Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 13, (2024)
https://www.jneonatalsurg.com

OPEN ACCESS

Development and validation of RP-HPLC method for the simultaneous estimation of sitagliptin phosphate and dapagliflozin in bulk and marketed formulations with forced degradation studies

Mohini A. Khatik[1*], Santosh D. Ghule[2], Sudha vengurlekar[3], Sachin Kumar Jain[4]

[1]*Research Scholar, Department of pharmacy, Oriental University, Indore, Madhya Pradesh, India.

[2]Professor, Faculty of Pharmacy, Oriental University, Indore, Madhya Pradesh, India.

[3]Professor & Dean, Faculty of Pharmacy, Oriental University, Indore, Madhya Pradesh, India

[4]Professor & Head, Faculty of Pharmacy, Oriental University, Indore, Madhya Pradesh, India

***Corresponding Author:**

Mohini A. Khatik,

Research Scholar, Institute of pharmacy, Oriental University, Indore, Madhya Pradesh, India.

Email ID : mohinikhatik2015@gmail.com

Cite this paper as: Mohini A. Khatik, Santosh D. Ghule, Sudha vengurlekar, Sachin Kumar Jain (2024) Development and validation of RP-HPLC method for the simultaneous estimation of sitagliptin phosphate and dapagliflozin in bulk and marketed formulations with forced degradation studies. *Journal of Neonatal Surgery*, 13, 1082-1102.

ABSTRACT

Introduction: The present study focuses on the development and validation of novel RP-HPLC method for the simultaneous estimation of Sitagliptin phosphate and Dapagliflozin in bulk drugs and marketed formulations. Method validation was carried out in accordance with International Council for Harmonisation (ICH) guidelines, assessing critical parameters such as accuracy, linearity, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ)[1,2]. Additionally, forced degradation studies were performed under acidic, alkaline, oxidative, photolytic, and thermal conditions to evaluate the stability-indicating capability of the developed methods. These validated analytical tools are expected to offer reliable and reproducible results for routine quality assurance, regulatory compliance, and stability testing of combination antidiabetic formulations.RP-HPLC methods for their estimation, along with assessing their stability through forced degradation studies.

Materials and Methods: The RP-HPLC method utilized a reversed-phase C18 column with a mobile phase composition optimized to achieve sharp, well-resolved peaks. Validation of method was performed according to ICH Q2(R1) guidelines, assessing parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), ruggedness, and robustness. Forced degradation studies were conducted under various stress conditions including acidic, alkaline, oxidative, photolytic, and thermal environments.

Results: The RP-HPLC method showed distinct retention times of 5.473 minutes for Sitagliptin and 8.611 minutes for Dapagliflozin. Both methods demonstrated high accuracy and precision with acceptable %RSD values (<2%). LOD and LOQ values confirmed the sensitivity of the methods. Among all stress conditions, the maximum degradation was observed under alkaline conditions, indicating that both drugs are less stable in basic environments.

Conclusion: The developed RP-HPLC method is simple, sensitive, and reliable for the simultaneous estimation of Sitagliptin Phosphate and Dapagliflozin in bulk and marketed formulations. Their successful validation and application to forced degradation studies underscore their suitability for routine quality control, formulation analysis, and stability testing in pharmaceutical industries

Keywords: *Sitagliptin Phosphate, Dapagliflozin, RP-HPLC, , Method Validation, Forced Degradation, Linearity, Stability Studies.*

1. INTRODUCTION

Diabetes is a long-term condition that happens when the pancreas either doesn't create enough insulin or the body isn't able to properly use the insulin it makes. When diabetes isn't managed, it often leads to high blood sugar levels (hyperglycemia), which can progressively damage the body's nerves and blood vessels.[3,4]Diabetes is a collection of conditions characterized by the body's inability to either produce sufficient insulin, effectively use the insulin it does produce, or both. This prevents

sugar from entering your cells from the bloodstream, resulting in elevated blood sugar levels. Glucose, the sugar in your blood, is a primary source of energy. When there's a lack of insulin or a buildup of blood sugar, it can lead to various health issues. [5.6]

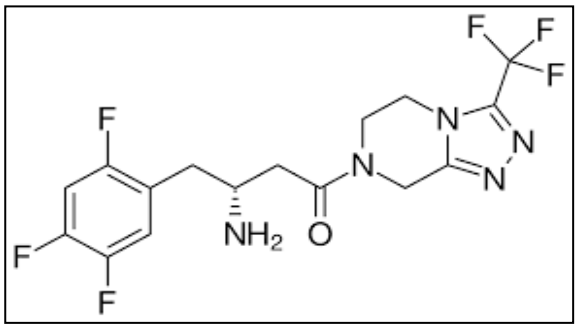

Figure 1: Structure of Sitagliptin

Type 2 diabetes occurs when your body can't use insulin effectively.While many people can control their blood sugar through a healthy diet and regular exercise, some also need to take medication.[7]

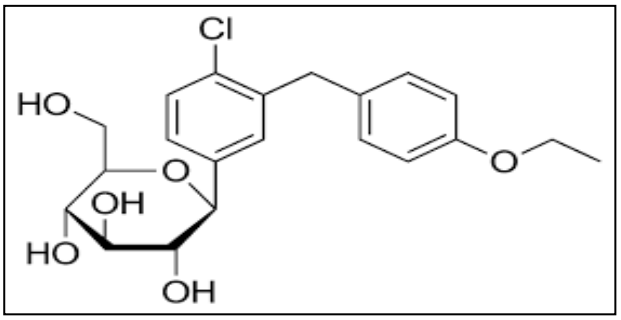

Figure 2: Structure of Dapagliflozin

Although Type 2 diabetes is more prevalent in the elderly, its incidence in younger populations has increased, a trend attributed to the rising rates of childhood obesity. The structures of DAPA and SITA are depicted in the figure 1 &2 [8]

. SITA works by increasing the levels of the hormones GLP-1 and GIP. This action leads to a boost in insulin production and a decrease in glucagon secretion from alpha cells. The reduction in glucagon ultimately results in less glucose being produced by the liver. In short, Sitagliptin enhances the body's natural processes to lower blood sugar. DAPA inhibiting SGLT2, DAPA blocks reabsorption of filtered glucose in the kidney, increasing urinary glucose excretion and reducing blood glucose levels. [6-8] By the literature survey it was found that analytical methods are available for estimation of DAPA and SITA alone and with other combination. [9-15]. So, there is thought to perform Stability indicating RP-HPLC method development and validation for simultaneous estimation of tablet dosage form. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability -indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, etc. and separation of drug from degradation products. [16] Thus, the objectives of this work are to develop a new sensitive stability indicating RP-HPLC method for simultaneous determination of DAPA and SITA. Also, it is validated for market product named UDAPA-S 10/100containing DAPA and SITA in tablet dosage form. [17]

T2DM.[7]

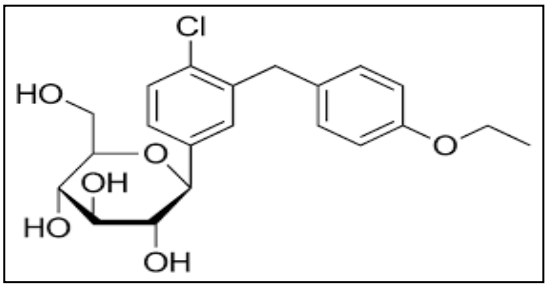

Figure 2:Structure of Dapagliflozin

2. MATERIAL AND METHODS:

Sitagliptin phosphate and Dapagliflozin (analytical grade) were received as gift samples from Wockhardt research centre. Chh. Sambhajinagar, Maharashtra, , India. Marketed combination tablets containing Sitagliptin (100 mg) and Dapagliflozin (10 mg) were purchased from a local pharmacy in Chh. Sambhajinagar, Maharashtra. HPLC-grade methanol, acetonitrile, were procured from Merck Life Science Pvt. Ltd., while orthophosphoric acid, sodium hydroxide, hydrochloric acid, and hydrogen peroxide (AR grade) were sourced from SD Fine-Chem Ltd., Mumbai. A 0.45 µm nylon membrane filter (Millipore) was used for filtration.

Instrumentation included Waters 2695 HPLC system with a 2996 PDA detector. A C18 column (250 mm × 4.6 mm, 5 µm) was used for separation. Analytical procedures were supported by a Shimadzu digital balance and a Eutech pH meter. Stock solutions were prepared in methanol and stored under suitable conditions for use in method development and validation.

RP-HPLC Analysis of Marketed Formulation (Janusmart D 100/10):

The marketed tablet containing 100 mg Sitagliptin Phosphate and 10 mg Dapagliflozin was analyzed by RP-HPLC method. The powdered tablet was dissolved in the selected solvent mixture (Phosphate buffer pH 5.8: Acetonitrile 60:40), sonicated, and diluted to prepare stock solutions (1000 µg/ml). Aliquots were further diluted to 100 µg/ml (Sitagliptin) and 10 µg/ml (Dapagliflozin), and their absorbance values were compared with standards to calculate % assay.[15,16]

RP-HPLC Method Development:

RP-HPLC was performed using a Shimadzu LC 2010 system with a C18 column. Different mobile phase combinations were tested, and the optimized phase—Phosphate buffer (pH 4.8): Acetonitrile: Methanol (50:40:10) was selected. The buffer was prepared with Na_2HPO_4 and KH_2PO_4, pH adjusted with orthophosphoric acid, filtered, and degassed. Standard solutions of both drugs were prepared in the mobile phase, ranging from 100–500 µg/ml for Sitagliptin and 10–50 µg/ml for Dapagliflozin, and analyzed under optimized chromatographic conditions.[17,18]

Method Validation by RP-HPLC

A robust RP-HPLC method was developed and validated for the simultaneous estimation of Sitagliptin Phosphate and Dapagliflozin in pharmaceutical dosage forms, following ICH Q2(R1) guidelines. The method was assessed for various validation parameters including system suitability, specificity, precision, accuracy, linearity, solution stability, robustness, and ruggedness.[19]

System Suitability

System suitability was verified by injecting five replicates of standard solutions containing 100 µg/ml of Sitagliptin Phosphate and 10 µg/ml of Dapagliflozin. Parameters such as retention time (RT), peak area, tailing factor, and theoretical plates were evaluated. All values were found to be within acceptable limits, confirming the suitability of the chromatographic system for analysis.[20]

Specificity

Specificity was established by injecting both individual and mixed standard solutions of Sitagliptin Phosphate (200 µg/ml) and Dapagliflozin (20 µg/ml). The chromatograms showed no interference at the retention times of either analyte, indicating the method's ability to selectively quantify the drugs in the presence of excipients, impurities, or degradation products.[21]

Precision

Precision was assessed at three levels: system precision, method precision, and intra-day/inter-day precision. System precision was confirmed by injecting six replicates of the mixed standard solution (200 µg/ml Sitagliptin and 20 µg/ml Dapagliflozin). Method precision involved analyzing six sample replicates of known concentrations. Intra-day precision was studied by analyzing three concentration levels (100, 200, 300 µg/ml for Sitagliptin and 10, 20, 30 µg/ml for Dapagliflozin) at 2-hour intervals over 12 hours. Inter-day precision was performed on three different days using the same concentration range. The %RSD values were within acceptable limits, confirming the method's reproducibility.[22]

Accuracy (Recovery Studies)

Accuracy was evaluated using the standard addition method at three concentration levels (80%, 100%, and 120%). Predefined amounts of Sitagliptin Phosphate (100, 200, 300 µg/ml) and Dapagliflozin (10, 20, 30 µg/ml) were spiked with known amounts of standard. The % recovery was calculated using the formula:

$$\% \text{ Recovery} = A / (B + C) \times 100$$

Where A is the total drug estimated, B is the weight of the sample drug, and C is the amount of pure drug added. The recovery results for both drugs were within acceptable limits, indicating good accuracy.

Linearity and Range

Linearity was assessed by analyzing standard solutions at five different concentrations ranging from 100–500 µg/ml for

Sitagliptin Phosphate and 10–50 µg/ml for Dapagliflozin. Each concentration was injected into the HPLC system, and the resulting peak areas were recorded. Calibration curves were constructed by plotting peak area versus concentration. Regression equations were generated, and correlation coefficients (r^2) were found to be close to 1, confirming the linearity of the method.[23]

Stability in Analytical Solution

The stability of Sitagliptin Phosphate and Dapagliflozin in solution was evaluated at two concentrations (100 and 300 µg/ml for Sitagliptin, 10 and 30 µg/ml for Dapagliflozin) over 24 hours under refrigerated (8°C) and room temperature conditions. The % assay values showed no significant changes, indicating that both drugs were stable under the tested conditions.[24,25]

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were determined using the standard deviation of the response (σ) and the slope (S) of the calibration curve. The equations used were:

$LOD = 3.3 \times \sigma / S$ and $LOQ = 10 \times \sigma / S$

The calculated values indicated that the method had good sensitivity for both Sitagliptin Phosphate and Dapagliflozin.

Robustness

Robustness was evaluated by making deliberate changes to the chromatographic conditions, specifically by altering the flow rate to 0.8 ml/min and 1.2 ml/min. The assay results and system suitability parameters remained consistent, demonstrating that the method was robust against minor variations in analytical conditions.[26]

Ruggedness

Ruggedness was tested by analyzing standard solutions at three different temperatures (25°C, 37°C, and 60°C). The %RSD of peak areas was calculated and found to be within acceptable limits, indicating that the method yields consistent results under varying environmental conditions.[27,28]

Force Degradation Study

A force degradation study was conducted to evaluate the stability and integrity of Sitagliptin Phosphate and Dapagliflozin under various stress conditions using RP-HPLC. This method effectively detects degradation products and impurities, providing insights into the drug's chemical behavior.[29-35]

Acidic Degradation:

Accurately weighed 100 mg each of Sitagliptin Phosphate and Dapagliflozin were dissolved in 80 ml HPLC-grade methanol and the volume adjusted to 100 ml using 5N HCl. This solution (in 1N methanolicHCl) was incubated at 55–60°C in a water bath shaker for 2 hours. Aliquots were neutralized with 1N NaOH and diluted to obtain test solutions of 100 µg/ml (Sitagliptin) and 10 µg/ml (Dapagliflozin) for chromatographic analysis.[36-38]

Alkaline Degradation:

For base degradation, 100 mg of each drug was dissolved in 80 ml methanol and made up to 100 ml with 1N NaOH (to yield 0.2N methanolic NaOH). The solution was heated at 55–60°C for 2 hours. Aliquots were withdrawn and diluted to obtain 100 µg/ml and 10 µg/ml of Sitagliptin and Dapagliflozin, respectively, and analyzed by RP-HPLC.[37]

Oxidative Degradation:

Oxidative stress testing involved dissolving 100 mg of each drug in methanol and adjusting the volume to 100 ml with 10% hydrogen peroxide. The solution was exposed to 55–60°C for 2 hours. Aliquots were taken, peroxide was neutralized, and the solution was diluted to the required concentrations for analysis.[39]

Thermal Degradation:

To assess thermal stability, 100 mg each of Sitagliptin and Dapagliflozin were kept in an oven at 55–60°C for 3 days. Samples equivalent to 10 mg of each drug were withdrawn at intervals, prepared to 100 µg/ml and 10 µg/ml concentrations, and analyzed to detect degradation.[38,39,40]

Photolytic (UV) Degradation:

Photostability testing involved exposing 100 mg of each drug to short and long wavelength UV light for 48 hours. Samples were taken at regular intervals, prepared as per protocol, and analyzed to determine the impact of UV light on drug stability.[41,42]

Analysis of Marketed Formulation by RP-HPLC

The marketed formulation *Janusmart D 100/10* (Roussel Laboratories Pvt. Ltd.), containing 100 mg of Sitagliptin Phosphate and 10 mg of Dapagliflozin, was evaluated for assay by RP-HPLC. The tablets were powdered, and quantities equivalent to

100 mg of Sitagliptin and 10 mg of Dapagliflozin were transferred to 100 ml and 10 ml volumetric flasks, respectively. The mobile phase was added, and the mixture was sonicated for 5–10 minutes before volume adjustment. From the stock solutions (1000 µg/ml), 1 ml and 0.1 ml aliquots were taken to prepare 100 µg/ml (Sitagliptin) and 10 µg/ml (Dapagliflozin) working solutions. The assay results were compared with standards, and the percentage content was calculated. According to the Indian Pharmacopoeia, the formulation should contain 90.0–110.0% of the labeled amounts of both drugs. The results confirmed compliance within acceptable limits.[43-46[

3. RESULTS AND DISCUSSION:

RP-HPLC Method Development and Optimization:

For effective separation and quantification of Sitagliptin Phosphate and Dapagliflozin, chromatographic conditions were optimized through multiple trials by varying one or two parameters at a time. Each trial was evaluated based on peak resolution, shape, and baseline stability. Trials showing poor resolution, distorted peaks, or baseline noise were discarded. The final optimized conditions were selected based on clear, sharp peaks and consistent performance.

Table 1: Various Trials and Optimization of Chromatographic Conditions

Trial No	HPLC System	ChromatographicConditions	Observations	Remarks
1	HPLC (Shimazdu LC 2010 with Uv detector)	**Mobile Phase-** Phosphate Buffer (pH 6.8): Methanol (40:60) **Column -**Inertsil C18 (4.6 x 250mm, 5µm) **Flow rate-** 1 ml/min **Injection Volume-** 20µl **Pump mode-**Isocratic **Columntemperature-**Ambient **Wavelength-**215 nm	Peaks werenot fully separated.peak shapes was not acceptable	**Rejected**
2	HPLC (Shimazdu LC 2010 with Uv detector)	**Mobile Phase-** Phosphate Buffer (pH 6.8): Acetonitrile (40:60) **Column -**Inertsil C18 (4.6 x 250mm, 5µm) **Flow rate-** 1 ml/min **Injection Volume-** 20µl **Pump mode-**Isocratic **Columntemperature-**Ambient **Wavelength-**215 nm	Peaks werenot fully separated but peak shapes was good as compared to first trial	**Rejected**
3	HPLC (Shimazdu LC 2010 with Uv detector)	**MobilePhase-** Phosphate Buffer (pH 5.8): Acetonitrile (50:50) **Column -**Inertsil C18 (4.6 x 250 mm, 5µm) **Flow rate-** 1 ml/min**Injection Volume-** 20µl **Pump mode-**Isocratic **Columntemperature-**Ambient **Wavelength-**215 nm	Peaks were separated but the peak shapes was not good	**Rejected**

4	HPLC (Shimazdu LC 2010 with Uv detector)	**MobilePhase-** Phosphate Buffer (pH 5.8): Acetonitrile: Methanol (60:20:20) **Column** -Inertsil C18 (4.6 x 250 mm, 5µm) **Flow rate-** 1 ml/min**Injection Volume-** 20µl **Pump mode-**Isocratic **Columntemperature-**Ambient **Wavelength-**215 nm	Peaks were separated but the first peak shape was not good at pH 5.8	**Rejected**
5	HPLC (Shimazdu LC 2010 with Uv detector)	**MobilePhase-** Phosphate Buffer (pH 4.8): Acetonitrile: Methanol in the ratio of 50:40:10 **Column** -Inertsil C18 (4.6 x 250 mm,5µm) **Flow rate-** 1 ml/min**Injection Volume-** 20µl **Pump mode-**Isocratic **Columntemperature-**Ambient **Wavelength-**215 nm	Peaks shapewere good,with goodresolutionandi ntensity at pH 4.8	**Accepted**

Blank Chromatogram

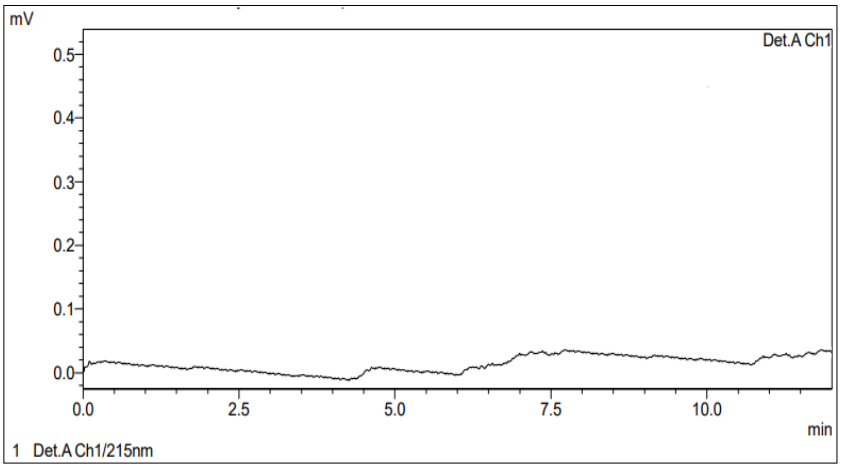

Figure 3: Blank Chromatogram

Optimized trial 5

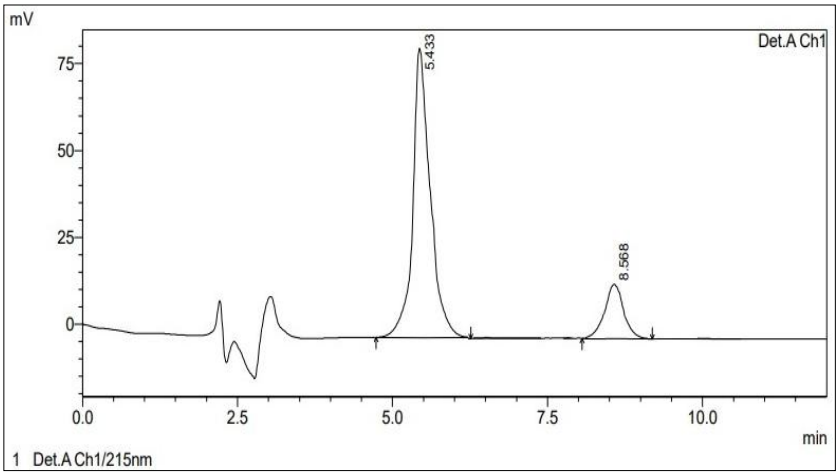

Figure 4: HPLC Fingerprinting of Sitagliptin Phosphate and Dapagliflozin

Table 2: Evaluation parameter of Optimized Sitagliptin Phosphate and Dapagliflozin trial

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Sitagliptin Phosphate	5.433	1987624	82764
2	Dapagliflozin	8.568	515628	15382

System Suitability

The HPLC method has been developed for the determination of the percentage assay of Sitagliptin Phosphate and Dapagliflozin in tablet dosage form. Parameters like Retention time, Peak area, tailing factor, and theoretical plates were found to be within acceptable limit.

Table 8: System Suitability Parameters of Sitagliptin Phosphate and Dapagliflozin

Replicates		Retention time	Peak area	Tailing Factor	Theoretical Plates
1	SGP	5.483	1986358	1.125	2267.014
	DGF	8.536	512384	1.358	2768.316
2	SGP	5.384	1996854	1.184	2586.648
	DGF	8.563	518463	1.268	2260.104
3	SGP	5.465	2010466	1.167	2473.357
	DGF	8.566	508462	1.206	2217.126
4	SGP	5.475	1989476	1.174	2346.117
	DGF	8.638	521571	1.384	2162.102
5	SGP	5.408	1976354	0.941	2476.568
	DGF	8.573	513269	1.276	2256.642

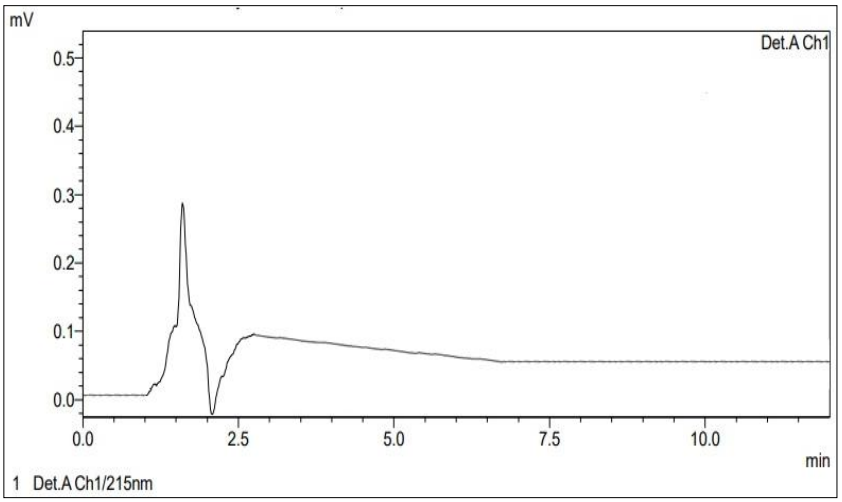

Figure 5: Blank Chromatogram

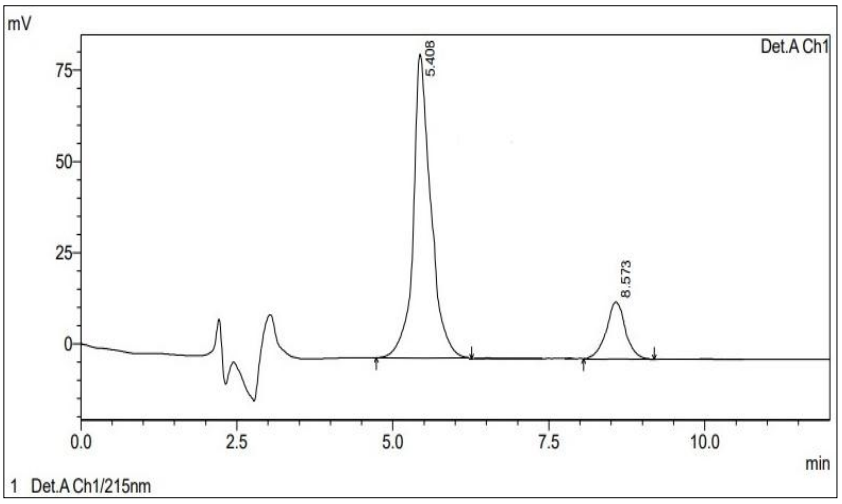

Figure6: System Suitability Chromatogram of Standard Sitagliptin Phosphate and Dapagliflozin 5

Specificity

The absence of additional peaks in the chromatogram indicates non- interference of excipients. There was no interference from the blank at the retention time of analyte peaks.The chromatograms of standard drugs alone and in their mixture.The Retention time for Sitagliptin Phosphate and Dapagliflozin was found to be 5.473 & 8.611 min respectively.

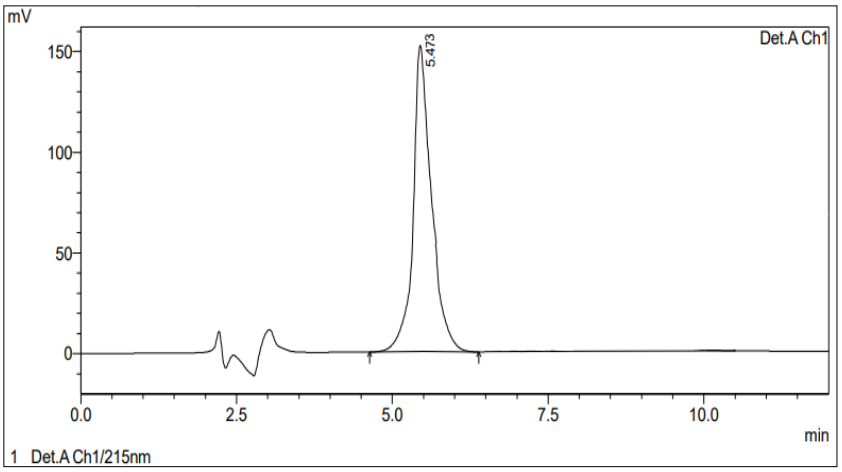

Figure7 :Standard Chromatogram of Sitagliptin Phosphate

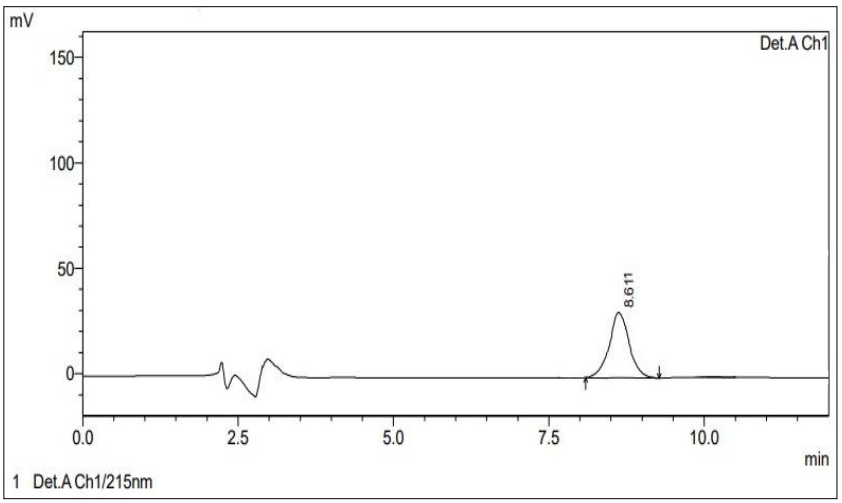

Figure 8: Standard Chromatogram of Dapagliflozin

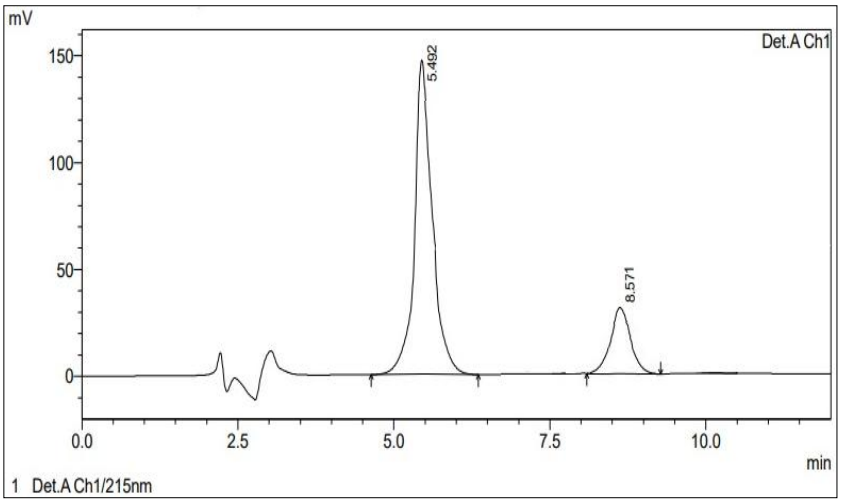

Figure 9: Standard Chromatogram of Mixture of Sitagliptin Phosphate and Dapagliflozin

Table 3: System Suitability Parameters for Sitagliptin Phosphate and Dapagliflozin

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Tailing Factor	Theoretical PlateCount
1	SGP	5.473	3778624	1.127	2452.314
2	DGF	8.611	1045627	1.364	2267.156
3	SGP & DGF	5.492	3684576	1.056	2381.208
		8.571	1043686	1.214	2286.015

Precision

a) System Precision

The system precision was performed by measuring the peak response for standard drugs solutions in six replicates. Peak areas, mean, standard deviation and % relative standard deviation (%RSD) for Sitagliptin Phosphate and Dapagliflozin was found to be 1.230% and 1.567 %. The results were found well within the acceptable criteria.

Table 4: System Precision Data of Sitagliptin Phosphate and Dapagliflozin

Sr.No.	**Peakareasof SGP**	**Peakareasof DGF**
1	3781654	1046827
2	3786584	1045762
3	3695201	1086384
4	3756484	1073268
5	3786879	1056891
6	3687549	1048967
Mean	**3749058.50**	**1059683.16**
SD(±)	**46130.69**	**16608.40**
RSD(%)	**1.230**	**1.567**

Acceptance criteria	**% RSDshouldnotbemorethan2**

b) Method Precision

The method precision was performed by measuring the peak response for sample solutions in six replicates. The % assay for Sitagliptin Phosphate and Dapagliflozin in six samples was calculated.

Table 5: Method Precision Data of Sitagliptin Phosphate and Dapagliflozin

Sr.No.	**% Assay of SGP (w/w)**	**% Assay of DGF (w/w)**
1	100.52	100.25
2	101.34	99.67
3	99.37	99.68
4	100.28	100.58
5	99.64	100.47
6	100.54	99.27
Mean	**100.28**	**99.98**
SD(±)	**0.705**	**0.522**
RSD(%)	**0.705**	**0.522**
Acceptancecriteria	**% RSDshouldnotbemorethan2**	

c) Intraday and Inter-day Precision

The % RSD in intraday precision for Sitagliptin Phosphate (100, 200, 300 µg/ml) was found to be 0.718, 1.242, 0.285% and for Dapagliflozin (10, 20, 30 µg/ml) was found to be 1.312, 1.599, 1.601 % respectively. In inter-day precision % RSD for Sitagliptin Phosphate (100, 200, 300 µg/ml) was found to be 00.926, 1.181, 1.126 % and for Dapagliflozin (10, 20, 30 µg/ml) was found to be 1.412, 0.829, 0.995 % respectively. % RSD in intraday and inter-day studies were found well within the acceptable limits.

Table 6: Intraday Precision data of Sitagliptin Phosphate and Dapagliflozin

Sitagliptin Phosphate					
Sr. no.	Conc. (µg/ml)	Area	meanpeakarea	SD(±)	%RSD
1	100	1983357	1980704	14210.913	0.718
		1939402			
		1965354			
2	200	3786248	3774592	46899.219	1.242
		3814564			
		3722964			
3	300	5808638	5794540	16534.439	0.285
		5798641			
		5776341			
Dapagliflozin					

Sr. no.	Conc. (µg/ml)	Area	meanpeakarea	SD(±)	%RSD
1	10	514568	516349	6779.290	1.312
		523841			
		510638			
2	20	1045682	1042124	16663.914	1.599
		1056721			
		1023968			
3	30	1498637	1479264	23694.322	1.601
		1486308			
		1452846			

Table 7: Inter-day Sitagliptin Phosphate and Dapagliflozin

Sitagliptin Phosphate						
Sr. no.	Day	Conc. (µg/ml)	Area	meanpeakarea	SD(±)	%RSD
1	Day 1	100	1975630	1991709.333	18459.500	0.926
	Day 2		2011867			
	Day 3		1987631			
2	Day 1	200	3845684	3796413	44862.521	1.181
	Day 2		3785631			
	Day 3		3757924			
3	Day 1	300	5806395	5847075.35	65880.754	1.126
	Day 2		5811746			
	Day 3		5923085			
Dapagliflozin						
Sr. no.	Day	Conc. (µg/ml)	Area	meanpeakarea	SD(±)	%RSD
1	Day 1	10	512684	510513.34	7215.19	1.412
	Day 2		502462			
	Day 3		516394			
2	Day 1	20	1042183	1044177.667	8662.00	0.829
	Day 2		1053663			
	Day 3		1036687			
3	Day 1	30	1472268	1456549	14499.30	0.995
	Day 2		1453681			

	Day 3		1443698			

Accuracy (Recovery Study)

The accuracy of the assay method was evaluated by standard addition method in triplicate at 100 % level of the labelled claim and the percentage recovery was calculated. The mean % recovery was found to be 100.25 % & 99.80 % for Sitagliptin Phosphate and Dapagliflozin respectively.

Table 8: Recovery study for Sitagliptin Phosphate and Dapagliflozin

Sitagliptin Phosphate							
Level	**Set**	**Amount added(µg/ml)**	**Amount found(µg/ml)**	**%Recovery**	**Mean**	**SD**	**%RSD**
80%	1	100	99.94	100.06	100.45	0.769	**0.765**
	2	100	100.05	99.95			
	3	100	98.68	101.33			
100%	1	200	199.96	100.02	100.30	0.391	**0.390**
	2	200	199.73	100.13			
	3	200	198.52	100.74			
120%	1	300	299.84	100.05	100.01	0.058	**0.058**
	2	300	300.17	99.94			
	3	300	299.88	100.04			
Dapagliflozin							
Level	**Set**	**Amount added(µg/ml)**	**Amount found(µg/ml)**	**%Recovery**	**Mean**	**SD**	**%RSD**
80%	1	10	9.93	99.36	99.46	1.017	**1.023**
	2	10	9.84	98.49			
	3	10	10.05	100.52			
100%	1	20	19.99	99.95	100.09	0.152	**0.152**
	2	20	20.01	100.07			
	3	20	20.05	100.25			
120%	1	30	29.95	99.86	99.96	0.100	**0.100**
	2	30	30.01	100.06			
	3	30	29.98	99.95			

Linearity and Range

Linearity for Sitagliptin Phosphate and Dapagliflozin was found to be in the range of 100-500 µg /ml and 10-50 µg/ml respectively with correlation coefficient value (r2) 0.993 for Sitagliptin Phosphate and 0.9994 for Dapagliflozin.

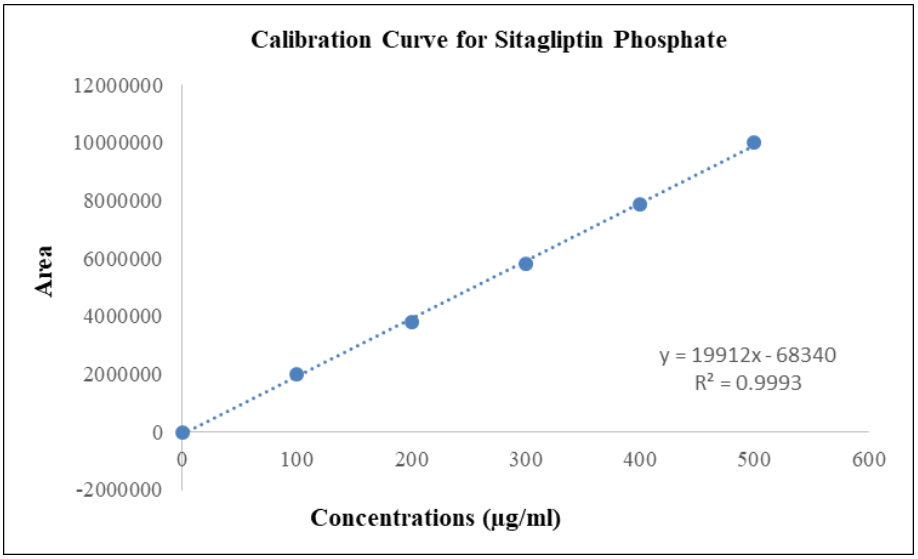

Figure 10: Standard Curve for Sitagliptin Phosphate

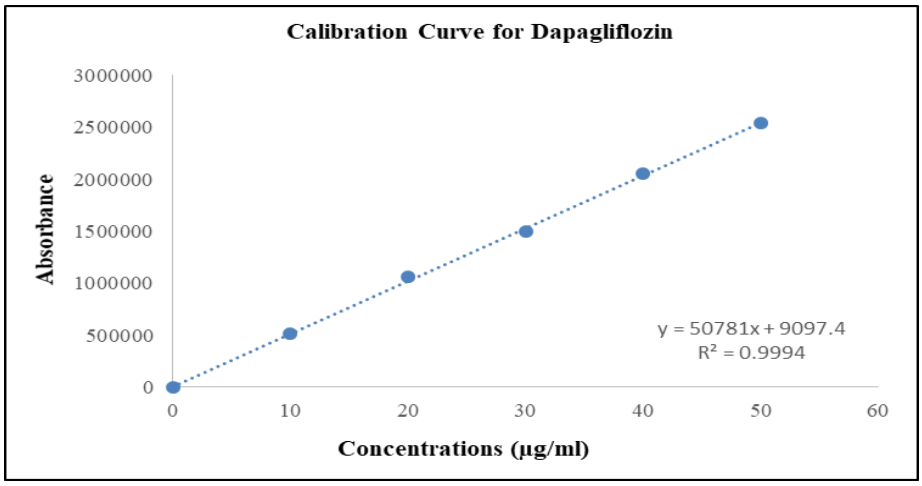

Figure 11: Standard Curve for Dapagliflozin

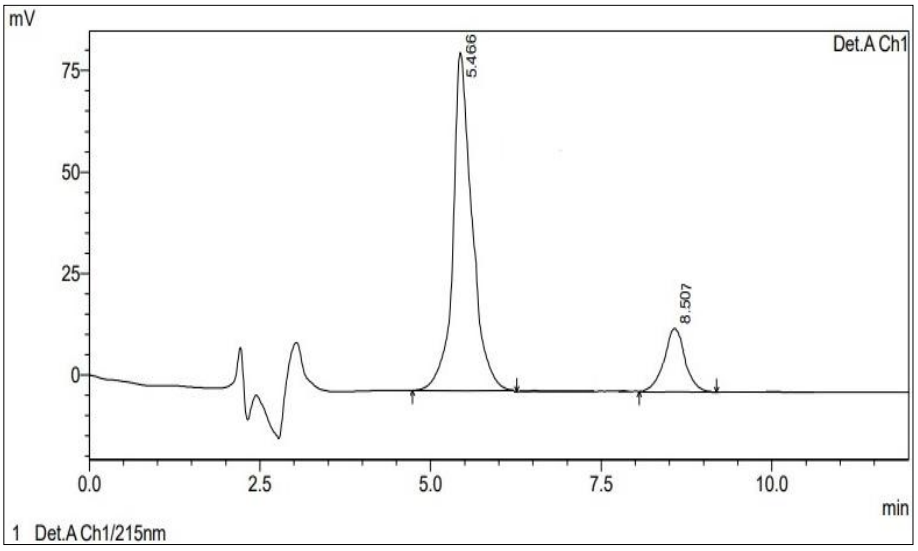

Figure 12: Standard Chromatogram for Linearity (STG 100 µg /ml & DGF 10µg /ml)

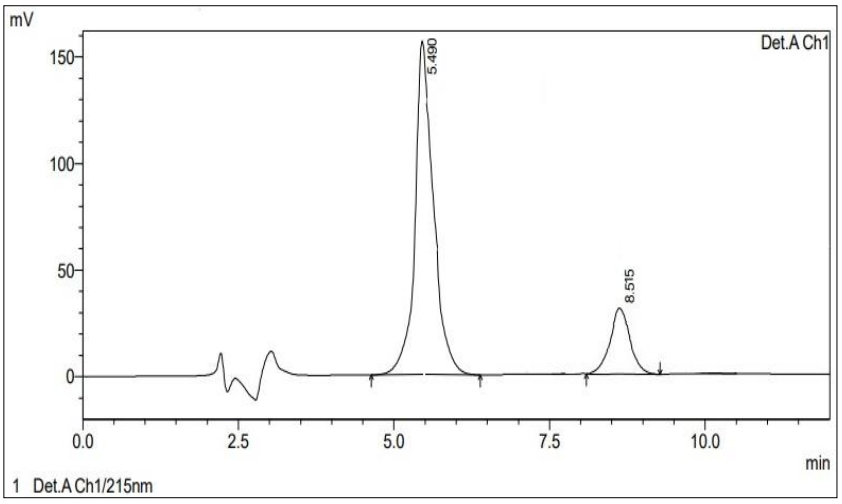

Figure 13: Standard Chromatogram for Linearity (STG 200 µg /ml & DGF 20µg /ml)

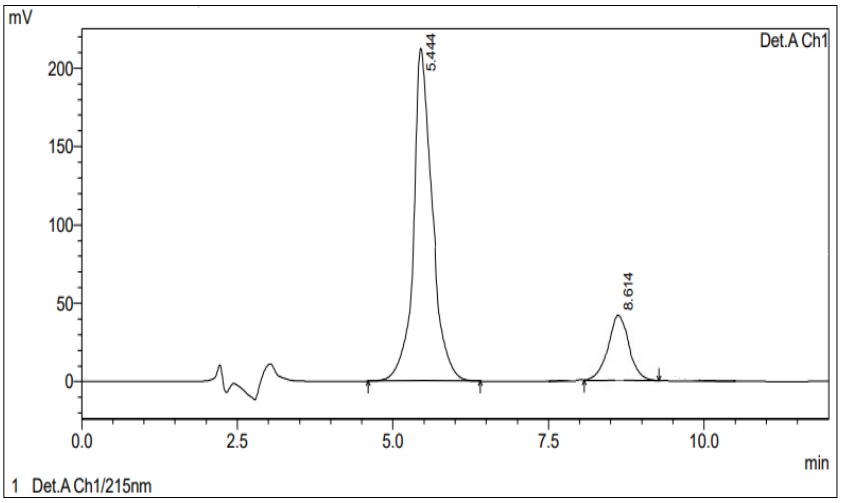

Figure 14: Standard Chromatogram for Linearity (STG 300 µg /ml & DGF 30µg /ml)

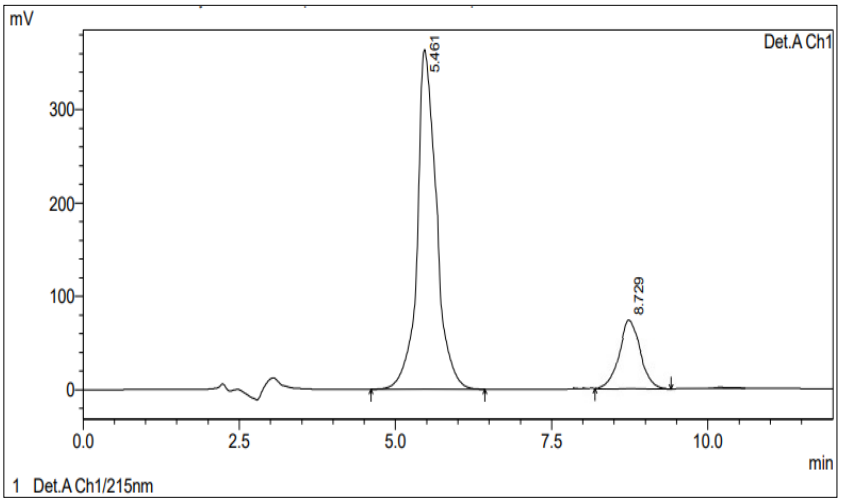

Figure 15: Standard Chromatogram for Linearity (STG 400 µg /ml & DGF 40 µg /ml)

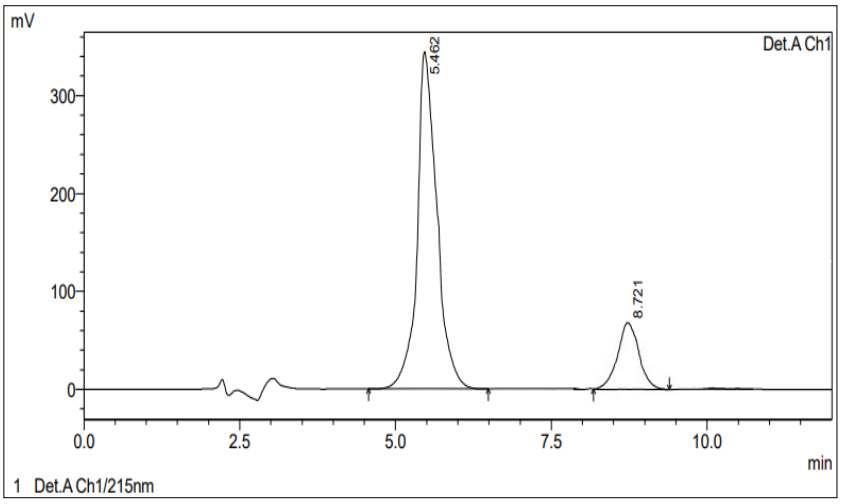

Figure 16: Standard Chromatogram for Linearity (STG 500 µg /ml & DGF 50 µg /ml)

Stability in Analytical Solution

No significant difference was found in the % Assay of both drugs before and after storing for 24 hrs in refrigerator and room temperature. This confirms the stability of the drugs in solutions.

Table 9: Solution Stability Data of Sitagliptin Phosphate and Dapagliflozin

Timelevel	**Refrigerator (25°C)**	**RoomCondition(37°C)**
Timeinhrs	%AssayofSGP	%Assayof DGF
Initial	100.20 (±0.34)	99.38 (±0.032)
After24hrs	101.45 (±0.58)	100.02 (±0.046)

**AverageofSixdetermination*

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

For Sitagliptin Phosphate LOD and LOQ were found to be 8.005 µg/ml and 2.426 µg/ml respectively. Whereas, for Dapagliflozin the LOD and LOQ were found to be 1.950 µg/ml and 5.908 µg/ml respectively. These values indicate that the method is suitable for the determination of the lower concentration and confirms that proposed method is sensitive for the determination.

Robustness

The robustness of an HPLC method is states about its capacity to remain unaffected by minor, deliberate alterations to its method parameters. This quality ensures the reliability of the method during routine usage. Percent (%) RSD at each condition was found less than 2. This indicates the robustness of the method.

Table 10: Robustness data of Sitagliptin Phosphate and Dapagliflozin at flow rate 0.8 ml/min

SGP				
Flow Rate (0.8ml/min)				
	Retention Time (min)	**Area**	**Theoretical Plate**	**Tailing factor**
	5.584	1984237	2484.12	1.126
	5.546	2008746	2463.02	1.145
	5.682	1978637	2554.26	1.158
Avg	**5.604**	**1990540**	**2500.47**	**1.142**
SD	**0.0701**	**16013.6**	**47.768**	**0.016**

%RSD	**1.252**	**0.804**	**1.910**	**1.401**
DGF				
Flow Rate (0.8ml/min)				
	Retention Time (min)	**Area**	**Theoretical Plate**	**Tailing factor**
	8.686	515684	2478.24	1.265
	8.614	514689	2456.11	1.234
	8.606	520863	2498.21	1.282
Avg.	**8.635**	**517079**	**2477.52**	**1.260**
SD	**0.044**	**3314.87**	**21.060**	**0.024**
%RSD	**0.510**	**0.641**	**0850**	**1.931**

Table 11: Robustness data of Sitagliptin Phosphate and Dapagliflozin at flow rate 1.2 ml/min

SGP				
Flow Rate (1.2 ml/min)				
	Retention Time (min)	Area	Theoretical Plate	Tailing factor
	5.293	1986384	2217.26	1.151
	5.364	1956204	2267.22	1.173
	5.376	1963578	2257.38	1.156
Avg.	5.344	1968722	2247.29	1.160
SD	0.044	15733.8	26.467	0.011
%RSD	0.839	0.799	1.177	0.994
DGF				
Flow Rate (1.2 ml/min)				
	Retention Time (min)	Area	Theoretical Plate	Tailing factor
	8.414	515274	2367.17	0.918
	8.434	513064	2364.66	0.929
	8.415	516587	2348.84	0.944
Avg.	8.421	514975	2360.22	0.930
SD	0.011	1780.43	9.937	0.013
%RSD	0.133	0.345	0.421	1.402

Ruggedness

The ruggedness parameter was determined by analyzing the different concentration at different temperature.

Table 12: Data of Ruggedness for Sitagliptin Phosphate and Dapagliflozin

SGP				
Change in Parameters	Area of Standard	Mean	SD	%RSD
25°C	1987364	1974169	15649.50	0.792
	1978263			
	1956879			
37°C	3786389	3788685	2569.21	0.067
	3791460			
	3788206			
60 °C	5808637	5841052	65425.70	1.120
	5798163			
	5916357			
DGF				
Change in Parameters	Area of Standard	Mean	SD	%RSD
25°C	515624	516660	4377.37	0.847
	521462			
	512893			
37°C	1059351	1058790	959.582	0.090
	1057682			
	1059337			
60 °C	1495226	145547	1871.72	0.125
	1493856			
	1497558			

Force Degradation studies of Sitagliptin Phosphate and Dapagliflozin

Forced degradation studies were conducted to evaluate the stability of Sitagliptin Phosphate and Dapagliflozin under various stress conditions using key parameters such as purity angle and purity threshold. These studies help identify potential degradation products and assess drug integrity. Among the tested conditions, the highest degradation was observed under basic conditions, with Sitagliptin Phosphate showing 10.25% degradation and Dapagliflozin showing 9.44% degradation, indicating their relative instability in alkaline environments.

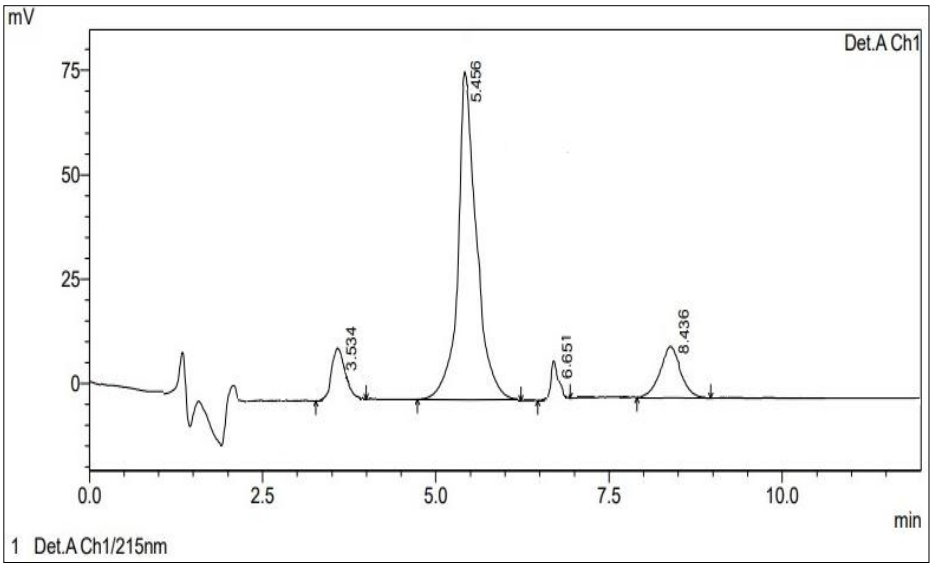

Figure 17: Chromatogram of SGP & DGF acidic stress condition

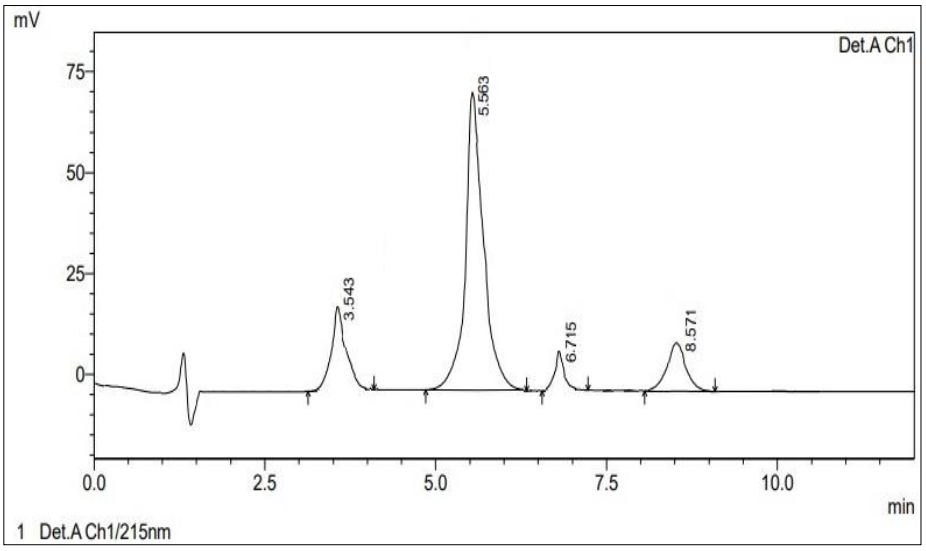

Figure 18: Chromatogram of SGP & DGF in basic stress condition

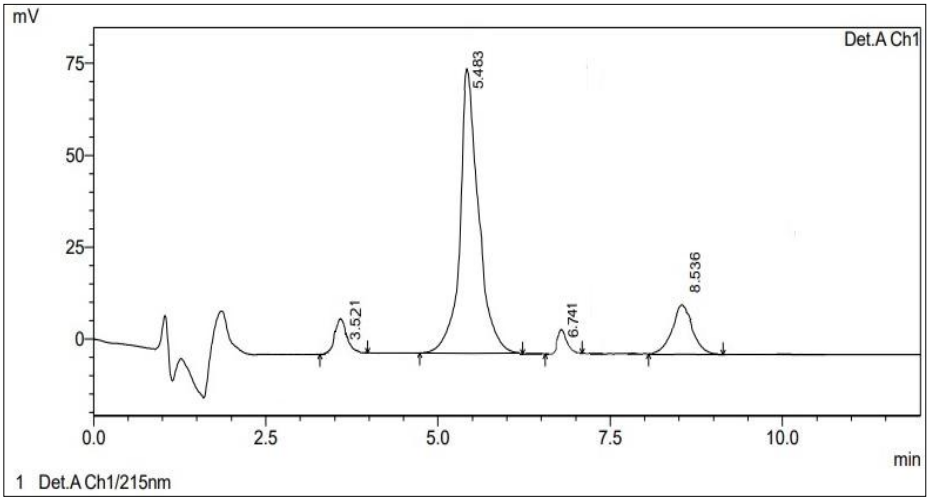

Figure 19: Chromatogram of SGP & DGF in oxidative stress condition

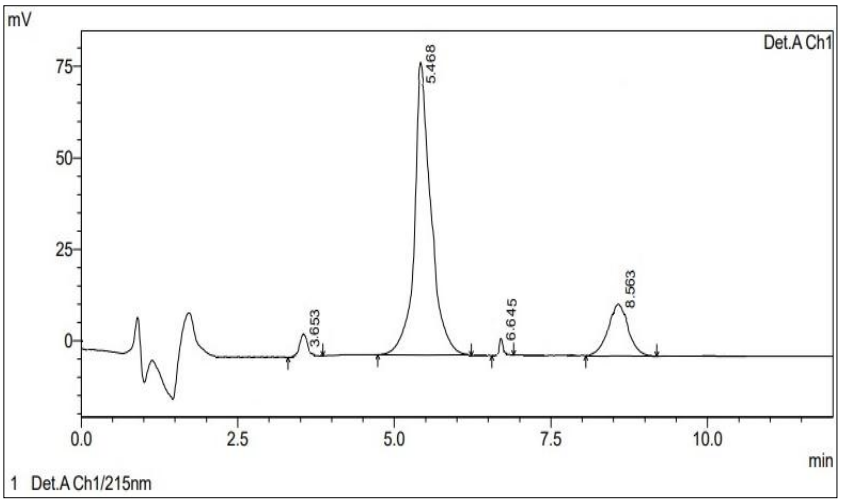

Figure 20: Chromatogram of SGP & DGF in thermal stress condition

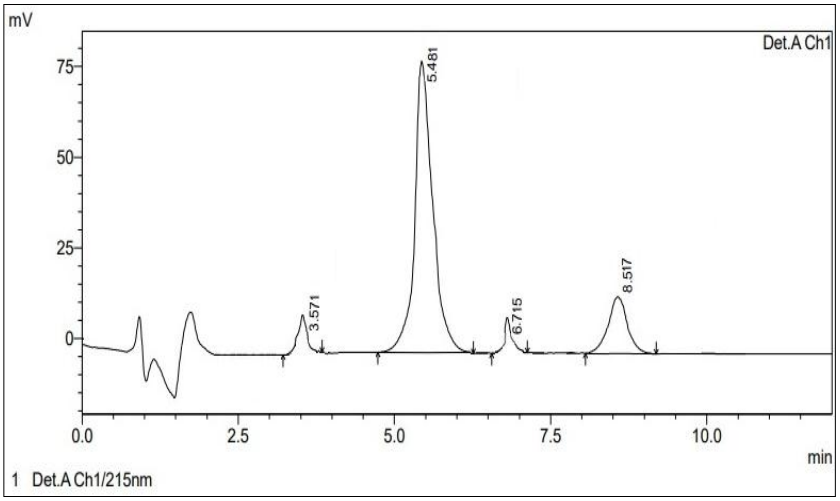

Figure 21: Chromatogram of SGP & DGF in Uv light stress condition

Analysis of Marketed Formulation

The results of Marketed formulation was found to be satisfactory. The percent assay of Janusmart D 100/10 tablet was found to be 97.31 % for Sitagliptin Phosphate and 98.43% for Dapagliflozin.

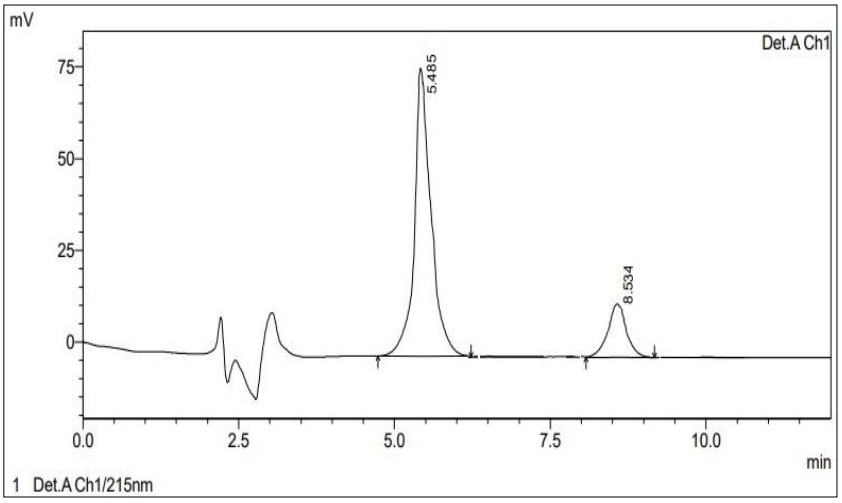

Figure 22: Chromatogram of Marketed Formulation (Janusmart D 100/10 Roussel Laboratories Pvt.Ltd.)

Table 13: Evaluation parameters of Marketed Formulation

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Tailing Factor	Theoretical PlateCount	% Assay
1	Marketed Formulation (Janusmart D 100/10 Tablet)	5.485 (SGP)	1933369 (SGP)	1.121 (SGP)	245.203 (SGP)	97.31
		8.534 (DGF)	507386 (DGF)	1.284 (DGF)	2367.764 (DGF)	98.43

4. CONCLUSION:

The study successfully developed and validated UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Sitagliptin Phosphate and Dapagliflozin in bulk and marketed formulations. Both methods demonstrated excellent linearity, accuracy, precision, sensitivity, and robustness. Forced degradation studies revealed higher degradation under alkaline conditions, indicating stability concerns in basic environments. The methods were effectively applied to marketed formulations, confirming their suitability for routine quality control and stability testing in pharmaceutical analysis.

5. CONFLICTS OF INTEREST:

Declare None.

6. ACKNOWLEDGEMENT:

The authors sincerely thank Oriental University, Indore, Madhya Pradesh, for providing the necessary facilities and support essential for the successful completion of this research project

REFERENCES

[1] International Conference on Harmonisation (ICH). ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology. Geneva: ICH; 2005.

[2] Chatwal GR, Anand KS. Instrumental Methods of Chemical Analysis. 5th ed. Mumbai: Himalaya Publishing House; 2014.

[3] International Conference on Harmonisation (ICH). Stability Testing of New Drug Substances and Products Q1A(R2). Geneva: ICH; 2003.

[4] Pawar AR, Patil SR. RP-HPLC method development and validation for simultaneous estimation of Sitagliptin and Dapagliflozin in tablet dosage form. Asian J Pharm Clin Res. 2018;11(3):208-11.

[5] World Health Organization. International Pharmacopoeia. General Methods and Stability Studies. Geneva: WHO; 2022. Available from: https://www.who.int/publications

[6] Singh S, Bakshi M. Guidance on conduct of stress tests to determine inherent stability of drugs. Pharm Technol Online. 2000;24(2):1-14.

[7] Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 7th ed. Boston: Cengage Learning; 2017.

[8] Meyer VR. Practical High-Performance Liquid Chromatography. 5th ed. Chichester: Wiley; 2010.

[9] Tiwari G, Tiwari R, Rai AK. Review on analytical method development and validation. Int J Pharm Life Sci. 2010;1(5):310–7.

[10] Sahu PK, Ramisetti NR, Cecchi T, Swain S, Patro CS, Panda J. An overview of experimental designs in HPLC method development and validation. J Pharm Biomed Anal. 2018;147:590–611.

[11] International Conference on Harmonisation (ICH). Q1B: Photostability Testing of New Drug Substances and Products. Geneva: ICH; 1996.

[12] Naik AA, Pawar D. UV and HPLC method development and validation for simultaneous estimation of Sitagliptin phosphate and Dapagliflozin. Int J Pharm ChemBiol Sci. 2017;7(2):70–5.

[13] Rane VP, Patil KR, Sangshetti JN, Yeole RD, Shinde DB. Stability-indicating LC method for the determination of Sitagliptin in bulk drug and pharmaceutical dosage form. Sci Pharm. 2010;78(3):477–92. doi:10.3797/scipharm.0910-15

[14] Dhalwal K, Shinde VM, Namdeo AG. Stability testing of phytopharmaceuticals: A review. Pharmacogn Rev. 2008;2(4):215–8.

[15] Vankalapati KR, Alegete P, Boodida S. Stability-indicating HPLC method development and validation for simultaneous estimation of metformin, dapagliflozin, and saxagliptin in bulk drug and pharmaceutical dosage form. Biomed Chromatogr. 2022;36(7):e5384. https://doi.org/10.1002/bmc.5384

[16] Gurrala S, Shivaraj C, Anumolu PD, Saraf G. Multivariate optimization of liquid chromatographic conditions for simultaneous analysis of dapagliflozin and saxagliptin in bulk and pharmaceutical dosage form. Future J Pharm Sci. 2021;7(1):85. https://doi.org/10.1186/s43094-021-00229-z

[17] Acharya H, Kotadiya R. Stability-indicating HPLC for remogliflozin, vildagliptin, and metformin: Method development, validation, and greenness. Ann Pharm Fr. 2024;82(6):1071–81. https://doi.org/10.1016/j.pharma.2024.06.006

[18] Vankalapati KR, Alegete P, Boodida S. Stability-indicating ultra performance liquid chromatography method development and validation for simultaneous estimation of metformin, linagliptin, and empagliflozin in bulk and pharmaceutical dosage form. Biomed Chromatogr. 2021;35(4):e5019. https://doi.org/10.1002/bmc.5019

[19] Sanagapati M, Dhanalakshmi K, Nagarjuna R. Novel univariate spectrophotometric determination of the recently approved anti-diabetic agent dapagliflozin in bulk and pharmaceutical formulations. SpectrochimActa A MolBiomolSpectrosc. 2019;213:86–90. https://doi.org/10.1016/j.saa.2019.01.056

[20] Vankalapati KR, Alegete P, Boodida S. Development and validation of simplified RP-HPLC method for determination of darunavir in bulk and pharmaceutical dosage form. Mater Today Proc. 2021;47:394–9. https://doi.org/10.1016/j.matpr.2021.04.097

[21] Bansal G, Singh M, Jindal K. Identification, isolation and characterization of potential process impurities and degradation products of glimepiride: Development of validated stability-indicating method. J Pharm Biomed Anal. 2015;115:312–22. https://doi.org/10.1016/j.jpba.2015.07.015

[22] Kumar V, Chawla G. Simple and rapid LC-MS/MS method for determination of sitagliptin in human plasma and its application to a pharmacokinetic study. J Pharm Biomed Anal. 2020;189:113426. https://doi.org/10.1016/j.jpba.2020.113426

[23] El-Bagary RI, Elkady EF, Ayoub BM. Second-derivative synchronous fluorimetry and time-programmed flow injection technique for simultaneous determination of vildagliptin and saxagliptin. J Pharm Biomed Anal. 2015;111:156–63. https://doi.org/10.1016/j.jpba.2015.03.011

[24] Bansal G, Singh M, Jindal K. RP-HPLC method for simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet formulation. J Pharm Biomed Anal. 2009;50(5):887–90. https://doi.org/10.1016/j.jpba.2009.06.019

[25] Gurrala S, Shivaraj C, Anumolu PD. Multivariate optimization of liquid chromatographic conditions for simultaneous quantification of dapagliflozin and saxagliptin: Application to in vitro dissolution and stability studies. Future J Pharm Sci. 2021;7(1):85. https://doi.org/10.1186/s43094-021-00229-z

[26] Sanagapati M, Dhanalakshmi K, Reddy NV. Novel univariate spectrophotometric determination of the recently approved antidiabetic agent dapagliflozin in bulk and pharmaceutical formulations. SpectrochimActa A MolBiomolSpectrosc. 2019;223:117353. https://doi.org/10.1016/j.saa.2019.117353

[27] Abdelrahman AE, Maher HM, Alzoman NZ. HPTLC method for the determination of metformin hydrochloride, saxagliptin hydrochloride, and dapagliflozin in pharmaceuticals. Curr Anal Chem. 2020;16(5):609–19. https://doi.org/10.2174/1573411015666190131123029

[28] Shakoor A, Ahmed M, Ikram R, Hussain S, Tahir A, Jan BM, et al. Stability-indicating RP-HPLC method for simultaneous determination of metformin hydrochloride and vildagliptin in tablet and biological samples. ActaChromatogr. 2020;32(1):39–43. https://doi.org/10.1556/1326.2019.00677