Nylon filter and degassed.

Chromatographic conditions:

Spursil C_{18} column (250mm X 4.6mm, 5µ) was used for the chromatographic separation at a detection wavelength of 225nm under 25°C temperature. Mobile phase of 0.1% OPA buffer pH 3.8, methanol and acetonitrile in the ratio 30: 60: 10 v/v/v which was degassed under ultra sonication was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was optimized to 1.0ml/min and injection volume 20µl was fixed upon the statisfactory results of various system suitability parameters such as retention time, column efficiency, tailing factor, asymmetry of the peaks. The results were shown in figure 3.

Table 1: Optimized Chromatographic Conditions

Parameters	Condition
Mobile Phase	0.1% OPA Buffer: Methanol: Acetonitrile (30:60:10 v/v/v)
pH	3.5
Column	Spursil C_{18} (250mm X 4.6mm) 5µ
Column Oven Temperature	25°C
Wavelength	225nm
Injection Volume	20µl
Flow rate	1.0ml/min

Retention Time	2.322 for Saxagliptin and 5.065 for Dapagliflozin
Run Time	10min

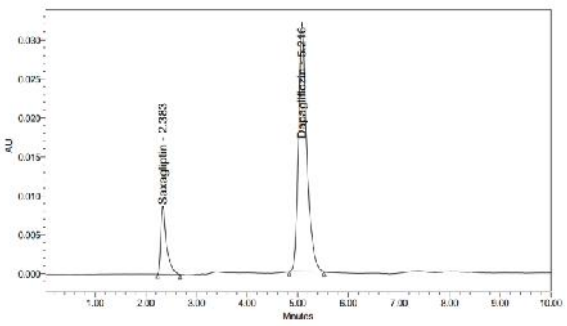

Fig 3: Optimized Chromatogram of Saxagliptin and Dapagliflozin

Method validation [5,6]

The method was validated by determining system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness by analyzing saxagliptin and dapagliflozin. The analytical method validation was carried as per ICH method guidelines.

System Suitability:

A system suitability test was performed to evaluate the chromatographic parameters (retention time, number of theoretical plates, capacity factor and asymmetry factor) before the validation runs. The results of system suitability parameter were given in table 2.

Linearity and Range:

The linearity of saxagliptin and dapagliflozin were evaluated at five concentration levels by diluting the standard solution to give solutions of saxagliptin and dapagliflozin in the concentration range from 10 t0 50 µg/ml and 20 to 100µg/ml. the regression analysis was carried out for the slope, intercept and correlation coefficient. The results were given in table 3 and 4.

Accuracy:

The accuracy of the method was determined by the standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed standard solution. The standard addition method was performed at 50%, 100% and 150% level of standard solution. The solutions were analyzed in triplicate at each level as per the proposed method. The corresponding results were recorded in table 5 and 6.

Precision:

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared saxagliptin and dapagliflozin test solution in the equipment. Record the chromatogram. The results were shown in table 7 and 8.

Robustness:

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms. The results were shown in table 9.

Limitof Detection (LOD) and Limit of Quantification (LOQ): The LOD is defined as the lowest concentration of an analyte in a sample that can be detected but not quantified. The LOQ is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy. The LOD and LOQ can be calculated based on the standard deviation of the response and the slope of calibration curve. LOD = 3.3σ/S and LOQ = 10σ/S where σ is the standard deviation of the intercept of the regression lines and S is the slope of the calibration curve.

Stability Studies

Stability testing was establishing for the allowed time span between sample collection and sample analysis. It is also important to evaluate an analytical methods ability to measure drug products in the presence of its degradation products. Forced degradation studies typically involve the exposure of samples of the drugs to the relevant stress conditions of acid, base, thermal, peroxide and UV. The results were shown in table 10.

Acid Degradation:

To 3.0ml of stock solution into a 10ml volumetric flask and 3ml of 1N HCl was added. Then the volumetric flask was kept at 60°C for 6 hours and then neutralized with 1N NaOH and Make up to 10ml with diluent. Filter the solution with 0.45µ syringe filter and place in vials. The chromatogram was recorded to assess the stability of drug substance. The results were shown in Figure 6.

Alkali Degradation:

To 3.0ml of stock solution into a 10ml volumetric flask and add 3ml of 1N NaOH. Then the volumetric flask was kept at 60°C for 6 hours and then neutralized with 1N HCl and make up to 10ml with diluent. Filter the solution with 0.45µ syringe filter and place in vials. The chromatogram was recorded to assess the stability of drug substance. The results were shown in figure 7.

Oxidative Degradation:

To 3.0ml of stock solution into a 10ml volumetric flask, 1.0ml of 3.0%v/v hydrogen peroxide is added the volume was made up to the mark with diluent. The solutions were kept for 30min at 60°C. The chromatogram was recorded to assess the stability of drug substance. The results were shown in figure 8.

Thermal Degradation:

The standard drug solution was placed in oven at 105°C for 6 hr to study dry heat degradation. The chromatogram was recorded to assess the stability of drug substance. The results were shown in figure 9.

Photo Degradation:

To 3.0ml of stock solution into a 10ml volumetric flask and expose to sunlight for 24 hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45µ syringe filter and place in vials. The chromatogram was recorded to assess the stability of drug substance. The results were shown in figure 10.

3. Results and Discussions

Method Development: Initially reverse phase liquid chromatography separation was tried to develop using

various ratios of methanol and buffer, water and acetonitrile, methanol acetonitrile and buffer as mobile phase, in which the drug did not respond properly. The organic content of the mobile phase was also investigated to optimize the elution of the drug. To improve the tailing factor, the pH of mobile phase becomes an important factor. Thereafter 0.1% OPA buffer pH 3.8, methanol and acetonitrile were taken in the ratio 30: 60: 10 and with a flow rate of 1.0ml/min was employed. Spursil C_{18} column (250mm X 4.6mm) 5µ was selected as stationary phase to reduce the tailing of the peak. 225nm was selected as the detection wavelength for UV detector. The retention time was found to about 2.383 min for saxagliptin and 5.216 min for dapagliflozin. The results were shown in table 1 and figure 3.

Analysis of Marketed Formulation by Developed Method: Assay of marketed tablet formulation containing 5mg of saxagliptin and 10mg of dapagliflozin was carried out by using this validated RP-HPLC method. Three injections of prepared sample and standard solutions were injected. The assay of the commercial sample was calculated by comparing the areas of standard and sample peaks. The assay of marketed formulation QTERN was found within the limit.

Method validation

System suitability:

An RP-HPLC method was developed by monitoring the system suitability parameter, i.e. tailing factor (T), the number of theoretical plates (N), the runtime and the cost effectiveness. System suitability method acceptable criteria set in each validation run were, tailing factor ≤ 2.0 and theoretical plates > 2000. In all cases the relative standard deviation (RSD) for the analytical peak area for consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution was presented. System suitability parameters were shown in Table 2. All the system suitability parameters are found to be satisfactory. The peak is reasonably symmetrical. High numbers of theoretical plates indicate the efficient performance of the column with reasonable retention times.

Linearity: The concentration range of 10 to 50µg/ml for saxagliptin and 20 to 100µg/ml of dapagliflozin were found to be linear with correlation coefficients of 0.9998 and 0.9991 saxagliptin and dapagliflozin respectively. The results were given in figure 4 and 5.

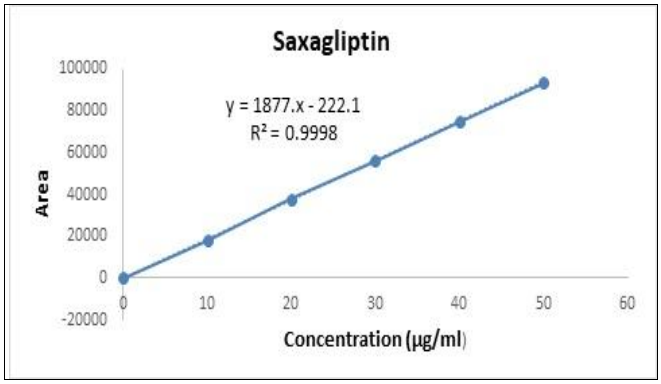

Fig 4: Calibration curve for Saxagliptin

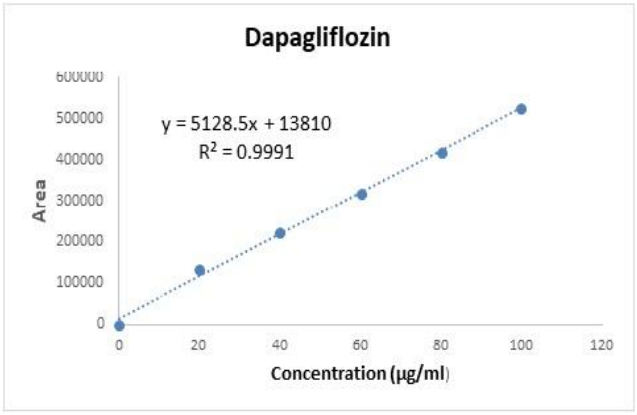

Fig 5: Calibration curve for Dapgliflozin

Accuracy:

The accuracy of an analytical method is the closeness of that results obtained by that method to the true value. Accuracy may often be expressed as percentage recovery by the assay of known added amount of analyte (50%, 100% and 150%). % Recovery should be in the range of 99.0% to 101.0%. The observed data was within the required range which indicates good recovery values and hence the method was accurate. The results were shown in table 5 and 6.

Precision:

The percentage relative standard deviation was calculated for the peak areas of the drug and it was found to be 0.8%, 0.3% and 0.5% the %RSD for the metronidazole, tetracycline and bismuth subcitrate respectively. The %RSD for the area of six standard injections was should be not be more than 2% and the method was found to be precised. The results were shown in table 7 and 8.

Degradation Studies

The degradation was determined by analyzed both drug solutions in the presence of acid, base, hydrogen peroxide, thermal and light or photo. The results of peak area of saxagliptin and dapagliflozin was changed hence the drug was degraded but the percentage degradation was less than 10%w/v. the results were within the limit as per ICH guidelines shown in table 10.

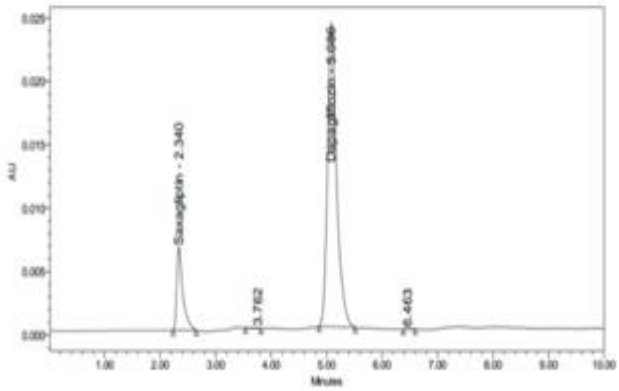

Fig 6: Typical Chromatogram of Acid Degradation for Saxagliptin and Dapagliflozin

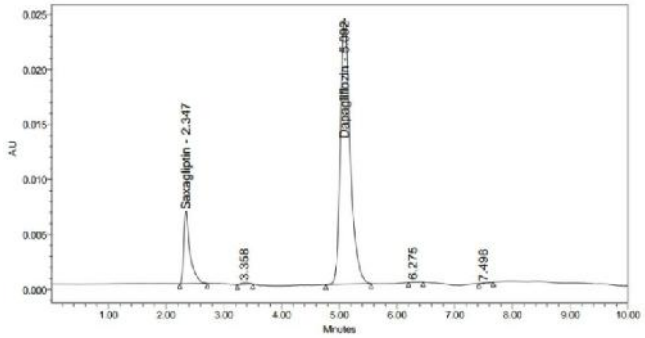

Fig 7: Typical Chromatogram of base Degradation for Saxagliptin and Dapagliflozin

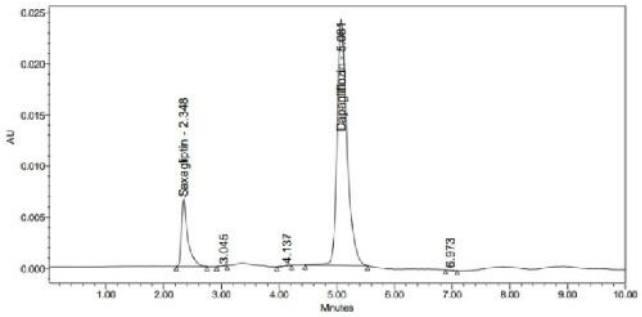

Fig 8: Typical Chromatogram of Peroxide Degradation for Saxagliptin and Dapagliflozin

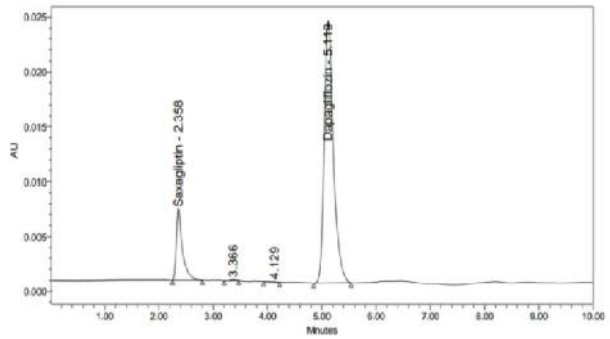

Fig 9: Typical Chromatogram of Thermal Degradation for Saxagliptin and Dapagliflozin

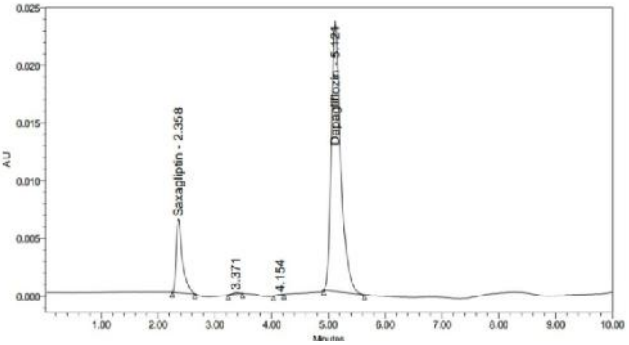

Fig 10: Typical Chromatogram of Photo Degradation for Saxagliptin and Dapagliflozin

Table 2: System Suitability Parameter

S.No	Parameters	Saxagliptin	Dapagliflozin
1	Retention time (min)	2.322	5.065
2	Area	56233	526461
3	Height	6832	41425
4	USP Resolution	--	11.49
5	USP Tailing	1.32	1.19
6	USP Plate Count	2552.18	4765.66

Table 3: Linearity data for Saxagliptin and Dapagliflozin

S.No	Saxagliptin		Dapagliflozin	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	10	17896	20	132359
2	20	37780	40	223105
3	30	56233	60	320315
4	40	74754	80	419173
5	50	93611	100	526461

Table 4: Regression Analysis of Calibration Curve

S.No	Parameters	Saxagliptin	Dapagliflozin
1	Correlation coefficient	0.9998	0.9991
2	Slope	1877	5128.5
3	Intercept	-222.1	13810

Table 5: Results of Recovery Study for Saxagliptin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	28244.7	5	5.01	100.26	99.86
100%	56457.3	10	9.89	99.76	

150%	85035.3	15	14.92	99.84	

Table 6: Results of Recovery Study for Dapagliflozin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	161058.3	10	10.04	100.39	100.64
100%	323719.3	20	20.18	100.89	
150%	484374.0	30	30.19	100.64	

Table 7: Results for Repeatability (Precision)

S.No	Area for Saxagliptin	Area for Dapagliflozin
1	56407	318752
2	56050	316862
3	56444	320903
4	56445	315150
5	56203	320979
6	56139	316258
Average	56281.3	318150.7
Stand Deviation	172.6	2457.0
% RSD	0.3	0.8

Table 8: Results for Intermediate Precision

S.No	Area for Saxagliptin	Area for Dapagliflozin
1	56082	316450
2	56734	318607
3	56133	316347
4	56124	319509
5	56948	319175
6	56919	317693
Average	56490.0	317963.5
Stand Deviation	419.8	1359.9
% RSD	0.7	0.4

Table 9: Robustness data for Saxagliptin and Dapagliflozin

Chromatographic Conditions	Resolution	Saxagliptin		Dapagliflozin	
		USP Tailing	USP Plate Count	USP Tailing	USP Plate Count
Flow rate (0.9ml/min)	12.44	1.29	3067.03	1.16	5361.12
Flow rate (1.0ml/min)	11.46	1.44	2589.12	1.19	4825.77
Flow rate (1.1ml/min)	11.48	1.27	2526.15	1.13	4766.36
Mobile phase composition (10% less)	12.97	1.19	3078.29	1.11	4573.25
Mobile phase composition (Actual)	11.46	1.44	2589.12	1.19	4825.77
Mobile phase composition (10% more)	9.37	1.19	2521.39	1.12	4756.36

Table 10: Stress testing data for Saxagliptin and Dapagliflozin

Nature of the Stress	Saxagliptin		Dapagliflozin	
Sample Name	Area	% Degraded	Area	% Degraded
Standard	56232.7		320211.3	
Acid	54275	3.48	295636	7.67
Base	52453	6.72	302783	5.44
Peroxide	53967	4.03	289767	9.51
Thermal	51867	7.76	316254	1.24
Photo	50162	10.80	286735	10.45

4. Conclusion

The observations and results obtained from this study including system suitability, linearity and range, accuracy, precision, robustness lie studies well within acceptable criteria. From the experimental, it can be concluded that the proposed method can be adopted fot the routine analysis of saxagliptin and dapagliflozin in their combined dosage form without interference of excipients and impurities.

5. Acknowledgement

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