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Analytical Method Development and Validation for the Ipragliflozine and Saxagliptin by using RP-HPLC in Pharmaceutical Dosage Forms as per ICH guidelines

Yallamellaiah Meghana[1], B. Sravanthi[2], Dr. Vijay Kumar Gampa*[3]
[1]Department of Pharmaceutical Analysis, KGR Institute of Technology and Management, Rampally, Keesara(m), Medchal-Malkajgiri District, Hyderabad -501302, Telangana, India.
[2]Assistant Professor, Department of Pharmaceutical Analysis, Rampally, Keesara(m), Medchal-Malkajgiri District, Hyderabad -501302, Telangana, India.
[3]Principal and Professor, KGR Institute of Technology and Management, Rampally, Keesara(m), Medchal-Malkajgiri District, Hyderabad-501302, Telangana, India.

Abstract
This research works the advancement & verification of a RP-HPLC technique for the concurrent quantification of Ipragliflozine & Saxagliptin in formulations. The optimal chromatographic conditions were established using an INTERSIL C18-EP column with a MP of ACN & potassium phosphate buffer (pH 5) in a 70:30 ratio, a flow of 1ml per min, & detection at 254 nm. The method was systematically verified for various parameters, including assay, linearity, precision, intermediate precision, accuracy, LOD, (LOQ, & robustness. The results demonstrated a strong correlation coefficient for linearity, with acceptable precision and accuracy across different concentrations. The LOD and LOQ for Ipragliflozine were determined to be 0.022 µg per ml and 0.076 µg per ml, respectively, while for Saxagliptin, they were 0.020 µg per ml and 0.075 µg per ml. Robustness testing confirmed method reliability under varied conditions, including flow speed & MP composition. Overall, the advancement HPLC technique is efficient, reproducible, and suitable for regular analysis of Ipragliflozine & Saxagliptin in QC laboratories, ensuring accurate dosage determination in preparations.
Keywords: Ipragliflozine, Saxagliptin, Hypersil C18 column, validation, reliability, RP-HPLC, ICH guidelines.

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Contents:

***Corresponding author**
Dr. Vijay Kumar Gampa
Principal and Professor,
KGR Institute of Technology and Management,
Rampally, Keesara(M), Hyderabad -501302, Telangana, India.

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1. Introduction

In the modern landscape of pharmaceutical sciences, the drive for precise, reliable, and efficient analytical methodologies has never been more critical. The development and validation of analytical methods are essential to ensure the quality, efficacy, and safety of pharmaceutical products. Among the diverse array of analytical techniques, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) stands out as a highly sensitive and specific method, particularly suited for the simultaneous estimation of multiple drug components. This study focuses on the analytical method development and validation for the concurrent identification of Ipragliflozine and Saxagliptin in pharmaceutical dosage forms, following the stringent guidelines set forth by the International Council for Harmonisation (ICH). Ipragliflozine, a sodium-glucose cotransporter-2 (SGLT-2) inhibitor, is widely used in the management of type 2 diabetes mellitus. It functions by inhibiting glucose reabsorption in the kidneys, thus promoting glucose excretion and lowering blood glucose levels. Saxagliptin, on the other hand, is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances the body's natural ability to regulate glucose by increasing incretin

levels, which in turn increase insulin release and decrease glucagon levels in the bloodstream. The combination of these two drugs offers a potent therapeutic approach to controlling hyperglycemia in diabetic patients, making their concurrent analysis in pharmaceutical formulations critical.

The integration of RP-HPLC in the analysis of Ipragliflozine and Saxagliptin provides several advantages, including high resolution, accuracy, and the ability to separate and quantify the drugs in complex matrices. The development of this method involves the meticulous optimization of various chromatographic parameters, such as the selection of the mobile phase, column type, flow rate, and detection wavelength. The choice of mobile phase, often comprising a combination of water, acetonitrile, and buffer solutions, plays a pivotal role in achieving optimal separation and peak resolution. The use of a C18 column is common in such analyses due to its efficiency in separating compounds with different polarities.

Method development is followed by a rigorous validation process to ensure the analytical method's reliability and compliance with ICH guidelines. Validation involves a comprehensive assessment of several key parameters, including specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness. Specificity refers to the method's ability to accurately identify and quantify the analytes in the presence of other components, such as excipients and degradation products. Linearity evaluates the method's capacity to produce results that are directly proportional to the analyte concentration over a specified range, which is crucial for accurate quantification.

Accuracy and precision are critical parameters that ensure the method produces reliable and reproducible results. Accuracy is determined by comparing the test results with those obtained from a reference method or known standards, while precision is evaluated by assessing the consistency of results obtained from multiple measurements of the same sample. The LOD and LOQ are crucial for determining the method's sensitivity, indicating the smallest amount of the analyte that can be reliably detected and quantified. Robustness testing involves evaluating the method's reliability under varied conditions, such as changes in pH, flow rate, and temperature, ensuring that the method remains consistent and accurate under different operational scenarios.

The successful development and validation of an RP-HPLC method for the concurrent identification of Ipragliflozine and Saxagliptin in pharmaceutical dosage forms hold significant implications for pharmaceutical quality control. It ensures that these critical antidiabetic drugs meet the required standards of efficacy, safety, and quality, thereby enhancing patient outcomes and contributing to public health. Additionally, this method can be adapted and applied to other drug combinations, further extending its utility in pharmaceutical analysis.

Drug Profile

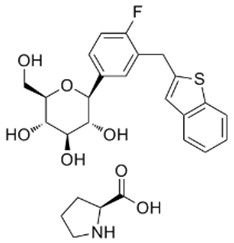

Fig.1 Ipragliflozin structure

IUPAC Name: (1S)-1,5-Anhydro-1-[3-(1-benzothiophen-2-ylmethyl)-4-fluorophenyl]-D-glucitol1
Molecular Formula: C21H21FO5S
Molecular Weight: 404.45 g/mol1
Melting Point: Approximately 110 °C1
pKa: Approximately 7.51
Category: Antidiabetic (SGLT2 inhibitor)1
Solubility: Poorly soluble in water1

Description

Ipragliflozin is an oral antidiabetic medication used to treat type 2 diabetes mellitus. It works by inhibiting the sodium-glucose co-transporter 2 (SGLT2) in the kidneys, which reduces glucose reabsorption and increases glucose excretion in the urine12.

Mechanism of Action

Ipragliflozin selectively inhibits SGLT2, a protein responsible for glucose reabsorption in the kidneys. By blocking this protein, it reduces blood glucose levels by promoting the excretion of glucose through urine12.

Pharmacodynamics

Ipragliflozin lowers blood glucose levels by increasing urinary glucose excretion. This action helps to improve glycemic control in patients with type 2 diabetes1.

Pharmacokinetics

- Absorption: Well absorbed from the gastrointestinal tract1.
- Distribution: Widely distributed in body tissues1.
- Metabolism: Metabolized primarily by UGT2B7, with minor contributions from UGT2B4, UGT1A8, and UGT1A91.
- Route of Elimination: Primarily excreted in the urine (67.9%) and feces (32.7%)1.
- Protein Binding: Approximately 94.6-96.5%.
- Half-Life: Around 14.97 ± 4.58 hours.

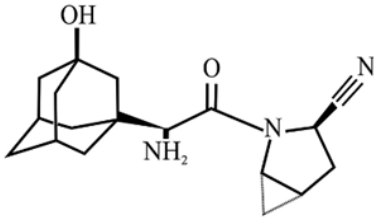

Fig.2 Saxagliptin Structure

IUPAC Name: (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxyadamantan-1-yl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile
Molecular Formula: $C_{18}H_{25}N_3O_2$
Molecular Weight: 315.41 g/mol1
Melting Point: Approximately 222-225 °C

pKa: Approximately 8.71
Category: Antidiabetic (DPP-4 inhibitor)1
Solubility: Poorly soluble in water1

Description

Saxagliptin is an oral antidiabetic medication used to improve blood sugar control in adults with type 2 diabetes mellitus. It is not used for treating type 1 diabetes12.

Mechanism of Action

Saxagliptin works by inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4). This inhibition increases the levels of incretin hormones, which help to regulate blood sugar by increasing insulin release and decreasing glucagon levels12.

Pharmacodynamics

Saxagliptin helps to lower blood sugar levels by enhancing the body's natural ability to control blood sugar levels, especially after meals.

Pharmacokinetics

Absorption: Well absorbed from the gastrointestinal tract.
Distribution: Widely distributed in body tissues.
Metabolism: Metabolized primarily by the liver.
Route of Elimination: Primarily excreted in the urine.
Protein Binding: Approximately 70%.
Half-Life: Around 2.5 hours.

Uses

Primary Use: Treatment of type 2 diabetes mellitus12.
Other Uses: Often used in combination with other antidiabetic agents1.

Dosage

Adults: Typically 2.5 mg or 5 mg once daily.
Children: Not typically used in pediatric patients.

Side Effects

Common side effects include upper respiratory tract infections, urinary tract infections, and headaches. Serious side effects can include severe allergic reactions, pancreatitis, and heart failure1.

Drug Interactions

Saxagliptin can interact with other medications that affect blood sugar levels or are metabolized by the liver.

Storage: Store at room temperature, away from moisture and heat

2. Materials and Methods

Choosing λmax:

UV spectrum of 10µg per ml Ipraglifozine & saxagliptinin diluents (MP ratio) was noted by examining in the scale of 200 to 400nm and the isobestic λmax of both the drugs obtained at 254 nm.

Optimization of Column:

Intersil C18-EP (4.6 x 250mm, 5µm) uncovered to be perfect as it produce excellent Gaussian shape of peak & R s at 1 ml per min flow speed.

Chromatographic Optimized Conditions

Instrument used : RP-HPLC having Auto Sampler and PDA detector
Temperature : Ambient
Column : Intersilc 18-EP (4.6x250mm,5µm)
MP ratio : ACN: KH2PO4 PH 5 (70:30ml)
Flow :1ml/min
λmax : 254 nanometers
volume Injected : 10 µl
Run duration : 10min.

Buffer & Mobile phase preparation:

Phosphate buffer pH 5Preparation:

By adding 6.4gm of phosphate buffer in 1L water. Adjust this solution to PH 5 by using OPA.

Composition of MP:

Mix a 300 ml KH2PO4 (30%) and ACN 700ml (70%) and remove gases in ultra-sonication water bath for 5 min. Filter using 0.45µ filter by vacuum filtration instrument.

Diluent: ACN: KH2PO4buffer PH 5(70:30)ratio.

System Suitability:

Tailing factor for Ipraglifozine & saxagliptin in Stad solution shouldn't > 2.0. For Standard solution Theoretical plates for the Ipraglifozine & Saxagliptin shouldn't 't< 2000. Calculation:(ForIpraglifozine and saxagliptin)

Method validation parameters:

Assay:

Standard Solution Preparation:

Precisely measure & poured 50mg of Ipragliflozine& 5mg Saxagliptin standard into a 25 ml VF add Diluents & to dissolve fully sonicate& get volume up till the margin Additional pipette out 0.3ml from solutions into a 10ml VF & dilute up till the margin using diluent. (60ppm Ipragliflozine, 6ppm Saxagliptin).

Sample Solution Preparation:

Precisely measure & poured equivalent to 50mg of Ipragliflozine and 5mg Saxagliptin equal wt of the sample into a 25ml VF add Diluents & to dissolve fully sonicate& get volume up till the margin Additional pipette out 0.3ml from solutions into a 10ml VF & dilute up till the margin using diluent. (60ppmIpragliflozine, 6ppm Saxagliptin).

Procedure:

Inject 10 mL of the standard, sample into the chromatographic system and measure the areas for the Ipragliflozine and Saxagliptin peaks.

Linearity:

Stock solution Preparation:

Precisely measure & poured 50mg of Ipragliflozine and 5mg Saxagliptin standard into a 25 ml VF add Diluents & sonication to dissolve fully & get volume up till the margin.

Level – I Preparation (Ipraglifozine 20ppm&Saxagliptin 2ppm): Pipette out 0.1ml from solution has taken in 10ml of VF dilute up till the margin using diluent.

Level–II Preparation (Ipraglifozine 40ppm& Saxagliptin 4ppm):

Pipette out 0.2ml from solution has taken in 10ml of VF dilute up till the margin using diluent.

Level–III Preparation (Ipraglifozine 60ppm & Saxagliptin 6ppm): Pipette out 0.3ml from solution has taken in 10ml of VF dilute up till the margin using diluent.

Level–IV Preparation (Ipraglifozine 80ppm & Saxagliptin 8ppm): Pipette out 0.4ml from solution has taken in 10ml of VF dilute up till the margin using diluent.

Level –V Preparation (Ipraglifozine 100ppm & Saxagliptin 10ppm): Pipette out 0.5ml from solution has taken in 10ml of VF dilute up till the margin using diluent.

Procedure: After Injecting every conc. into the HPLC & get the data of peaks. Construct a CC graph (Calibration curve) area of peak on X-axis vs conc. on Y-axis & find out R2.

Precision:

Stock Solution Preparation:

Precisely measure & poured 50mg of Ipragliflozine and 5mg Saxagliptin standard into a 25 ml VF add Diluents and to fully dissolve sonicate &get volume up till the margin using the solvent. Additional pipette out 0.3mlfrom stock solutions into a 10ml VF & get up till the margin with Diluent. (60ppmIpragliflozine, 6ppm Saxagliptin).

Procedure:

Injecte the std solution for 6 times & we get all 6 replicas area's in HPLC. The %RSD for the areas of 6 replicas was calculated to be under the limits.

Intermediate precision/ruggedness:

To examine the ID method precision, it was done on separate days under the lab.

Stock solution Preparation:

Precisely measure & poured 50mg of Ipragliflozine and 5mg Saxagliptin standard into a 25 ml VF add Diluents and to fully dissolve sonicate& get required volume with solvent. Additional pipette out 0.3mlfrom solutions into a 10ml VF & get up till the margin with Diluent. (60ppmIpragliflozine, 6ppm Saxagliptin).

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

Injecte 6 times the standard solution & get the for all 6 injections area in HPLC. The %RSD for the peak area's of 6 replicas injections was calculated to be under the boundaries.

Stock solution Preparation:

Precisely measure & poured 50mg of Ipragliflozine and 5mg Saxagliptin standard into a 25 ml VF diluents &sonicate to fully dissolve & get required volume with solvent with the same solvent. Additional take out 0.3ml from solution into a 10ml VF &get up till the margin using diluent.(60ppmIpragliflozine, 6ppm Saxagliptin).

Sample solutions Preparation:

50% solution preparation: Precisely measure & poured 25mg of Ipragliflozine and 2.5mg Saxagliptin standard into a 25 ml VF add Diluents & to fully dissolve sonicate& make required volume with solvent. Additional take out 0.3ml from solutions into a 10ml VF and get up till the margin using diluents.

For preparation of 100% solution:

Precisely measure & poured 50mg of Ipragliflozine& 5mg Saxagliptin standard into a 25 ml VF add Diluents & to fully dissolve sonicate& make required volume with solvent. Additional take out 0.3ml from solutions into a 10ml VF and get up till the margin using diluents. (60ppm Ipragliflozine, 6ppm Saxagliptin).

For preparation of 150% solution:

Precisely measure & poured75mg of Ipragliflozine and 7.5mg Saxagliptin standard into a 25ml VF add diluents and sonicate to dissolve fully & get volume up till the margin with the solvent. Additional take out 0.3ml from solution into a 10ml VF & make up till the margin using solvent.

Procedure:

Introduce standard solution, Accuracy -50%, 100% & 150% solutions. Find out the amount found & added for Docetaxel & Fosamprenavir & find the separate l recovery &Avg recovery values.

Limit of detection:

0.022µg/ml Ipraglifozine solution Preparation:

Precisely measure & poured 55 mg of Ipraglifozine standard into a 25 ml VF add diluents and to fully dissolve sonicate get volume up till the margin.

- Additional take out 1ml from solution into a 10ml VF & get up till the margin using diluents.
- Additional take out 1ml from solution into a 10ml VF & get up till the margin using diluents.
- Additional take out 1ml from solution into a 10ml VF & get up till the margin using diluents.
- Additional take out 0.2 ml from solution into a 10ml VF & get up till the margin using diluents. (0.02ppm)

0.020µg/ml saxagliptin solution Preparation:

Precisely measure & poured 25 mg of saxagliptin standard into a two 25 ml VF add Diluents &sonicate to dissolve fully & get volume up till the margin with the solvent.

- Additional take out 1ml from solution into a 10ml VF and get up till the margin using diluents.
- Additional take out 1ml from solution into a 10ml VF and get up till the margin using diluents.
- Additional take out 1ml from solution into a 10ml VF and get up till the margin using diluents.
- Additional take out 0.2ml from solution into a 10ml VF and get up till the margin using diluents. (0.02ppm)

Limit of quantification:

0.076µg/ml Ipraglifozine solution Preparation:

Precisely measure & poured 25 mg of Ipraglifozine and saxagliptin standard into a 25 ml VF add diluents & to fully dissolve sonicate& get volume up till the margin using the solvent. Additional take out 1 ml from solution into a 10ml VF & get up till the margin using diluents.

- Additional take out 1mlfrom solution into a 10ml VF & get up till the margin using diluents.
- Additional take out 1mlfrom solution into a 10ml VF & get up till the margin using diluents.
- Additional take out 0.7mlfrom solution into a 10ml VF & get up till the margin using diluents.(0.07ppm)

0.075µg/ml saxagliptin solution Preparation:

Precisely measure & poured25 mg of saxagliptin working standard into a 25 ml VF add diluents &sonicate to fully dissolve & get volume up till the margin with the solvent

- Additional take out 1 ml of the above stock solution into a 10ml volumetric flask and dilute uptothemark with Diluents.
- Additional take out 1mlfrom solution into a 10ml VF &get up till the margin using diluents.
- Additional take out 1mlfrom solution into a 10ml VF &get up till the margin using diluents.
- Additional take out 0.7mlfrom solution into a 10ml VF &get up till the margin using diluents. (0.07ppm)

Robustness:

Robustness, is a deliberate change in the Flow speed, MP ratos, Temperature changes was done to check the affect on this technique

- The speed of flow speed was differ at 0.8 ml per min to 1.2 ml per min.
- Standard solution 30µg/ml of Ipraglifozine and saxagliptin prepared and an alyzedmade & analyzed using the different flow speed across with actual flow.
- The ratios of Organic in the MP was differ from 40% - 60%.
- Standard solution 30 µg/ml of Ipraglifozine and saxagliptin fosamprenavir was made and analyzed using the different MP ratios across with the real MP rato in the method.

3. Results and Discussion

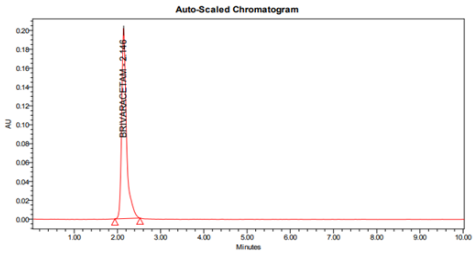

Figure 3: Chromatogram for system suitability

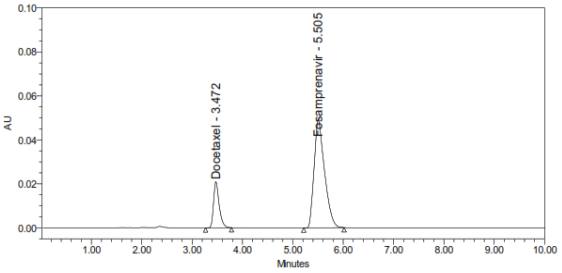

Figure 4: Standard Chromatogram

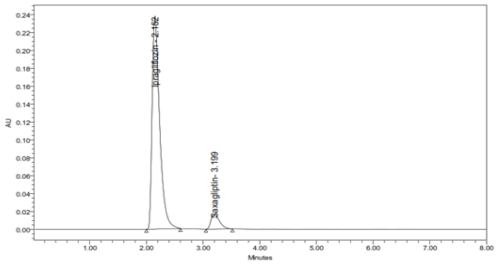

Figure 5: Sample Chromatogram

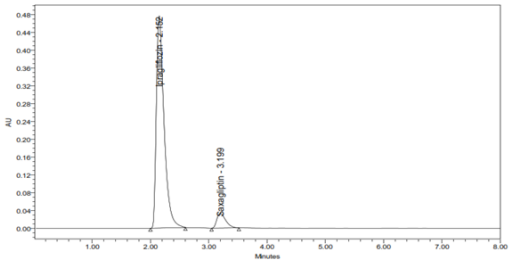

Figure 6: linearity-5 Chromatograph

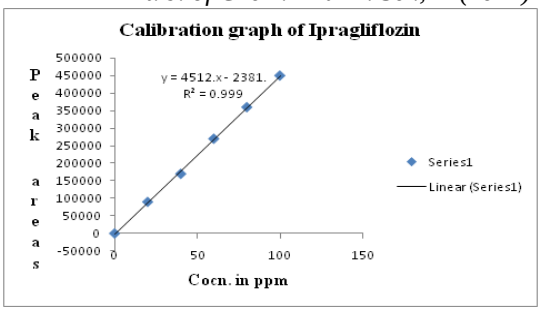

Figure 7: Calibration graph for Ipraglifozine

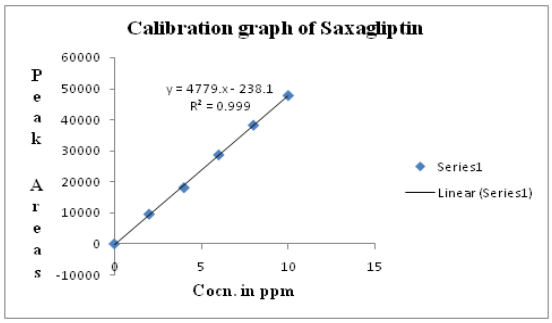

Figure 8: Calibration graph for saxagliptin

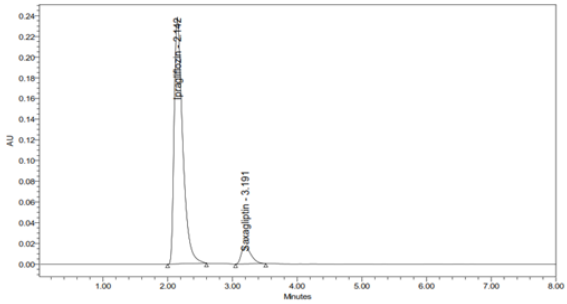

Figure 9: Precision Chromatogram

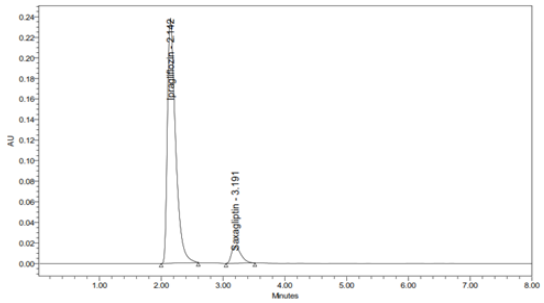

Figure 10: ID Precision Chromatogram

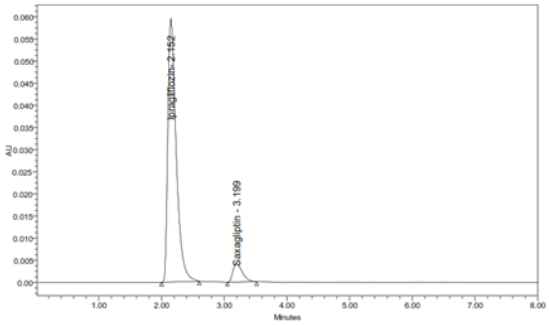

Figure 11: Accuracy 50% Chromatogram

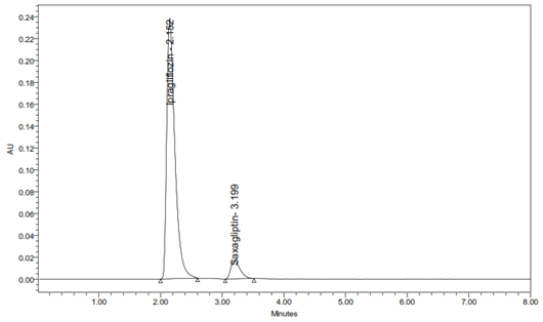

Figure 12: Accuracy 100% Chromatogram

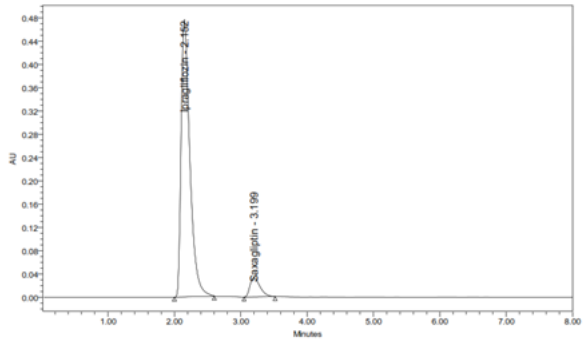

Figure 13: Accuracy 150% Chromatogram

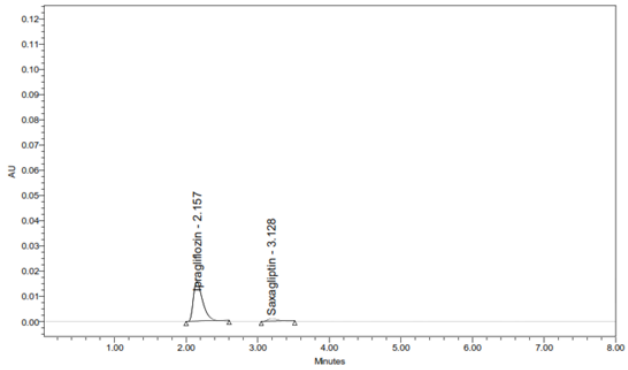

Figure 14: Ipraglifozine and saxagliptin depicting LOD Chromatograph

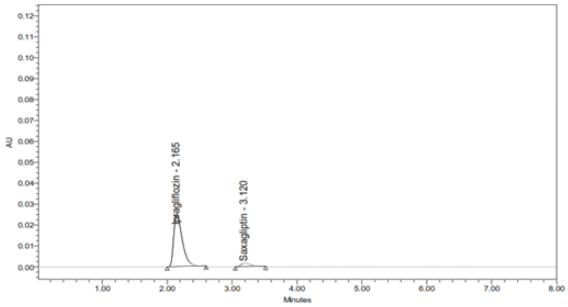

Figure 15: Ipraglifozine and saxagliptin dipicting LOQ Chromatograph

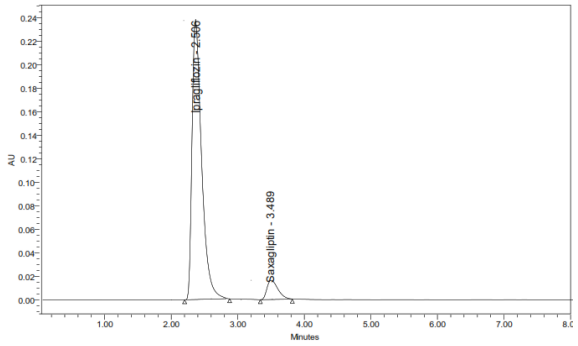

Figure 16: Less flow depicting Chromatograph

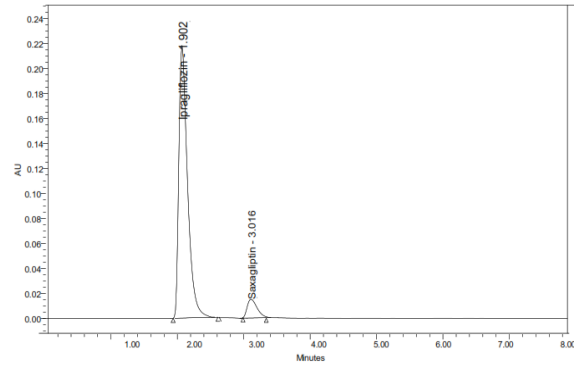

Figure 17: More flow depicting Chromatograph

Table 1: Results of system suitability parameters

Sample Name's	RT'S	Area (µV sec)	Height (µV)	Resolution	tailing USP	plate count USP
Ipragliflozin	2.152	269899	743634	3.69	1.00	6809
Saxagliptin	3.199	28590	74363		1.06	2568

Table 2: Assay Table

Std Name's	RT'S	Area (µV sec)	Height (µV)	Resolution	tailing USP	plate count USP
Ipragliflozin	2.152	269789	743627	4.21	1.12	6818
Saxagliptin	3.199	28495	74358		1.04	2562

Sample Name's	RT'S	Area (µV sec)	Height (µV)	Resolution	tailing USP	plate count USP
Ipragliflozin	2.152	269899	743634	3.69	1.00	6809
Saxagliptin	3.199	28590	74363		1.06	2568

Table 3: Outcomes of Precision for Ipraglifozine and saxagliptin

Injection	Ipragliflozin Area's	Saxagliptin Area's
1 Injection	259899	27590
2 Injection	268899	28690
3 Injection	269799	28790
4 Injection	269889	28590
5 Injection	269899	28590
6 Injection	269899	28590
Avg	268047.3	28473.33
Standard Deviation	4010.9	440.0
%RSD	1.4	1.5

Table 4: Intermediate precision Results for Ipraglifozine and Saxagliptin

Injection	Area's of Ipraglifozine	Area's of Saxagliptin
1 Injection	268899	28690
2 Injection	268899	28690
3 Injection	269799	28790
4 Injection	269889	28590
5 Injection	269899	28590
6 Injection	269899	28590
Avg	269547.3	28656.67
Standard Deviation	503.6	81.6
%RSD	0.1	0.2

Table 5: Recovery(Accuracy) data for Ipraglifozine & Saxagliptin

%Conc. Ipraglifozine (at specified Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Avg Recovery
50%	134949	25	24.8	99.2	99.26
100%	269899	50	49.9	99.8	
150%	404848	75	74.9	98.8	

%Conc. saxagliptin (at specified Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Avg Recovery
50%	14295	2.5	2.45	98.0	98.57
100%	28590	5	4.92	98.4	
150%	42885	7.5	7.45	99.33	

Table 6: LOD Results

Drug's names	Baseline noise(µV)	Signal attained(µV)	Signal/Noise ratio	Conc. In ppm
Ipraglifozine	95	282	2.96	0.022
saxagliptin	95	260	2.94	0.020

Table 7: Results of LOQ

Drug's name's	Baseline noise(µV)	Signal attained(µV)	S/N ratio	Conc. In ppm
Ipraglifozine	95	945	9.99	0.076
saxagliptin	95	942	9.91	0.075

Table 8: Outcomes of difference in flow speeds for Ipragliflozin

S.No	Flow (ml per min)	SST outcomes of Ipragliflozin	
		Plate Count USP	Tailing USP
1	0.8	6780	0.92

2	1	6809	1.0
3	1.2	6978	1.21

Table 9: Outcomes of difference in flow speeds for Saxagliptin

S.No	Flow (ml per min)	SST outcomes of Saxagliptin	
		Plate Count USP	Tailing USP
1	0.8	2534	1.02
2	1	2568	1.06
3	1.2	2634	1.20

4. Conclusion

The Advancement RP-HPLC technique for the concurrent identification of Ipragliflozin & Saxagliptin in dosage forms proved to be efficient, accurate, and reproducible. The technique exhibited strong linearity, precision, and accuracy, meeting all validation criteria set by ICH regulations. With LOD and LOQ, the technique is highly sensitive and suitable for regular QC and analysis in the pharmaceutical industry. This verified technique ensures compliance with regulatory standards and can be applied for the consistent evaluation of combination dosage forms containing Ipragliflozin and Saxagliptin.

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