

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

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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 1 ml 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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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

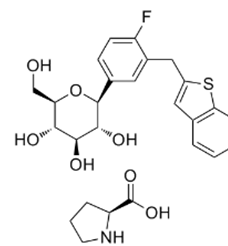


Fig.1 Ipragliflozin structure

IUPAC Name: (1S)-1,5-Anhydro-1-[3-(1-benzothiophen-2-ylmethyl)-4-fluorophenyl]-D-glucitol

Molecular Formula: C₂₁H₂₁FO₅S

Molecular Weight: 404.45 g/mol

Melting Point: Approximately 110 °C

pKa: Approximately 7.51

Category: Antidiabetic (SGLT2 inhibitor)

Solubility: Poorly soluble in water

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.

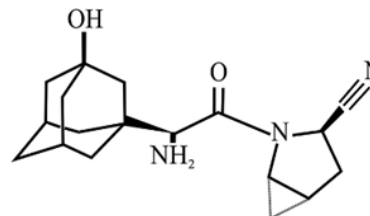


Fig.2 Saxagliptin Structure

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: C₁₈H₂₅N₃O₂

Molecular Weight: 315.41 g/mol

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 λ_{max} :

UV spectrum of 10 μ g per ml Ipragliflozine & saxagliptin diluents (MP ratio) was noted by examining in the scale of 200 to 400nm and the isobestic λ_{max} of both the drugs obtained at 254 nm.

Optimization of Column:

Intersil C18-EP (4.6 x 250mm, 5 μ m) 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,5 μ m)

MP ratio : ACN: KH₂PO₄ PH 5 (70:30ml)

Flow : 1ml/min

λ_{max} : 254 nanometers

volume Injected : 10 μ l

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 0.45 μ filter by vacuum filtration instrument.

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

System Suitability:

Tailing factor for Ipragliflozine & saxagliptin in Stad solution shouldn't > 2.0. For Standard solution Theoretical plates for the Ipragliflozine & Saxagliptin shouldn't < 2000. Calculation:(ForIpragliflozine 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 (Ipragliflozine 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 (Ipragliflozine 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 (Ipragliflozine 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 (Ipragliflozine 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 (Ipragliflozine 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 0.3ml from stock solutions into a 10ml VF & get up till the margin with Diluent. (60ppm Ipragliflozine, 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 0.3ml from solutions into a 10ml VF & get up till the margin with Diluent. (60ppm Ipragliflozine, 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. (60ppm Ipragliflozine, 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 & poured 75mg 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 recovery & Avg recovery values.

Limit of detection:

0.022µg/ml Ipragliflozine solution Preparation:

Precisely measure & poured 55 mg of Ipragliflozine 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 Ipragliflozine solution Preparation:

Precisely measure & poured 25 mg of Ipragliflozine 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 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 0.7ml from solution into a 10ml VF & get up till the margin using diluents. (0.07ppm)

0.075µg/ml saxagliptin solution Preparation:

Precisely measure & poured 25 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 upto the mark with 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 0.7ml from 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 ratios, 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 Ipragliflozine 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 Ipragliflozine and saxagliptin fosamprenavir was made and analyzed using the different MP ratios across with the real MP ratio in the method.

3. Results and Discussion

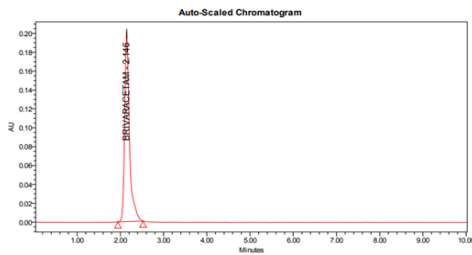


Figure 3: Chromatogram for system suitability

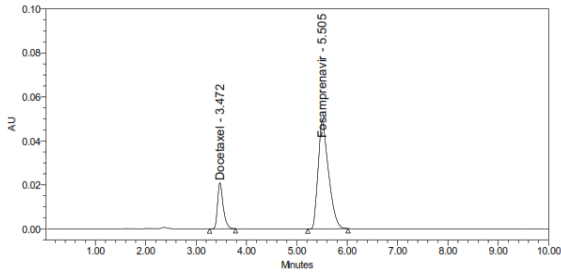


Figure 4: Standard Chromatogram

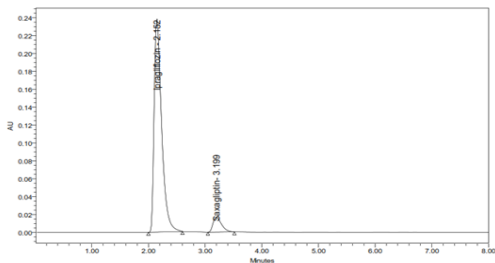


Figure 5: Sample Chromatogram

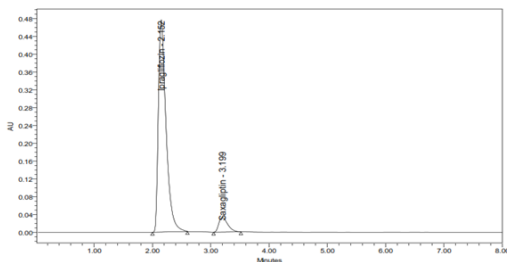


Figure 6: linearity-5 Chromatograph

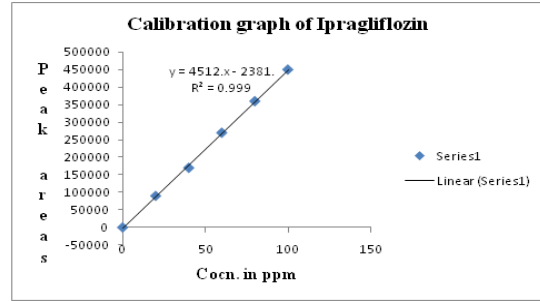


Figure 7: Calibration graph for Ipragliflozine

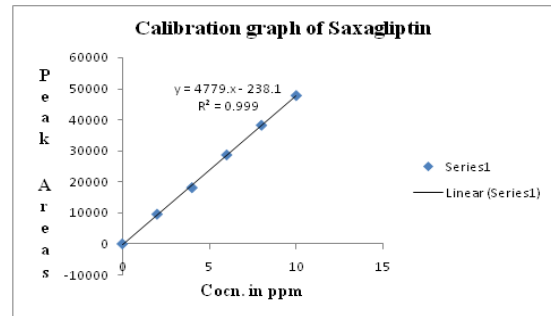


Figure 8: Calibration graph for saxagliptin

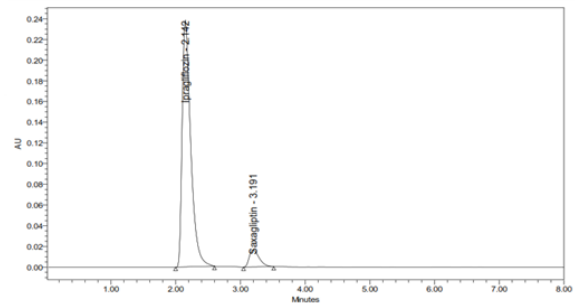


Figure 9: Precision Chromatogram

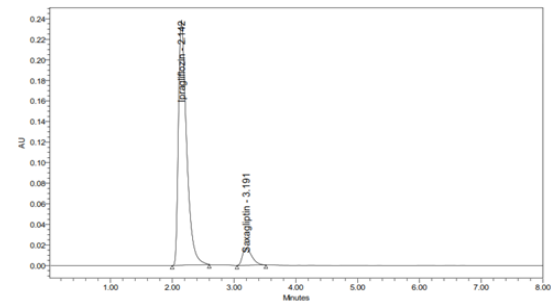


Figure 10: ID Precision Chromatogram

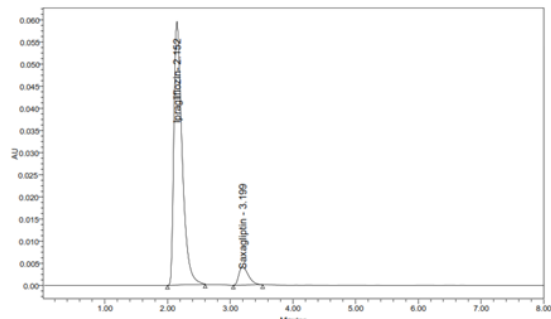


Figure 11: Accuracy 50% Chromatogram

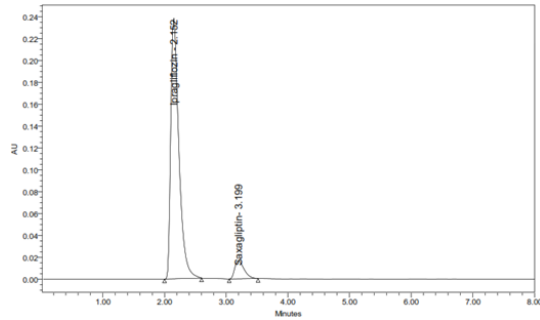


Figure 12: Accuracy 100% Chromatogram

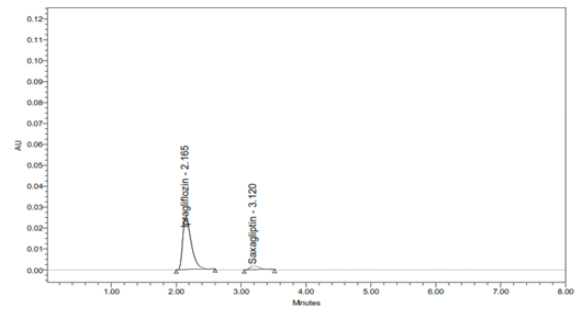


Figure 15: Ipragliflozine and saxagliptin depicting LOQ Chromatograph

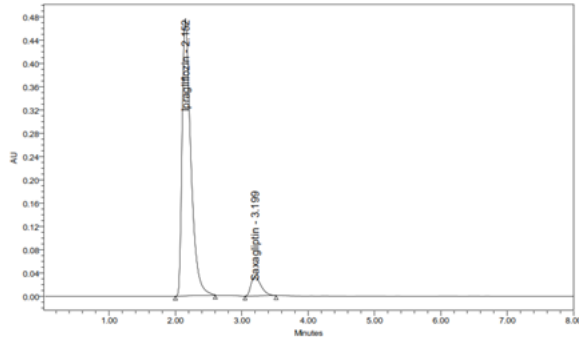


Figure 13: Accuracy 150% Chromatogram

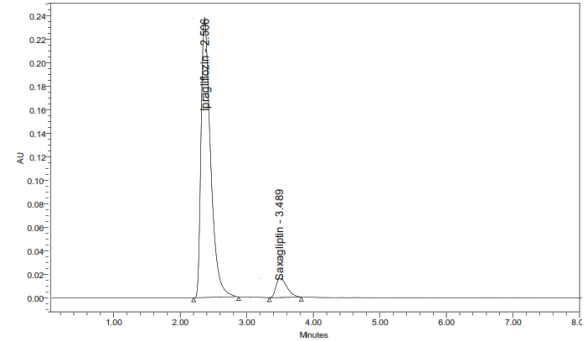


Figure 16: Less flow depicting Chromatograph

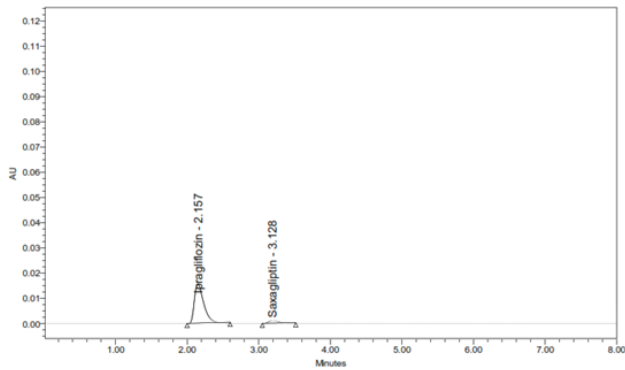


Figure 14: Ipragliflozine and saxagliptin depicting LOD Chromatograph

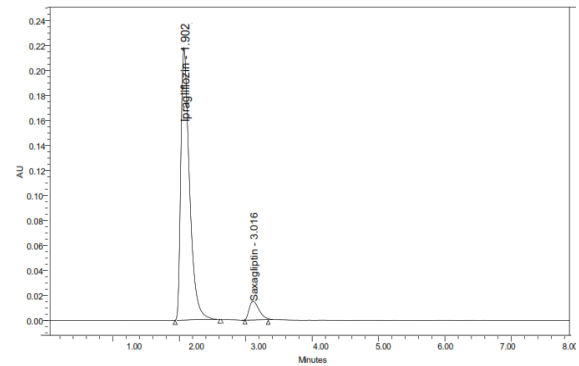


Figure 17: More flow depicting Chromatograph

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 Ipragliflozine and saxagliptin

| Injection | Ipragliflozine 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 Ipragliflozine and Saxagliptin

| Injection | Area's of Ipragliflozine | 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 Ipragliflozine & Saxagliptin

| % Conc. Ipragliflozine (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(μ V) | Signal attained(μ V) | Signal/Noise ratio | Conc. In ppm |
|----------------|--------------------------|---------------------------|--------------------|--------------|
| Ipragliflozine | 95 | 282 | 2.96 | 0.022 |
| saxagliptin | 95 | 260 | 2.94 | 0.020 |

Table 7: Results of LOQ

| Drug's name's | Baseline noise(μ V) | Signal attained(μ V) | S/N ratio | Conc. In ppm |
|----------------|--------------------------|---------------------------|-----------|--------------|
| Ipragliflozine | 95 | 945 | 9.99 | 0.076 |
| saxagliptin | 95 | 942 | 9.91 | 0.075 |

Table 8: Outcomes of difference in flow speeds for Ipragliflozine

| S.No | Flow (ml per min) | SST outcomes of Ipragliflozine | |
|------|-------------------|--------------------------------|-------------|
| | | 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.

5. References

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