wjpmr, 2025, 11(6), 343-357

SJIF Impact Factor: 6.842

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

A RESEARCH ON SIMULTANEOUS ESTIMATION OF SITAGLIPTIN AND DAPAGLIFLOZIN BY RP-HPLC METHOD BY COMBINE TABLET DOSAGE FORM

[1]*Yash B. Gagare, [2]Sagar S. Gawade, [3]Pradip R. Mandhare, [4]Yuvraj B. Guldagad and [5]Manoj L. Chaudhari

[1,2,3,4]Students, (Mrs. Saraswati Wani College of Pharmacy, Ganegaon).
[5]Leacturer, (Mrs. Saraswati Wani College of Pharmacy, Ganegaon).

***Corresponding Author: Yash B. Gagare**

Students, (Mrs. Saraswati Wani College of Pharmacy, Ganegaon).

Article Received on 17/04/2025 **Article Revised on 07/05/2025** **Article Published on 28/05/2025**

ABSTRACT

A simple and unique reversed-section high-performance liquid chromatography Method became developed and proven for the simultaneous determination of Sitagliptin (sita) and Dapagliflozin (dapa) in bulk and pharmaceutical dosage form. Chromatography turned into achieved on zorobax eclipse plus phenyl hexyl c18 (250 mm × four.6 mm, 5 µ particle length) column containing cell segment of Buffer: methanol: acetonitrile inside the ratio of 40:35:25% v/v. (1ml of Triethylamine is brought and ph is adjusted to a few.7 with zero.1% orthophosphoric Acid) at a drift fee of 1. Zero ml/minute. The analyte become monitored the use of image Diode array detector (pda) at 267 nm. The retention time turned into discovered to be 2.38 min. and 3.88 Minutes for met Sitagliptin and Dapagliflozin Respectively. Validation parameters specificity, linearity, accuracy, precision and Robustness had been found to be suited over the concentration stages of 100 µg/ml & 10 µg/ml for Sitagliptin (sita) and dapagliflozin (dapa) respectively. The Technique advanced has been statistically established in step with ich guidelines. The consequences of analysis had been proven as in line with international conference on Harmonization (ich) recommendations. Consequently the optimized approach can be Effectively applied for the simultaneous willpower of Sitagliptin and dapagliflozin inside the recurring quality Manipulate evaluation. Significance of evolved method is that, it is able to be utilized for Habitual or unknown sample evaluation of assay of Sitagliptin and dapagliflozin in pharmaceutical dosage form. Evolved by using diverse pharmaceutical industries. The proposed method become Located to be fast, correct, specific, particular, strong, rugged and cost-efficient.

KEYWORDS: Sitagliptin and dapagliflozin.

INTRODUCTION

Diabetes currently affects 463 million people worldwide and is expected to reach 700 million people by 2045. Diabetes mellitus is complex and chronic disease that necessarily involves ongoing medical attention along with multifactorial risk reduction strategies in addition to glycemic control.

Sitagliptin is a new oral hypoglycemic of the new dipeptidyl peptidase4 (DPP-4) inhibitor class of drugs. The drug works to competitively inhibit a protein/ enzyme, dipeptidyl peptidase 4 (DPP-4), that results in an increased amount of active incretins (GLP-1 and GIP), reduced amount of release of glucagon (diminishes its release) and increased release of insulin. Dapagliflozin is a competitive, reversible, and SGLT2 inhibitor with excellent selectivity the cotransporter SGLT2 is responsible for renal glucose reuptake; inhibiting the cotransporter enables higher renal glucose excretion, which results in lower plasma glucose level.

Analytical chemistry may be defined as the science and art of determining the composition of material in terms of elements or compounds contained in it.

In chromatographic separation, HPLC and HPTLC methods have widely been exploited in pharmaceutical analysis because of its simplicity, precision, accuracy and reproducibility of results. High Performance Liquid Chromatography High performance liquid chromatography is basically a highly improved form of column chromatography

HPLC instruments consist of a reservoir of mobile phases, pumps, an injector, a separation column, a detector and a data control and processor. Solvents must be degassed to eliminate formation of bubbles.

1. Tablet Dosage form

A tablet is a pharmaceutical oral dosage form (*oral solid dosage*, or OSD) or solid unit dosage form. Tablets may

be defined as the solid unit dosage form of medication with suitable excipients.

1. Ideal properties of tablet dosage form

1. It must be release active pharmaceutical ingredient in the body in an obvious way
2. It should have proper mechanical strength, does not fragile during transportation
3. Does not alter or change any physical and chemical properties under any environmental condition
4. It must have suitable chemical stability over a period of time.

2. HPLC Efficiency Parameters System suitability criteria

The theory of chromatography has been used as the basis for System Suitability tests, which are set of quantitative criteria that test the suitability of the chromatographic system to identify and quantify drug related samples by HPLC at any step of the pharmaceutical analysis.

The retention time is longer when the solute has higher affinity to the stationary phase due to its chemical nature. Efficiency: Plate Count N and Peak Capacity Pc the efficiency is calculated in terms of HEPT (Height Equivalent Theoretical Plate) and Theoretical plate count.

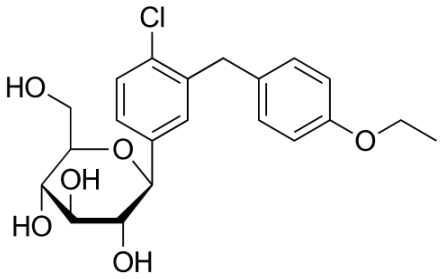
The sharpness of a peak is relevant to the limit of detection and limit of quantification of the chromatographic system. The sharper the peak for a specific area, the better is its signal-to-noise; hence the system is capable of detecting lower concentrations.

DRUG PROFILE

1. DAPAGLIFLOZINE

- **Molecular Formula** - C21H25Cl O6
- **Molecular weight** - 408.88 g·mol−1
- **IUPAC Name** - -2-[4-Chloro-3-(4-ethoxybenzyl) phenyl]-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol

Molecular Structure



- **Route of Administration** - Oral
- **Elimination Time –** 12.9 hours
- **Excretion –** Urine 75% and Face 25%
- **Category** – Anti-Diabetes
- **Bioavailability** – 78% (after 10mg dose)

Dapagliflozin, sold under the brand names Farxiga (US) and Forxiga (EU) Manufactured and Selle By AstraZeneca. among others, is a medication used to treat type 2 diabetes. It is also used to treat adults with heart failure and chronic kidney disease. It reversibly inhibits sodium-glucose co-transporter.

2 (SGLT-2) in the renal proximal convoluted tubule to reduce glucose reabsorption and increase urinary glucose excretion.

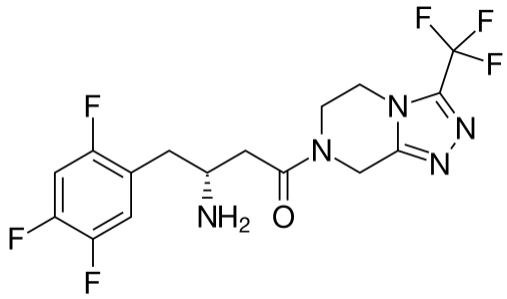
Common side effects include hypoglycaemia (low blood sugar), urinary tract infections, genital infections, and volume depletion (reduced amount of water in the body). Diabetic ketoacidosis is a common side effect in people with type 1 diabetes. Serious but rare side effects include Fournier gangrene.

Dapagliflozin is used along with diet, exercise, and usually with other glucose-lowering medications, to improve glycemic control in adults with type 2 diabetes. Dapagliflozin, in addition to other SGLT2-inhibitors, was shown to reduce the rate of decline in kidney function and kidney failure in non-diabetic and type 2 diabetic adults when added to the existing treatment regimen. Dapagliflozin is a highly potent (inhibitory constant 0.55 nmol/L) and reversible SGLT2 inhibitor that I > 1400 times more selective for SGLT2 than SGLT1, the main transporter responsible for glucose absorption in the gut. Dapagliflozin increased the amount of glucose excreted in the urine and improved both fasting (FPG) and post-prandial plasma glucose levels in patients with T2D.

2. SITAGLIPTIN

- **Molecular Formula –** C16H15F6N5O
- **Molecular weight –** 407.320 g·mol−1
- **IUPAC Name** - (R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4] triazolo[4,3-a]pyrazin-7(8H)-y(2,4,5-trifluorophenyl) butan-2-amine

Molecular Structure



- **Route of Administration –** Oral / By Mouth
- **Category** –Type 2 -Diabetes
- **Excretion –** Kidney (80%)
- **Bioavailability** –87%

Sitagliptin, sold under the brand name Januvia Manufactured and Selle By CTX

Lifesciences. among others, is an anti-diabetic medication used to treat type 2 diabetes. It is in the dipeptidyl peptidase-4 (DPP-4) inhibitor class and works by increasing the production of insulin and decreasing the production of glucagon by the pancreas. In the United Kingdom it is listed as less preferred than metformin or a sulfonylurea. It is taken by mouth. It is also available in the fixed-dose combination medication sitagliptin/metformin (Janumet, Janumet XR).

It is taken by mouth. It is also available as the fixed-dose combinations of sitagliptin/metformin (Janumet, Janumet XR) and sitagliptin/simvastatin. Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal.

MATERIALS AND METHODS

1. Materials

The chemicals were procured from Micro Lab Ltd. and are of AR grade. Drug samples were obtained for research purpose.

Table no. 1: List of chemicals used with their grade.

Sr. No	Material	Grade
1.	Dapagliflozin	AR
2.	Sitagliptin	AR
3.	Acetonitrile	AR
4.	Methanol	AR
5.	Phosphate	AR

Table no. 2: List of Equipment.

Sr.No.	Instrument	Manufacturer	Model
1.	Electronic Weighing Balance	Shimadzu	S-13V
2.	pH meter	Equip-Tronic	EQ-610
3.	HPLC	Shimadzu	LC-2050

1. Electronic Weighing Balance

Principle- The force exerted by the load situated on the balance pan is transmited to the load cell which in turn emits an electric signal whose intensity is proportional with the force.

Fig. no. 1: Electronic Weighing Balance.

2. pH Meter

Principle- A pH meter measures the acidity or basicity of a solution by detecting the electrical potential difference generated between a reference electrode and a measuring electrode placed in the solution**.**

Fig no. 2: pH meter.

3. HPLC

Principle- High-Performance Liquid Chromatography (HPLC) is a versatile analytical technique used to separate, identify, and quantify components in a mixture.

Fig no. 3: HPLC.

MATERIALS AND METHODS

1. Instrument

Chromatographic separation is achieved using HPLC System (Agilent HPLC 1100Series) containing Quaternary pump solvent manager, autosampler and

PDA detector. The output Signal is monitored and processed using Ezchrom Elite Software. A Zorobax eclipse Plus Phenyl Hexyl column (250 x 4.6mm, Id 5µ) is used as stationary phase. Isocratic mode is used for the elution.

2. Chemicals and reagents

Sitagliptin phosphate and Dapagliflozin as active pharmaceutical ingredients (API). Methanol, Acetonitrile, Triethylamine & 0.1% Orthophosphoric acid, Water, and potassium dihydrogen ortho-phosphate buffer of HPLC grade were obtained from Micro lab Ltd.

3. Preparation of Buffer pH 3.7

Dissolve 3.4 g of potassium dihydrogen ortho-phosphate in 1000 mL of HPLC grade water. 1ml Triethylamine is added and Sonicated to dissolve and pH is adjusted to 3.7 with 0.1% ortho phosphoric acid and filtered through 0.45µ filter.

4. Mobile phase (Diluent) Preparation

Initially, trials were carried out using various solvents in various proportions on HPLC to obtain the desired peak shape for drug. After few trials Buffer: Methanol: Acetonitrile (40:35:25% v/v) was fixed as the mobile phase which gave acceptable peak parameters.

400mL of Buffer pH 3.7, 350mL Methanol & 250 mL Acetonitrile Mixed well and degassed.

Table no. 3: Chromatographic Condition.

HPLC System	.	Agilent 1100 Series with PDA Detector
Mobile Phase	:	Buffer: Methanol: Acetonitrile (40:35:25% v/v)
Column Used	:	Zorobox eclipse Plus Phenyl Hexyl (250 x4.6mm, id 5µ)
Column Temperature	:	30°C
Flow Rate	:	1.0mL/Minutes
Injection Volume	:	20µL
Detection Wavelength	:	267nm
Mode	:	Isocratic elution
Run Time	:	15 minutes

5. Preparation of Stock (Standard) Solution

Weigh and transfer about 100 mg of standard Sitagliptin phosphate in 100 ml volumetric flask. Add abut 30 ml of diluent and sonicate to dissolve. Cool the solution and dilute up to the mark with diluent (1mg/mL=1000ppm).

Weigh and transfer about 100 mg of standard Dapagliflozin in 100 ml volumetric flask. Add about 30 ml of diluent and sonicate to dissolve. Cool the solution and dilute up to the mark with diluent (1mg/mL=1000ppm). Pipette out standard 10ml of Dapagliflozin solution and transfer in 50ml volumetric flask and dilute up to the mark with diluent (0.2mg/mL= 200ppm).

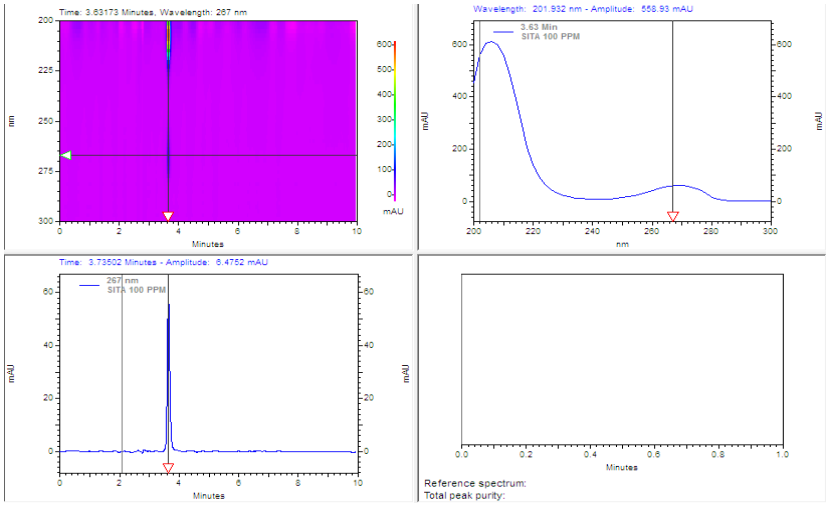
6. Sample solution Preparation (Control Sample)

Twenty tablets each containing 100mg of Sitagliptin Phosphate were weighed and powdered. Transfer the powder equivalent to 100mg of Sitagliptin phosphate to 100 ml volumetric flask. Add 70 ml of diluent and sonicate for 15min. with intermittent shaking. Cool the solution and dilute up to the mark with diluent. Filter through 0.45micron nylon syringe filter, first few ml of Sitagliptin phosphate is discarded and collected as stock sample solution.

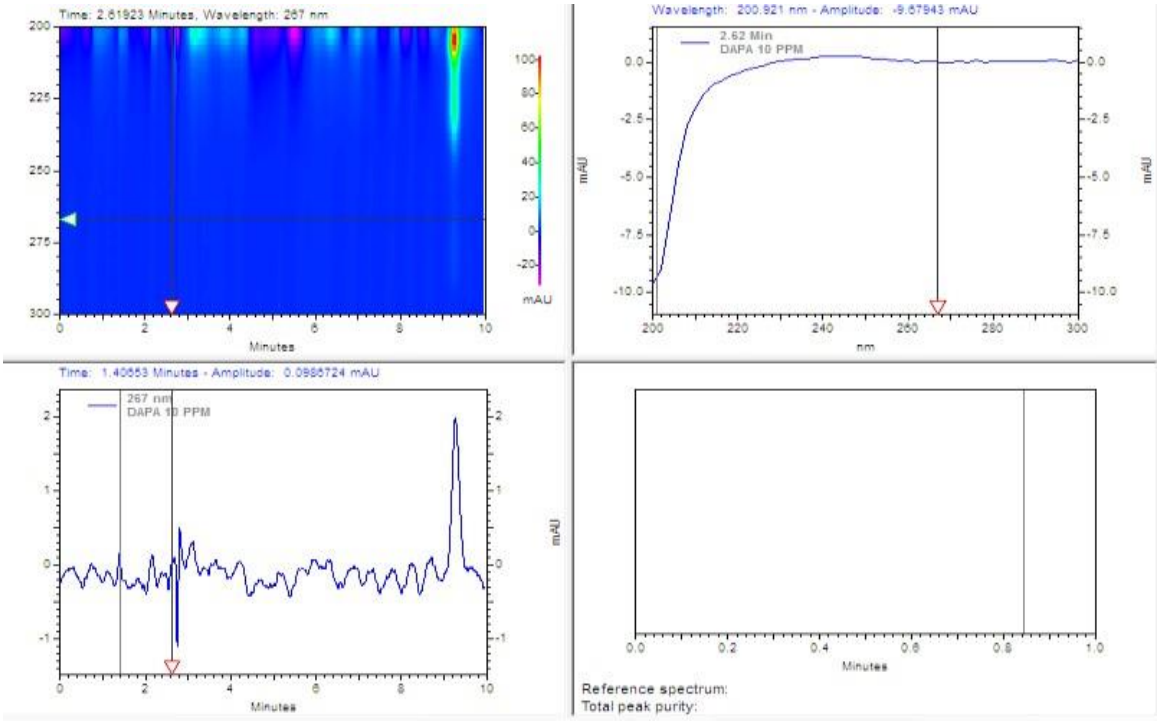
Twenty tablets each containing 10mg of Dapagliflozin were weighed and powdered. Transfer the powder equivalent to 10mg of Dapagliflozin to 100 ml volumetric flask. Add 70 ml of diluent and sonicate for 15min. with intermittent shaking. Cool the solution and dilute up to the mark with diluent. Filter through 0.45micron nylon syringe filter, first few ml of Dapagliflozin is discarded and collected as stock sample solution. Pipette out standard 10ml of Dapagliflozin solution and transfer in 50ml volumetric flask and dilute up to the mark with diluent (0.2mg/mL= 200ppm).

7. Selection of wavelength

Scan the standard solution in HPLC between 200 nm and 400 nm on spectrum scan mode, the three drugs show λ max at 267 nm for Sitagliptin phosphate and Dapagliflozin.



Graph -Wavelength scan of Sitagliptin.



Graph- Wavelength scan of Dapagliflozin.

1. System suitability studies

ICH recommendations and USP standards conducted the system suitability investigations. Calculations were performed to determine parameters such as tailing factor, capacity factor, asymmetry factor, and number of theoretical plates.

Table 4: System Suitability parameters.

Parameters	Sitagliptin	Dapagliflozin
Retention time (min)	2.3860	3.886
Tailing Factor (*T*)	2.09	2.3
Resolution (min)	1.5	1.5

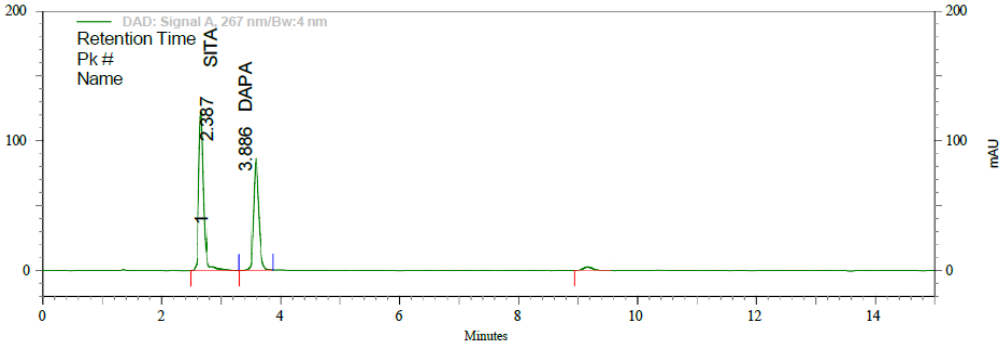
Table no. 5: Suitability study of Sitagliptin.

Sr.no	Standard Drug conn[n]	Peak area
1	Sitagliptin	1008850
2	Sitagliptin	1008767
3	Sitagliptin	1008675
4	Sitagliptin	1008667
5	Sitagliptin	1008990
6	Sitagliptin	1008454
	Mean	1008733

	S.D.	42.48529
	%RSD	4.211750

Table no. 6: Suitability study of Dapagliflozin.

Sr.no	Standard Drug conn[n]	Peak area
1	Dapagliflozin	67521
2	Dapagliflozin	67416
3	Dapagliflozin	67358
4	Dapagliflozin	67572
5	Dapagliflozin	67838
6	Dapagliflozin	67714
	Mean	67569
	S.D	2.2360
	%RSD	3.3092



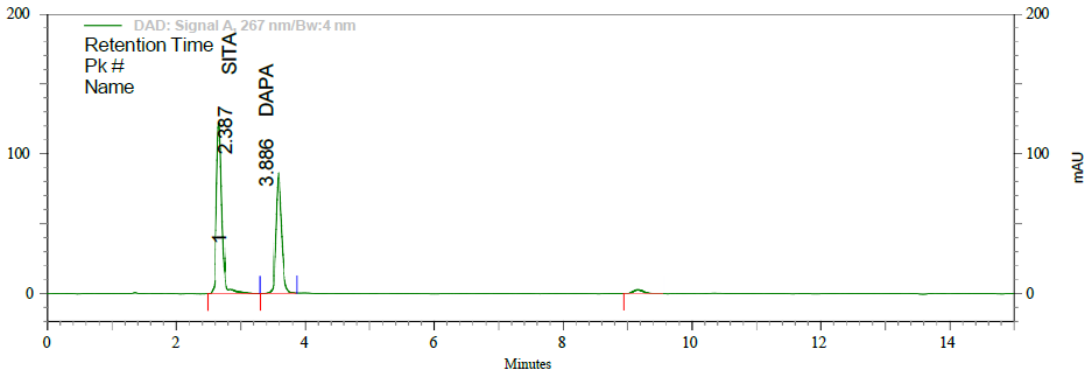
Graph -Suitability study of Sitagliptin and Dapagliflozin.

2. Specificity

The determination of method specificity can be achieved in two ways, first and most desirable, all potential interfering compounds can be tested to demonstrate their separation from the peak (S) of interest with a specified resolution (usually RS ≥ 2) A second method for achieving a specificity is the use of selective detectors especially for co eluting compounds For example a selective detectors (e.g. electrochemical and radioactivity) will respond some compounds but not to others.

Table No. 7: Specificity Study Sitagliptin and Dapagliflozin.

Sr.No.	Solution	Interference
1	Standard solution	No Interference Observed
2	Sample solution	No Interference Observed
3	Placebo solution	No Interference Observed



Graph: Specificity Study Sitagliptin and Dapagliflozin.

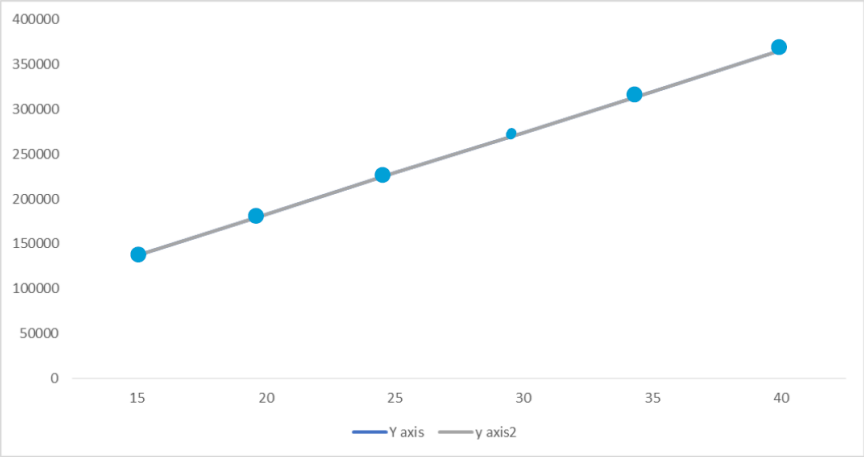
3. LOD and LOQ

An investigation of linearity was performed in triplicate. Utilizing the calibration curve method, the LOD and LOQ were determined. An average of the slope and intercept was used to establish the Limit of Detail (LOD) and Limit of Quantification (LOQ).

LOD-Limit of Detection

Table No 8: Limit of Detection Study of Sitagliptin.

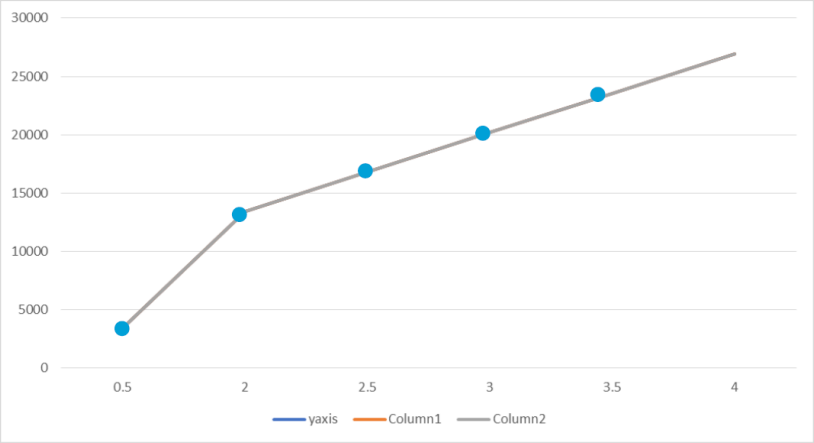
Sr.No.	Conc^{n} in µl	Peak Area
1	15	137152
2	20	182588
3	25	228870
4	30	274305
5	35	320023
6	40	365740
Mean		251446
S.D.		0.7071
%RSD		0.0281



Graph: LOD Study of sitagliptin.

Table No 10: Limit of Detection Study of Dapagliflozin.

Sr.No.	Conc^{n} in µl	Peak Area
1	0.5	3366.50
2	2.0	13450.7
3	2.5	16833.4
4	3.0	20200.1
5	3.5	23555.7
6	4.0	26948.4
Mean		17392.4
S.D.		0.28284
%RSD		1.626



Graph: LOD Study of Dapagliflozin.

4. LOQ- Limit of Quantification

Table No 11: Limit of Quantification Study of Sitagliptin.

SR NO	DRUG In 30%	PEAK AREA
1	Sitagliptin	302660
2	Sitagliptin	302600
3	Sitagliptin	302645
4	Sitagliptin	302630
5	Sitagliptin	302621
6	Sitagliptin	302646
MEAN		302633
S.D.		3.7416
%R.S.D.		0.00123

Table No 12: Limit of Quantification Study of Dapagliflozin.

SR NO	DRUG In 30%	PEAK AREA
1	Dapagliflozin	20200.1
2	Dapagliflozin	20225.8
3	Dapagliflozin	20156.3
4	Dapagliflozin	20186.4
5	Dapagliflozin	20202.8
6	Dapagliflozin	20225.9
MEAN		20199.5
S.D		0.24469
%R.S.D		0.000445

LOD and LOQ

Sr. no	Drug	LOD	LOQ
1	Sitagliptin	0.2	0.12
2	Dapagliflozin	0.16	0.44

5. Linearity

Linearity of an analytical method is its ability to elicit test results that are directly or by a well- defined mathematical transformation, proportional to the concentration of drug in samples within a given range.

From standard stock solution of Sitagliptin phosphate and Dapagliflozin shown in table no- 04, the different concentration of solution is prepared by using calibrated micropipette (10- 100 µL & 100- 1000 µL). The final concentration of this solution was observed in the range of 50–150 µg/mL for SITA and 5–15 µg/mL for DAPA respectively. Calibration curves were plotted with observed peak areas against concentration to obtain the calibration curve and correlation coefficients. Characteristics parameters for regression equation (y = mx+c) of the method and these parameter were used to confirm the good linearity of the method.

Table no. 13: Preparation of Solution of Sitagliptin.

Sr No.	Solution in %	Wt. of Standard	Dilution	ppm
1	50%	50	100	500
2	80%	80	100	800
3	90%	90	100	900
4	100%	100	100	1000
5	110%	110	100	1100
6	120%	120	100	1200
7	150%	150	100	1500

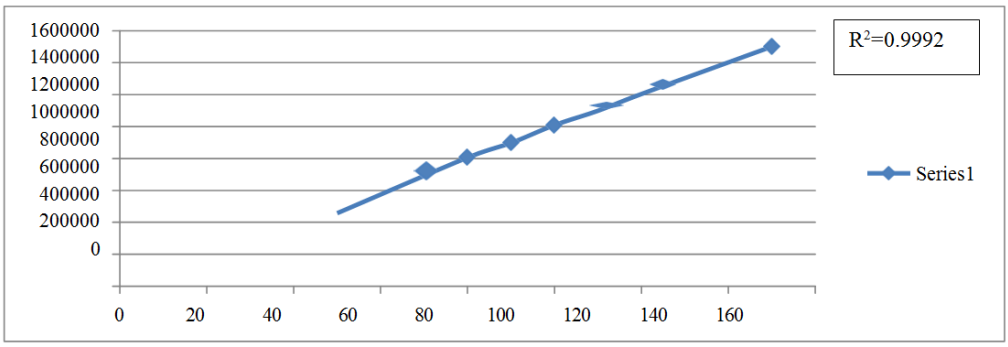
Table no. 14: Preparation of Solution of Dapagliflozin.

Sr No.	Solution in %	Wt. of Standard	Dilution	ppm
1	50%	5	50	100
2	80%	8	50	160

3	90%	9	50	180
4	100%	10	50	200
5	110%	11	50	220
6	120%	12	50	240
7	150%	15	50	300

Table no. 15: Linearity data of Sitagliptin.

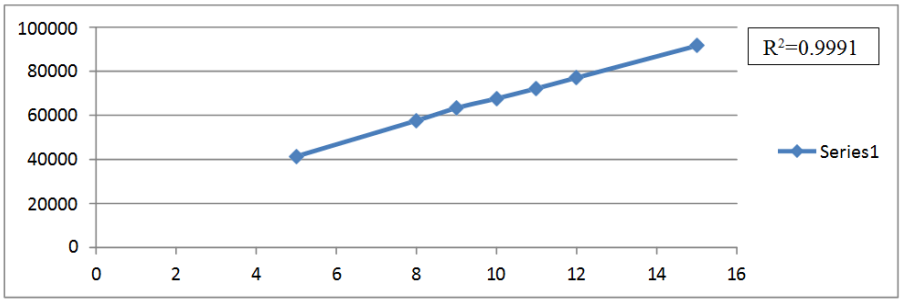
Sr No	Solution %	Conc in ppm	Area
1	50%	500	457176
2	80%	800	805692
3	90%	900	896992
4	100%	1000	1008887
5	110%	1100	1098604
6	120%	1200	1203707
7	150%	1500	1500744
		Correlation Coefficient	0.9992



Graph: Linearity curve of Sitagliptin.

Table no 16: Linearity data of Dapagliflozin.

Sr No	Solution %	Conc in ppm	Area
1	50%	5	41377
2	80%	8	57611
3	90%	9	63329
4	100%	10	67623
5	110%	11	72219
6	120%	12	77080
7	150%	15	91595
		Correlation Coefficient	0.9991



Graph: Linearity curve of Dapagliflozin.

Results

It was observed that the proposed method was found to be having linearity in the concentration range of 50–150 µg/mL for SITA and 5–15 µg/mL for DAPA respectively.

The retention time was found to be 3.560 minutes for Sitagliptin and 9.060 for Dapagliflozin respectively.

The proposed method was found to be having linearity in the concentration range of 50-150 µg/ml for SITA (R^2= 0.9992) and 5-15 µg/ml for DAPA (R^2= 0.9991) respectively.

6. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as present recovery by the assay of known added amounts of analyte.

From standard stock solution of SITA & DAPA show the different concentration of solution is prepared by using calibrated micropipette (10- 100 µL & 100- 1000 µL pipette). The accuracy was determined for SITA and DAPA (equivalent to 100 mg of SITA and 10 mg of DAPA) (50%, 100%, and 150% of the label claimed respectively) to the quantity equivalent to average weight of marketed tablets. The resulting mixtures were analyzed in triplicates. The % recovery of added drug is taken as a measure of accuracy.

Table No. 17: Preparation of Solution.

SR NO	Solution %	Conc in ppm	Sita Std µl	Dapa Std µl	Diluent µl	Final volume in µl
1	50%	500	50	5	675	1000
2	100%	1000	100	10	350	1000
3	150%	1500	150	15	25	1000

Table no. 18: Accuracy data of Sitagliptin.

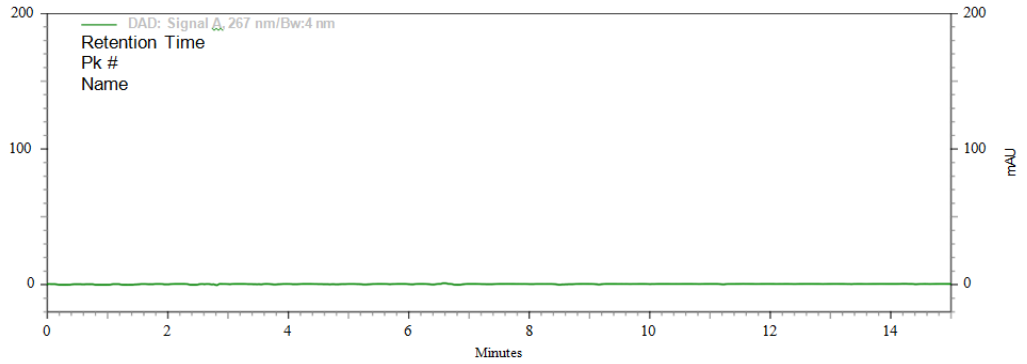
SITA		Wt in mg	Conc.	ppm
std wt.		100.0	Std conc.	1000
Sample wt.		100.0	50 % Sample conc.	500
			100 % Sample conc.	1000
			150 % Sample conc.	1500
No Of Inj.	Std Area	50% Sample Area	100% Sample Area	150% Sample Area
Inj-1	1008714	503368	1018730	1515880
Inj-2	1028747	509688	1022687	1529152
Inj-3	1011193	501251	1005785	1507322
Mean	1017187	504769	1015734	1517451
Std. Dev.	8005.6	4389.5	8840.3	10999.5
RSD	0.787	0.870	0.870	0.725
%		99.25	99.86	99.45
Formula		$\frac{\text{Sample area x Standard conc.}}{\text{Standard area x sample conc.}}$ X 100		

Table no. 19: **Accuracy data of Dapagliflozin.**

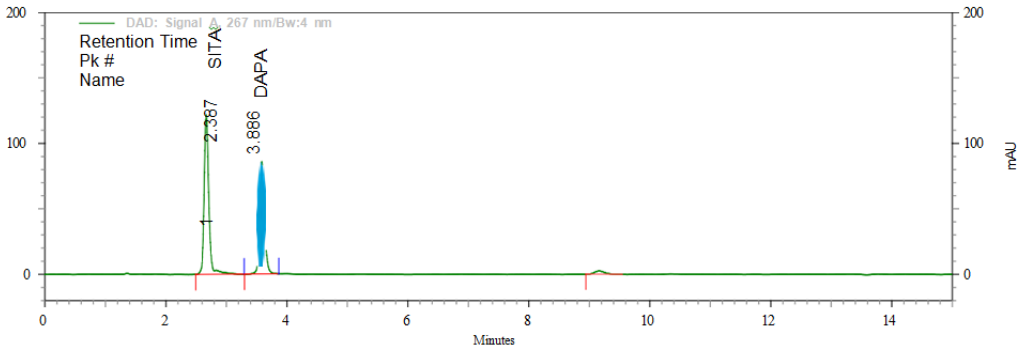
DAPA		Wt in mg	Conc.	ppm
std wt.		100.0	Std conc.	200
Sample wt.		100.0	50 % Sample conc.	100
			100 % Sample conc.	200
			150 % Sample conc.	300
No of Inj.	Std Area	50% Sample Area	100% Sample Area	150% Sample Area
Inj-1	53863	26755	54032	81987
Inj-2	54763	26977	53692	81050
Inj-3	53510	26910	54798	81625
Mean	54020	26881	54174	81554
Std. Dev.	504.6	113.9	566.5	472.5
RSD	0.934	0.424	1.046	0.579
%		99.52	100.28	100.65
	Formula	$\frac{\text{Sample area x Standard conc.}}{\text{Standard area x sample conc.}}$		X 100

Results

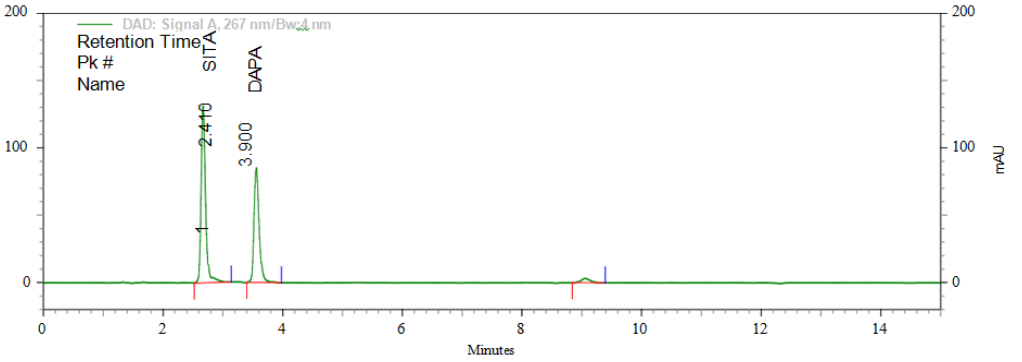
It was observed that the mean % recoveries obtained were found to be 98.00-102.00% for SITA and 98.00 - 102.00% for DAPA respectively.



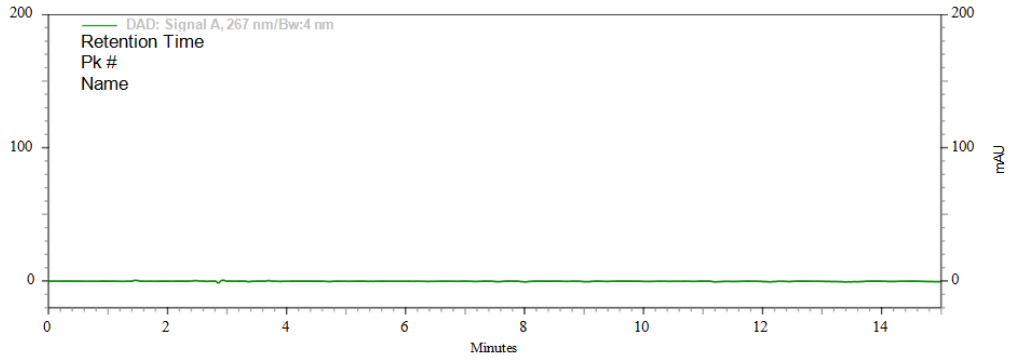
The representative chromatogram obtained for Blank in Linearity of SITA and DAPA in figure 1



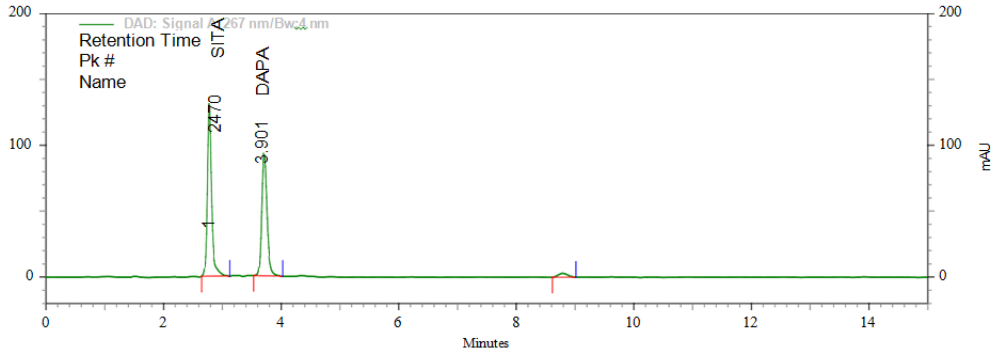
The representative chromatogram obtained for standard solution shown in linearity of SITA & DAPA in figure 2.



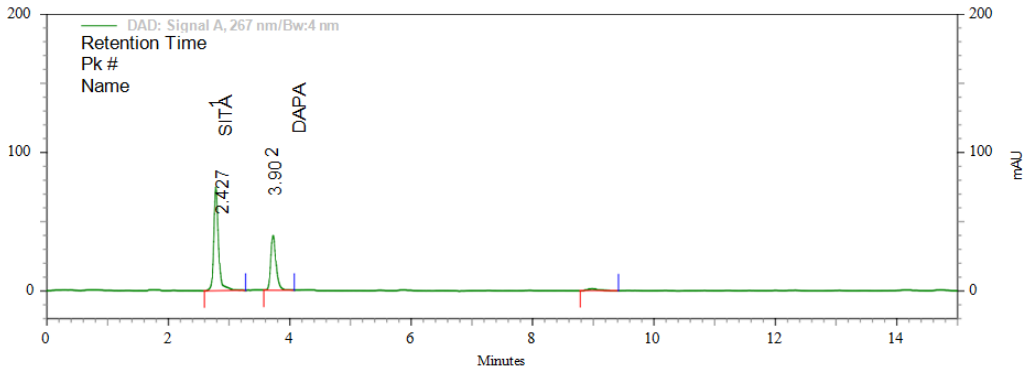
The representative chromatogram obtained for sample solution shown in linearity of SITA & DAPA in figure 3.



The representative chromatogram obtained for blank in accuracy of SITA & DAPA shown in figure 4.



The representative chromatogram obtained for standard solution shown in accuracy of SITA & DAPA in figure 5.



The representative chromatogram obtained for sample solution shown in accuracy of SITA & DAPA in figure 6.

7. Repeatability

A repeatability study in RP-HPLC involves running the same sample multiple times under identical conditions to assess the precision of the method. It ensures the method can consistently produce similar results when the same sample is analyzed repeatedly by a single analyst.

Table no. 20: Repeatability study of Sitagliptin.

Sr.no	Drug	Peak area
1	Sitagliptin	1008800
2	Sitagliptin	1008867
3	Sitagliptin	1008775
4	Sitagliptin	1008567
5	Sitagliptin	1008890
6	Sitagliptin	1008654
	Mean	1008758
	S.D	1.00000
	%RSD	0.000099

Table no.21: Repeatability study of Dapagliflozin.

Sr.no	Drug	Peak area
1	Dapagliflozin	67525
2	Dapagliflozin	67456
3	Dapagliflozin	67388
4	Dapagliflozin	67542
5	Dapagliflozin	67878
6	Dapagliflozin	67754
	Mean	67590
	S.D	96.150
	%RSD	0.14225

Result

Table No. 22: Repeatability data for Dapagliflozin and Sitagliptin.

Sr no	Area of Dapa	Area of Sita
1	67525	1008800
2	67456	1008867
3	67388	1008775
4	67542	1008567
5	67878	1008890
6	67754	1008654
Mean	67590	1008758
S.D	96.150	1.00000
%RSD	0.14225	0.000099

8. Ruggedness

Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. The percentage RSD value for analyst I found to be0.001401 and 0.0010 for Sitagliptin and Dapagliflozin. The percentage RSD values for analyst II found to be 0.00035 and 0.0020 for Sitagliptin and Dapagliflozin. The results were shown in table.

Table NO. 23: Ruggedness data for Sitagliptin.

Sr. No.	Different Condition	
	Analyst-I	Analyst-II
1	1008875	1008865
2	1008752	1008830
3	1008658	1008690
Mean	1008761	1008795
S.D.	1.414243	3.53553
%RSD	0.001401	0.00035

Table NO 24: Ruggedness data for Sitagliptin.

Sr. No.	Different Condition	
	Analyst-I	Analyst-II
1	67629	67675
2	67675	67612
3	67695	67620
Mean	67666	67635
S.D.	0.7071	1.4142
%RSD	0.0010	0.0020

Result

Table No. 25: Ruggedness data for Dapagliflozin and Sitagliptin.

Drug Name	Different conditions	Mean%	SD	%RSD
Sitagliptin	Analyst-I	10087.61	1.41423	0.001401
	Analyst-II	10087.95	3.53553	0.00035
Dapagliflozin	Analyst-I	676.66	0.7071	0.0010
	Analyst-II	676.35	1.4142	0.0020

9. Robustness

Changes in chromatographic conditions such as flow rate (± 0.1) and column temperature (± 3°C) were studied to determine the robustness of the method which should have %RSD ≤2%. Developed method was robust with a %RSD <1%.

HPLC System	:	Agilent 1100 Series with PDA Detector
Mobile Phase	:	Buffer: Methanol: Acetonitrile (40:35:25% v/v)
Column Used	:	Zorobax eclipse Plus Phenyl Hexyl (250 x4.6mm, id 5µ)
Column Temperature	:	30°C
Flow Rate	:	1.0mL/Minutes
Injection Volume	:	20µL
Detection Wavelength	:	267nm
Mode	:	Isocratic elution
Run Time	:	15 minutes

Table no. 26: Robustness study of sitagliptin.

Sr no	Parameter	R1	R2	R3	Mean	S. D	%RSD
1	Flow 1.1µl	1008865	1008768	1008687	1008773	0.7071	0.07
2	Flow 0.9µl	1008945	1008789	1008756	1008830	0.0000	0.00
3	Temperature 33^{o}c	1008500	1008567	1008678	1008581	1.0000	9.91
4	Temperature 27^{o}c	1008597	1008614	1008720	1008643	1.0000	9.91

Table no. 27: Robustness study of Dapagliflozin.

Sr no	Parameter	R1	R2	R3	Mean	S. D	%RSD
1	Flow 1.1µl	67875	67798	67567	67746	4.0	0.059
2	Flow 0.9µl	67987	67890	67765	67880	1.0	0.014
3	Temperature 33^{o}c	67590	67574	67810	67698	7.74	0.011
4	Temperature 27^{o}c	67639	67786	67987	67790	96.23	0.014

RESULT AND DISCUSSION

Table No. 28: Result Table.

Sr no	Parameter	Sitagliptin	Dapagliflozin
1	**System Suitability**	2.3860	3.886
2	**Specificity**	No Inference Observed	No Inference Observed
3	**LOD**	0.2	0.16
4	**LOQ**	0.12	0.44
5	**Linearity**	0.9992	0.9991
6	**Accuracy**	98-102%	98-102%
7	**Repeatability**	0.000099	0.14205
8	**Ruggedness**	0.00035	0.0020

9	**Robustness**	-	-
9.1	Flow 1.1µl	0.07	0.059
9.2	Flow 0.9µl	0.00	0.014
9.3	Temperature 33^{o}c	9.91	0.011
9.4	Temperature 27^{o}c	9.91	0.014

CONCLUSION

RP-HPLC methods for simultaneous estimation of dapagliflozin and sitagliptin in combined tablet dosage forms are rapid, accurate, sensitive, robust, and validated according to international guidelines.

The reported LOD (0.160 µg/mL for dapagliflozin; 0.20 µg/mL for sitagliptin) and retention times (2.3860 min for dapagliflozin; 3.886 min for sitagliptin) confirm the sensitivity and selectivity of the methods.

No significant interference from tablet excipients or matrix effects has been reported, confirming the specificity and suitability of the RP-HPLC methods for routine quality control.

Robustness and ruggedness are confirmed through consistent assay performance under deliberate parameter variations.

REFERENCES

1. Analytical Procedure Development Q14 International Council For Harmonisation of Technical Requirement For Pharmaceuticals For Human Use, 2022; 1–64.
2. Anderson, S. L. Dapagliflozin efficacy and safety : a perspective review. Therapeutic Advances in Drug Safety, 2014; 5(6): 242–254.
3. Characterization of Renal Glucose Reabsorption in Response to Dapagliflozin in Healthy Subjects and Subjects With Type 2 Diabetes, 2013; 36.
4. Gundala, A., Kvsrg, P., & Koganti, B. Application of quality by design approach in RPHPLC method development for simultaneous estimation of saxagliptin and dapagliflozin in tablet dosage form. Brazilian Journal of Pharmaceutical Sciences, 2019; 55: 1–10.
5. Gupta, A., & Mishra, S. K. A novel analytical method for simultaneous quantification of dapagliflozin and sitagliptin by reverse phase high performance liquid chromatography. Journal of Medical Pharmaceutical and Allied Sciences, 2021; 10(3): 2931–2936.
6. Patil S, Dwivedi S, Bagade S, "Development Of Spectrophotometric Method For The Estimation Of Pioglitazone Hcl Fromtwo Different Marketed Brands" American Journal of PharmTech Research, 2011; 1(4): 264-275.
7. Hashem, H., & El-sayed, H. M. Quality by design approach for development and validation of a RP-HPLC method for simultaneous determination of co-administered levetiracetam and pyridoxine HCl in prepared tablets. Microchemical Journal, 2018; 143(7): 55–63.
8. Jeyabalan, G., & Nyola, N. Simultaneous estimation of sitagliptin phosphate monohydrate and metformin hydrochloride in bulk and pharmaceutical formulation by RP-HPLC, 2012; 3(2): 24-28.
9. Kant, R., Bodla, R. B., Kapoor, G., & Bhutani, R. Bioorganic Chemistry Optimization of a single HPLC-PDA method for quantifying Metformin , Linagliptin and Teneligliptin using central composite design. Bioorganic Chemistry, 2019; 91(4): 103-111.
10. Kavitha, D., Sahoo, S. K., P, V. R., Nagamani, M., & Ch, B. Development and validation of RP-HPLC method for determination of Metformin and Sitagliptin in bulk and pharmaceutical dosage form, 2017; 5(2348): 34–39.
11. Practical HPLC Method Development, Lloyd R. Snyder, Joseph J. Kirkland, Joseph L. Glajch.
12. Nadpara, N. P., Thumar, R. V, Kalola, V. N., & Patel, P. B. Review Article QUALITY BY DESIGN (QBD) : A COMPLETE REVIEW, 2012; 17(2): 20–28.
13. Nicholson, M. K., Asswad, R. G., & Wilding, J. P. H. Dapagliflozin for the treatment of type 2 diabetes mellitus – an update. Expert Opinion on Pharmacotherapy, 2021; 22(17): 2303–2310.
14. Patel, K. Y., Dedania, Z. R., Dedania, R. R., & Patel, U. QbD approach to HPLC method development and validation of ceftriaxone sodium. Future Journal of Pharmaceutical Sciences, 2021; 4(7): 1–10.
15. Patel, P., Patel, Y., Jani, S., & Detholia, K. Quantitative Computation of Synthetic Mixture Comprising Sitagliptin and Dapagliflozin from Synthetic Mixture by Vierdot 's Method. Journal of Pharmaceuticals Sciences and Drug Discovery, 2022; 1(2): 1–5.
16. Roy, S. S., Upadhyay, R., & Dalwadi, P. M. Quality by Design (QbD): A Comprehensive Review, 2022; 7(5): 1116–1138.
17. Sanka, S. K., Sythana, S., Jhansi, A., & Shanmugasundharam, P. Development and Validation for Simultaneous Estimation of Sitagliptin and Metformin in Pharmaceutical Dosage Form using RP-HPLC Method, 2013; 5(4): 1736–1744.
18. Sebaiy, M. M., Adl, S. M. El, Baraka, M. M., Hassan, A. A., & Sayed, H. M. El. Quality by design approach for development and validation of a RP - HPLC method for simultaneous estimation of xipamide and valsartan in human plasma. BMC Chemistry, 2022; 1–13.
19. Sharma, H., Sapkota, H. P., & Dangi, N. B. A Brief Review of Analytical Methods for the Estimation of Allopurinol in Pharmaceutical Formulation and

Biological Matrices. International Journal of Analytical Chemistry, 2021; 1-12.
20. Standards of Medical Care in Diabetes d. (2014). Diabetes Care, 2014; 37: 14–80.
21. Suthar, A. M., Prajapati, L. M., Joshi, A. K., Patel, J. R., Kharodiya, M. L., & Educational, P. Estimation of Saxagliptin hydrochloride monohydrate and Dapagliflozin propendiol monohydrate in combined dosage form. Journal of Innovations in Applied Pharmaceutical Science (JIAPS) e-ISSN, 2018; 3(2): 1–7.
22. Umekar, M. J., Mante, G. V, Hemke, A. T., & Umekar, M. J. RP-HPLC Method for Estimation of Dapagliflozin from its Tablet. Article in International Journal of ChemTech Research, 2021; 11(01): 242–248.
23. Subbarayan, S., & Kipnes, M. (2011). Sitagliptin: a review. *Expert opinion on pharmacotherapy*, *12*(10), 1613-1622.
24. Rydén L, Standl E, Bartnik M, Van den Berghe G, Betteridge J, De Boer MJ, *et al*. Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary. *European Heart Journal*. 2007; 28(1): 88–136.
25. Barnett AH. Treatment options for type 2 diabetes: introducing the incretin-based therapies. *Practical Diabetes International*. 2009; 26(5): 179–83.
26. Miller SA, St. Onge EL. Sitagliptin: a dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *Annals of Pharmacotherapy*. 2006; 40(7–8): 1336–43.