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RP-HPLC METHOD AND ITS VALIDATION FOR ANALYSIS OF SAXAGLIPTIN AND METFORMIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:
A rapid, precise, and validated Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method was developed for the simultaneous estimation of Saxagliptin and Metformin in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a Symmetry ODS C18 column (4.6 mm × 150 mm, 5 µm particle size) maintained at a temperature of 38°C. The mobile phase consisted of Methanol and 0.1% Orthophosphoric acid (64:36 v/v), delivered at a flow rate of 1.0 ml/min. Detection was carried out at a wavelength of 224 nm using a Waters Alliance 2695 HPLC system equipped with a PDA Detector (996 model). The injection volume was 20 µl, and the total run time was 7 minutes. The method was validated according to ICH Q2(R1) guidelines and demonstrated excellent linearity, accuracy, precision, specificity, robustness, and acceptable limits of detection and quantification for both drugs. The results confirm that the method is suitable for the simultaneous determination of Saxagliptin and Metformin in both active pharmaceutical ingredients (API) and finished dosage forms. This validated method can be reliably employed in routine quality control and stability testing within pharmaceutical laboratories.

Keywords: *RP-HPLC, Saxagliptin, Metformin, Symmetry ODS C18 column, simultaneous estimation, validation.*

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INTRODUCTION:

High performance liquid chromatography (also known as high pressure liquid chromatography) is a type of column chromatography used to separate, identify, and quantify active ingredients in biochemistry and analysis[1].HPLC mainly utilizes a column that holds packaging material (stationary phase), a pump that moves the mobile phase through the column and a detector that shows the retention time of the molecule. Retention time varies depending on the interaction between the stationary phases the molecule being analysed, and the solvent used.[2]A known amount of the material to be analysed is added to the mobile phase stream and evaluated by a chemical or physical interaction with the stationary phase. The amount of retardation is determined by the type of the analyte as well as the stationary and mobile phase composition. Retention time is the time it takes for a certain analyte to elute (come out of the end of the column). Any miscible combination of water and organic liquids is the most common mobile phase utilised (the most common are methanol & acetonitrile).Gradient elution is used to change the mobile phase composition during the study.[3]

TYPES OF HPLC:

The phase system employed in the process determines the type of HPLC.[3, 4] The following HPLC types are commonly used in analysis:

Normal phase chromatography:

This approach separates analytes based on polarity and is also known as Normal phase HPLC (NP-HPLC). A polar stationary phase and a non-polar mobile phase are used in NP-HPLC. The polar analyte interacts with the polar stationary phase and is retained by it. As the polarity of the analyte rises, so does the adsorption strength, and the interaction between the polar analyte and the polar stationary phase lengthens the elution time.

Reversed phase chromatography:

Reversed phase high performance liquid chromatography (RP-HPLC) consists of a non-polar stationary phase and amoderately aqueous polar mobile phase. RP-HPLCworks on the principle of hydrophobic interactions, the non-polar stationary phase is formed by repulsive forces between a polar eluent, the comparatively non-polar analyte, and the non-polar eluent. When the analyte molecule associates with the ligand in the aqueous eluent, the contact surface area around the non-polar segment of the analyte molecule is proportional to the contact surface area around the non-polar segment of the analyte molecule.

Size exclusion chromatography:

Size Exclusion chromatography, also known as gel permeation chromatography or gel filtration chromatography, is a type of chromatography that separates particles based on their size. It can also be used to figure out the quaternary and tertiary structures of proteins and amino acids. This method is often used to determine the molecular weight of polysaccharides.

Ion exchange chromatography:

Ion-exchange chromatography (IEC) depend on the attraction between solute ions and charged sites bound to the stationary phase. The ion exchange chromatography is mainly used for the purification of water

Bio-affinity chromatography:

In this method separation based on specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, retains proteins with interaction to the column-bound ligands. Proteins bound to a bio affinity column can be eluted in two ways:

• Biospecific elution: inclusion of free ligand in elution buffer which competes with column bound ligand.

• Aspecific elution: change in pH, salt, etc. which weakens interaction proteinwith column-bound substrate

6. EXPERIMENTAL METHODS

INSTRUMENTS USED

1	HPLC WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.	
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil

CHEMICALS USED:

1	Saxagliptin	Provided by Sura Pharma labs
2	Metformin	Provided by Sura Pharma labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck
5	Telma-LN 40	Glenmark

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Saxagliptin and Metformin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air

completely and make volume up to the mark with the same Methanol.

Further pipette 0.6ml of Saxagliptin and 1ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

7. RESULTS AND DISCUSSION:

(Optimized Condition)

Mobile phase : Methanol: 0.1% Orthophosphoric acid (64:36% v/v)
Column : Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size
Flow rate : 1 ml/min
Wavelength : 224 nm
Column temp : 38ºC
Sample Temp : Ambient
Injection Volume : 20 µl
Run time : 7 minutes

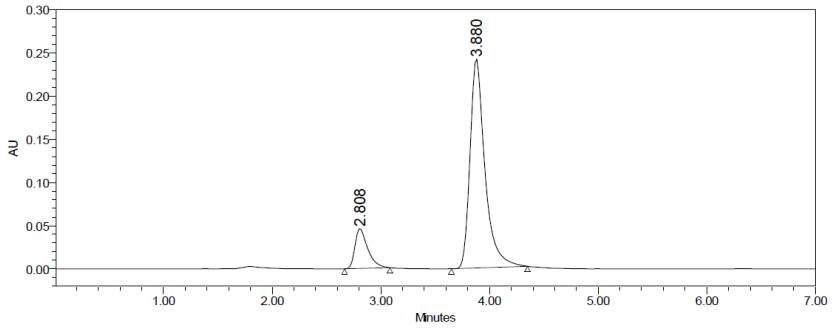

Figure-18: Chromatogram for Trail 5

Table 16: - Peak Results for Trail 5

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Saxagliptin	2.808	65258	4326		1.08	5685.4
2	Metformin	3.880	8659854	659823	5.68	1.42	6895.7

Observation:
From the above chromatogram it was observed that the Saxagliptin and Metforminpeaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

SYSTEM SUITABILITY:

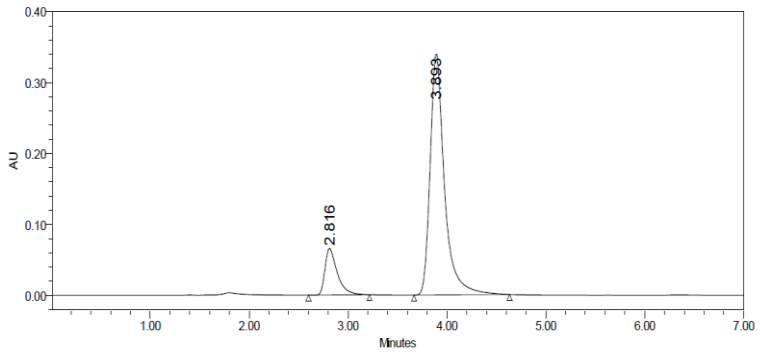

Figure 19: Chromatogram for system suitability

Table-17: Results of system suitability parameters for Saxagliptin and Metformin

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Saxagliptin	2.816	65358	4536		1.08	5689.6
2	Metformin	3.893	8658746	658985	5.69	1.42	6892.4

Acceptance Criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

SPECIFICITY:

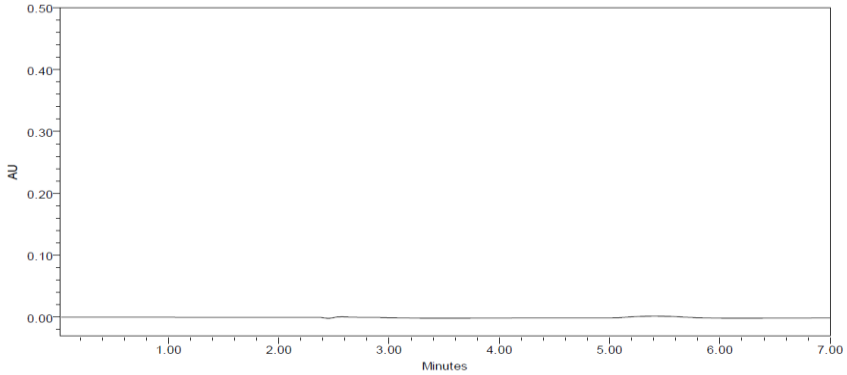

Fig-20: Chromatogram showing blank (mobile phase preparation)

METHOD VALIDATION PARAMETERS:

Assay (Standard):

Table-18: Showing assay standard Results

S. No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Saxagliptin	2.813	65684	4365		1.08	5632.4	1
2	Metformin	3.886	8659824	659824	5.69	1.42	6859.2	1
3	Saxagliptin	2.813	65985	4329		1.09	5682.3	2
4	Metformin	3.886	8645872	658266	5.68	1.43	6824.1	2
5	Saxagliptin	2.813	65784	4426		1.08	5692.8	3
6	Metformin	3.886	8657847	6589412	5.69	1.43	6895.4	3

Assay (Sample):

Table-19: Showing assay sample results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Saxagliptin	2.799	66859	4458		1.09	5785.4	1
2	Metformin	3.863	8756854	669585	5.69	1.43	6956.7	1
3	Saxagliptin	2.799	66258	4462		1.10	5789.5	2
4	Metformin	3.861	8769582	663598	5.68	1.44	6945.2	2
5	Saxagliptin	2.799	66435	4438		1.09	5784.1	3
6	Metformin	3.863	8754985	668548	5.69	1.44	6927.7	3

Table-20: Showing Assay Results

S.No.	Name of Compound	Label Claim	Amount Taken (from Combination Tablet)	% Purity
1	Saxagliptin	10mg	59.84	99.68%
2	Metformin	40mg	499.63	99.46%

The retention time of Saxagliptin and Metformin was found to be 2.808mins and 3.880mins respectively. The % purity of Saxagliptin and Metforminin pharmaceutical dosage form was found to be 99.68% and 99.46% respectively.

Precision:

Precision of the method was carried out for both sample and standard solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Table-21: Results of method precision for Saxagliptin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Saxagliptin	2.808	65898	4365	5682.2	1.08
2	Saxagliptin	2.808	65487	4375	5628.6	1.09
3	Saxagliptin	2.808	65324	4395	5649.7	1.08
4	Saxagliptin	2.808	65982	4328	5638.4	1.09
5	Saxagliptin	2.808	65248	4371	5698.3	1.08
6	Saxagliptin	2.808	65734	4391	5682.7	1.09
Mean			65612.17			
Std. Dev			304.8425			
% RSD			0.464613			

Table-22: Results of method precision for Valproate

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	3.880	8659824	658784	6859.4	1.42	5.68
2	Metformin	3.880	8658547	657489	6824.6	1.43	5.69
3	Metformin	3.880	8659824	652368	6829.3	1.42	5.68
4	Metformin	3.880	8659875	658745	6892.7	1.43	5.69
5	Metformin	3.880	8658745	658213	6875.2	1.42	5.68
6	Metformin	3.880	8659862	652354	6859.8	1.42	5.69
Mean			8659446				
Std. Dev			623.2924				
% RSD			0.007198				

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate Precision/Ruggedness:

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

DAY 1:

Table-23: Results of Intermediate precision for Saxagliptin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Saxagliptin	2.808	66895	4468	5784.2	1.09
2	Saxagliptin	2.808	66986	4523	5835.1	1.09
3	Saxagliptin	2.808	66258	4475	5864.4	1.10
4	Saxagliptin	2.808	66457	4514	5864.6	1.09
5	Saxagliptin	2.808	66539	4489	5784.9	1.10
6	Saxagliptin	2.808	66298	4565	5748.5	1.10
Mean			66572.17			
Std. Dev			304.536			
% RSD			0.457452			

Table-24: Results of Intermediate precision for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	3.882	8758568	669583	6982.4	1.43	
2	Metformin	3.882	8756982	665984	6935.3	1.44	5.69
3	Metformin	3.882	8746925	665345	6984.7	1.44	
4	Metformin	3.882	8723654	665325	6952.8	1.43	5.70
5	Metformin	3.882	8754982	669852	6898.9	1.44	
6	Metformin	3.882	8754698	665874	6976.5	1.43	5.69
Mean			8749302				
Std. Dev			13188.56				
% RSD			0.150738				

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

DAY 2:

Table-25: Results of Intermediate precision for Saxagliptin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Saxagliptin	2.799	66510	4310	5711.6	1.01
2	Saxagliptin	2.813	66216	4219	5826.2	1.03
3	Saxagliptin	2.808	66501	4316	5715.1	1.05
4	Saxagliptin	2.816	66129	4501	5756.0	1.06
5	Saxagliptin	2.860	66016	4468	5891.6	1.09
6	Saxagliptin	2.824	66519	4419	5892.8	1.08
Mean			66315			
Std. Dev			222.72			
% RSD			0.3358			

Table-26: Results of Intermediate precision for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	3.861	8761210	668200	6952.1	1.44	
2	Metformin	3.886	8721601	666111	6971.5	1.43	5.70
3	Metformin	3.880	8739120	664626	6990.4	1.43	
4	Metformin	3.893	8742810	664462	6960.1	1.44	5.71
5	Metformin	3.949	8784519	665511	6941.2	1.44	
6	Metformin	3.914	8712915	668440	6950.9	1.44	5.70
Mean			8743695				
Std. Dev			26194.05				
% RSD			0.299				

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Accuracy Standard:

Table-27: Results of Accuracy standard values

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Saxagliptin	2.860	65359	4358		1.09	5698.5	1
2	Metformin	3.949	8659825	659862	5.68	1.42	6859.4	1
3	Saxagliptin	2.860	65874	4395		1.08	5672.4	2
4	Metformin	3.949	8659875	653485	5.68	1.43	6824.2	2
5	Saxagliptin	2.860	65398	4382		1.08	5683.1	3
6	Metformin	3.949	8674587	6587458	5.69	1.42	6875.6	3

Table-28: Accuracy (recovery) data for Saxagliptin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	35921.67	30	30.134	100.446%	100.30%
100%	70894.33	60	60.205	100.341%	
150%	105654.7	90	90.093	100.103%	

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0%.

Table-29: Accuracy (recovery) data for Metformin

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	4276302	50	50.208	100.416%	100.21%
100%	8484717	100	100.148	100.148%	
150%	10160609	150	150.091	100.060%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (97-103%).

ACCURACY

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

ACCURACY 50%:

Table-30: Results of Accuracy sample 50% values

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Saxagliptin	2.816	35929	3896		0.98	4896.2	1
2	Metformin	3.893	4274645	578452	5.08	1.28	6895.1	1
3	Saxagliptin	2.816	35989	3958		0.99	4874.3	2
4	Metformin	3.893	4275698	586592	5.09	1.29	6826.7	2
5	Saxagliptin	2.816	35847	3874		0.99	4879.4	3
6	Metformin	3.893	4278563	586874	5.08	1.28	6895.3	3

Accuracy 100%:

Table-31: Results of Accuracy sample 100% values

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Saxagliptin	2.860	70989	4485		1.09	5698.8	1
2	Metformin	3.949	8488468	659822	5.70	1.43	6985.4	1

3	Saxagliptin	2.860	70896	4398		1.10	5786.9	2
4	Metformin	3.949	8478696	658952	5.71	1.44	6975.4	2
5	Saxagliptin	2.860	70798	4458		1.09	5864.7	3
6	Metformin	3.949	8486987	658754	5.70	1.43	6898.9	3

Accuracy 150%:

Table-32: Results of Accuracy sample 150% values

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Saxagliptin	2.824	105753	6528		1.24	6587.4	1
2	Metformin	3.914	12695265	752454	6.82	1.68	8695.3	1
3	Saxagliptin	2.824	105584	6584		1.25	6582.2	2
4	Metformin	3.914	12689898	752658	6.83	1.69	8759.6	2
5	Saxagliptin	2.824	105627	6539		1.24	6538.6	3
6	Metformin	3.914	12694574	753689	6.82	1.68	8698.5	3

LINEARITY:

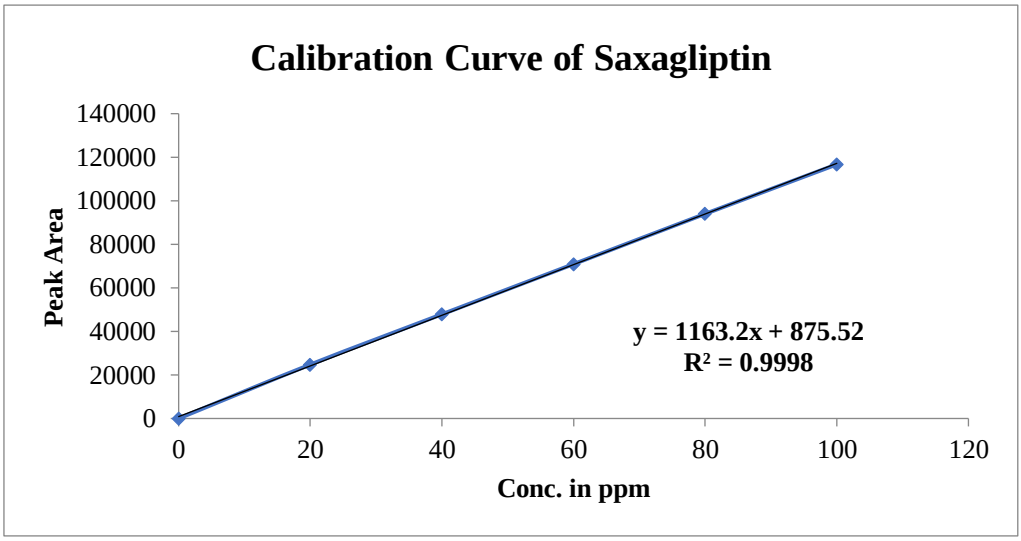

Figure 63: Calibration graph for Saxagliptin

Table 33: Linearity Results: (Saxagliptin)

Concentration (ppm)	Area
20	24759
40	47859
60	70898
80	93985

100	116698

Acceptance Criteria: Correlation coefficient should be not less than 0.999.

Linearity Results: (for Valproate)

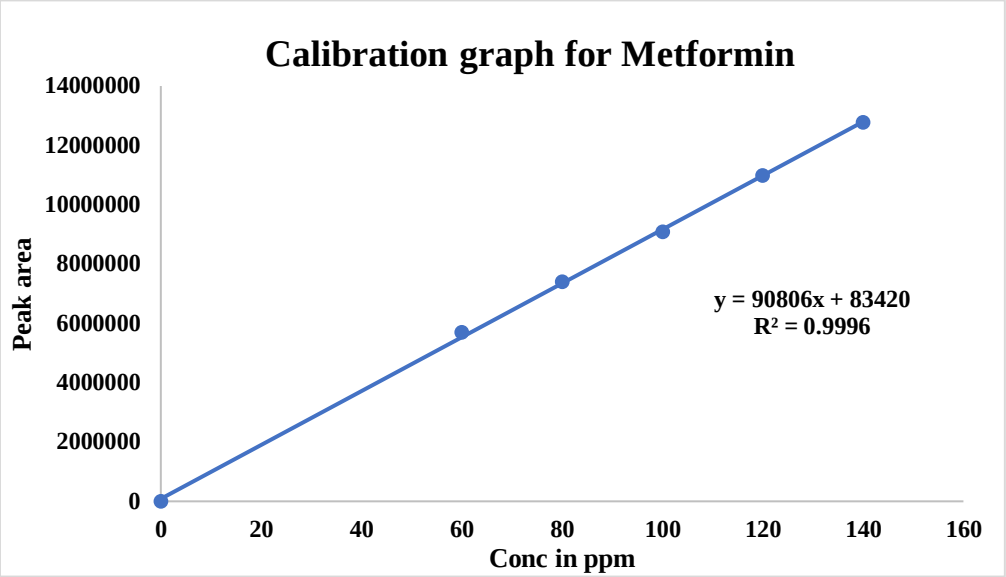

Figure 64: Calibration graph for Metformin

Table 34: Linearity Results (for Valproate)

Concentration(ppm)	Area
60	5687842
80	7389878
100	9085847
120	10969854
140	12769854

Acceptance Criteria:

- Correlation coefficient should be not less than 0.99.

Table-35: Analytical performance parameters of Saxagliptin and Metformin

Parameters	Saxagliptin	Metformin
Slope (m)	1163.2	90806
Intercept (c)	875.52	83420
Correlation coefficient (R^2)	0.999	0.999

Acceptance Criteria:

Correlation coefficient (R^2) should not be less than 0.999.

LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Saxagliptin :

Result:

= 0.97µg/ml

Valproate:
Result:
= 2.06µg/ml

QUANTITATION LIMIT
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.
LOQ=10×σ/S
Where
σ = Standard deviation of the response
S = Slope of the calibration curve
Saxagliptin:
Result:
=2.91µg/ml
Valproate:
Result:
= 6.18µg/ml

ROBUSTNESS:

Table-36: System suitability results for Saxagliptin:

S.No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
1	0.9	5784.6	1.06	3.091
2	1.0	5685.4	1.08	2.813
3	1.1	5869.5	1.09	2.553

* Results for actual flow (1.0 ml/min) have been considered from Assay standard.

Table-37: System suitability results for Valproate:

S.No	Flow Rate (ml/min)	System Suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
1	0.9	6698.3	1.46	4.274
2	1.0	6895.7	1.42	3.886
3	1.1	6983.6	1.49	3.538

* Results for actual flow (1.0ml/min) have been considered from Assay standard.

Variation of mobile phase organic composition:

Table-38: System suitability results for Saxagliptin

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
1	10% less	5895.3	1.12	3.301
2	*Actual	5685.4	1.08	2.813
3	10% more	5964.2	1.16	2.469

Table-39: System suitability results for Valproate:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
1	10% less	6785.2	1.46	4.344
2	*Actual	6895.7	1.42	3.886
3	10% more	6982.4	1.49	3.508

8. SUMMARY AND CONCLUSION:

Summary:

A simple, accurate, precise, and reproducible Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Saxagliptin and Metformin in bulk drug and pharmaceutical dosage forms. The chromatographic separation was achieved using a Symmetry ODS C18 column (4.6 mm × 150 mm, 5 µm particle size) maintained at a temperature of 38ºC. The mobile phase consisted of Methanol and 0.1% Orthophosphoric acid in the ratio of 64:36% v/v, delivered at a flow rate of 1.0 ml/min. Detection was carried out at a wavelength of 224 nm using a Waters Alliance 2695 HPLC system equipped with a PDA Detector (996 model). The injection volume was 20 µl, and the total run time was 7.0 minutes. The method was validated according to ICH Q2(R1) guidelines, and all critical validation parameters were evaluated. The method showed good specificity, with no interference from excipients or impurities. Linearity was observed over a suitable concentration range with high correlation coefficients.

CONCLUSION:

The developed RP-HPLC method is simple, reliable, and efficient for the simultaneous estimation of Saxagliptin and Metformin in bulk and tablet dosage forms. The method was successfully validated in accordance with ICH guidelines and found to be specific, accurate, precise, robust, and suitable for routine quality control and stability testing. Its short analysis time and good resolution make it ideal for regular pharmaceutical analysis in research, development, and manufacturing environments.

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