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**R E S E A R C H A R T I C L E**

# Development of new simultaneous RP-HPLC method for the estimation of Dapagliflozin and Saxagliptin in tablet dosage form

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**A B S T R A C T**

The aim of present research work made to develop and validate RP-HPLC method for the simultaneous estimation of Dapagliflozin and Saxagliptin in bulk and combined dosage form. The optimized mobile phase was consists of 0.1% OPA: Methanol: Acetonitrile (30: 60: 10) and chromatographic separation was carried on Xterra C 18, column (4.6*150mm, 5µ). The detection of absorption maxima was monitored at 225nm. The flow rate was maintained at 1.0 ml/min. The linear concentration Dapagliflozin and Saxagliptin were found to be from 20-100 µg/ml and 10-50 µg/ml with regression coefficient was 0.999 for both drugs. The values of % RSD are less than 2% indicating accuracy and precision of the method. The mean percentage recovery was found to be 100.64% of Dapagliflozin and 100.36% of Saxagliptin. The proposed method is precise, simple and accurate to determine the amount of Dapagliflozin and Saxagliptinin formulation. So the method can be useful in the routine quality control of these drugs.

**Keywords:** Dapagliflozin and Saxagliptin, RP-HPLC, Mobile phase, Accuracy, Regression coefficient

**A R T I C L E I N F O**

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## 1. Introduction

Dapagliflozin is indicated for the management of diabetes mellitus type 2, and functions to improve glycemic control in adults when combined with diet and exercise. Dapagliflozin is a sodium-glucose co transporter 2 inhibitor, which prevents glucose reabsorption in the kidney. Using dapagliflozin leads to heavy glycosuria (glucose excretion in the urine), which can lead to weight loss and tiredness. Dapagliflozin was approved by the FDA on Jan 08, 2014. Dapagliflozin is not recommended for patients with type 1 diabetes mellitus or for the treatment of diabetic ketoacidosis[1].

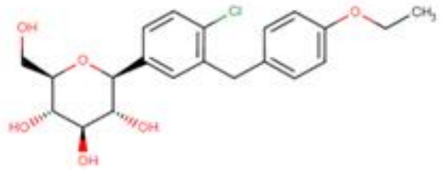

**Fig 1:** Chemical structure of Dapagliflozin

Saxagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor antidiabetic for the treatment of type 2 diabetes. DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones in the body called incretins. Incretins decrease blood sugar by increasing consumption of sugar by the body, mainly through increasing insulin production in the pancreas, and by reducing production of sugar by the liver. DPP-4 is a membrane associated peptidase which is found in many tissues, lymphocytes and plasma. DPP-4 has two main mechanisms of action, an enzymatic function and another mechanism where DPP-4 binds adenosine deaminase, which conveys intracellular signals via dimerization when activated. The inhibition of DPP-4 increases levels active of glucagon like peptide 1 (GLP-1), which inhibits glucagon production from pancreatic alpha cells and increases production of insulin from pancreatic beta cells[2].

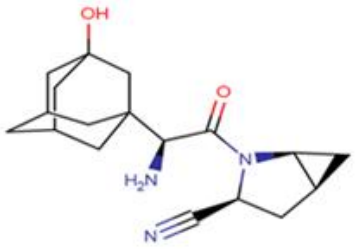

**Fig 2:** Chemical structure of Saxagliptin

Dapagliflozin and Saxagliptin existing drugs. Literature reveals different methods for their analysis in their formulations3,4,5. But our present plan is to develop a new, simple, precise& accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated.

## 2. Materials and Methods

**Instruments used:**

The following instruments are used to determination of Dapagliflozin and Saxagliptin.

**Table 1:**List of Instruments

| S. No | Instrument | Model |
|---|---|---|
| 1 | HPLC | WATERS, software: Empower, 2695 separation module.2487 UV detector. |
| 2 | UV/VIS spectrophotometer | LABINDIA UV 3000$^+$ |
| 3 | pH meter | Adwa – AD 1020 |
| 4 | Weighing machine | Afcoset ER-200A |

**Chemicals used:**

The following chemicals are used to determination of Dapagliflozin and Saxagliptin.

**Table 2:** List of Chemicals

| S. No | Chemical | Company Name |
|---|---|---|
| 1 | Saxagliptin | PHARMATRAIN |
| 2 | Dapagliflozin | PHARMATRAIN |
| 3 | $KH_2PO_4$ | FINER chemical LTD |
| 4 | Water and Methanol for HPLC | LICHROSOLV (MERCK) |
| 5 | Acetonitrile for HPLC | MOLYCHEM |
| 6 | Ortho phosphoric Acid | MERCK |

**Wave length selection:**

UV spectrum of 10 µg/ml Saxagliptin and 10 µg/ml Dapagliflozin in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 225 nm. At this wavelength both the drugs show good absorbance.

**HPLC Method Development**

**Preparation of mobile phase:**

Accurately measured 300 ml (30%) of 0.1% OPA Buffer, 600 ml (60%) of Methanol and 100 ml (10%) of Acetonitrile were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

**Standard Solution Preparation:**

Accurately weigh and transfer 10 mg of Saxagliptin and 20 mg of Dapagliflozin working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3.0 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

**Sample Solution Preparation:**

Accurately weigh and transfer equivalent to 10 mg of Saxagliptin and 20 mg of Dapagliflozin sample into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Saxagliptin and Dapagliflozin.

**Optimized Chromatographic Conditions**:

Instrument used **:** HPLC with auto sampler and UV detector.
Temperature **:**Ambient (25°C)
Mode of separation **:**Isocratic mode
Column **:** Xterra C 18, column (4.6*150mm, 5µ)
Buffer **:**0.1% OPA
Mobile phase **:**0.1% OPA: Methanol: Acetonitrile (30: 60: 10)
Flow rate **:** 1 ml per min
Wavelength **:**225 nm
Injection volume **:** 20 µl
Run time **:** 15 min.

**System Suitability:**

System suitability is an integral part of many analytical procedures. The system suitability parameters such as theoretical plates, tailing factor and resolution. Tailing factor for the peaks due to Saxagliptin and Dapagliflozin in Standard solution should not be more than 2.0. Theoretical plates should not be less than 2000. Resolution should not be less than 2.

**Method Validation**

Method validation was done for the according ICH guidelines Q2 (R1). The validation parameters like linearity, specificity, accuracy, precision, LOD & LOQ and robustness[11,12].

**Linearity:**

For determination of linearity five different concentrations i.e. 25%, 50%, 100%, 125%, 150% were prepared and injected in triplicate. Then plotting the graph concentration Vs peak area and measure the correlation coefficient. It should not more than 0.999.

**Precision:**

The standard and sample solutions were injected into the five times in intraday and inter day, the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak area.

**Accuracy:**

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same. Each solution was injected three times under optimized conditions and then calculates the mean percentage recovery.

**LOD & LOQ:**

The sensitivity of the proposed method for measurement of dapagliflozin and saxagliptin were estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ).The LOD and LOQ were calculated by using the slope and SD of response (intercept).The mean slope value and SD of response were obtained after plotting six calibration curves.

**Robustness:**

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

**The flow rate was varied at 0.9 ml/min to 1.1ml/min:**

Standard solution 30 ppm of Saxagliptin & 60 ppm of Dapagliflozin was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

**The Organic composition in the Mobile phase was varied from ±10%:**

Standard solution 30 ppm of Saxagliptin & 60 ppm of Dapagliflozin was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

## 3. Results and discussion

**Assay:**

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown in fig 3 & 4.

**Table 3:** Assay Results

| Drug | Label Claim (mg) | % Assay |
|---|---|---|
| Dapagliflozin | 10 | 100.53 |
| Saxagliptin | 5 | 100.10 |

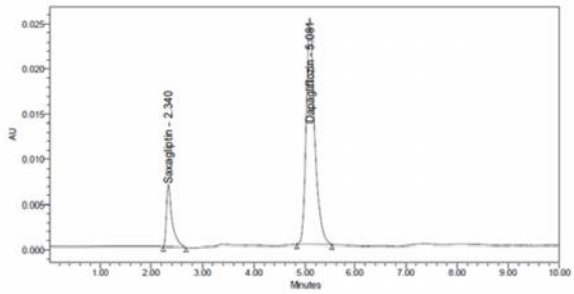


**Fig 3:** Chromatogram for Standard

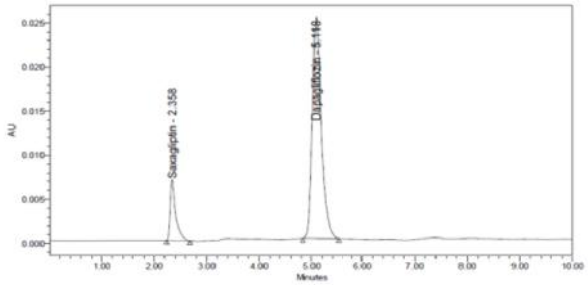


**Fig 4:** Chromatogram for Sample

**System Suitability:**

The system suitability of the method was checked by injecting five different preparations of the Dapagliflozin and Saxagliptin standard. The parameters of system suitability were checked. It was found from above data that all the system suitability parameters for developed method were within the limit. The results were shown in table 4.

**Linearity:**

The linearity range was found to lie from 20µg/ml to 100µg/ml of Dapagliflozin, 10µg/ml to 50µg/ml of Saxagliptin and chromatograms are shown in table 5.

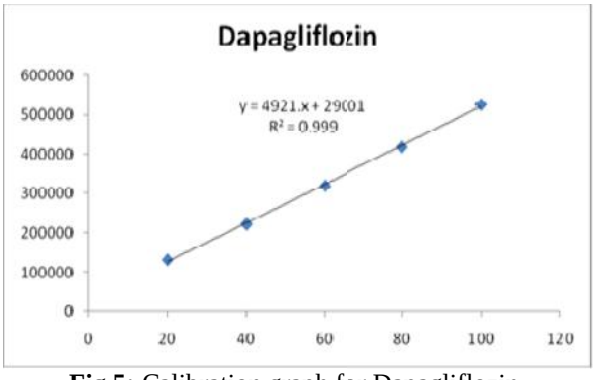


**Fig 5:** Calibration graph for Dapagliflozin

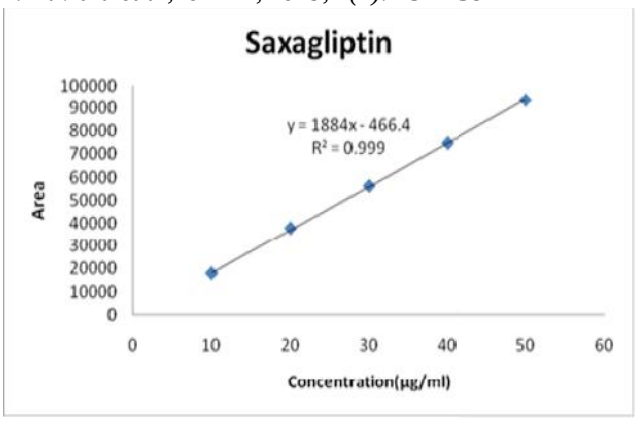


**Fig 6:** Calibration graph for Saxagliptin

**Precision:**

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results were reported in table 6.

**Accuracy:** Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated. The results are shown in table 7 & 8.

**Robustness:**

The standard and samples of Dapagliflozin and Saxagliptin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The results are shown in table 10 &11.

**Table 4:** Results of system suitability parameters

| S. No. | Name | RT (min) | Area (µV sec) | Height (µV) | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | Saxagliptin | 2.347 | 56445 | 6857 | 11.53 | 1.41 | 2593.29 |
| 2 | Dapagliflozin | 5.086 | 320903 | 25250 | | 1.18 | 4843.11 |

**Table 5:**Area of different concentration of Dapagliflozin and Saxagliptin

| S. No | Dapagliflozin | | Saxagliptin | |
|---|---|---|---|---|
| | Concentration (µg/ml) | Area | Concentration (µg/ml) | Area |
| 1 | 20 | 132359 | 10 | 17896 |
| 2 | 40 | 223105 | 20 | 37780 |
| 3 | 60 | 320315 | 30 | 56233 |
| 4 | 80 | 419173 | 40 | 74754 |
| 5 | 100 | 526461 | 50 | 93611 |
| Slope (m) | | 4921 | - | 1884 |
| Intercept (c) | | 29001 | - | 466.4 |
| Correlation coefficient ($R^2$) | | 0.999 | - | 0.999 |

**Table 6:** Results of Precision for Dapagliflozin and Saxagliptin

| Injection | Intraday precision | | Inter day precision | |
|---|---|---|---|---|
| | Area for Dapagliflozin | Area for Saxagliptin | Area for Dapagliflozin | Area for Saxagliptin |
| Injection-1 | 318752 | 56407 | 316450 | 56082 |
| Injection-2 | 316862 | 56050 | 318607 | 56734 |
| Injection-3 | 320903 | 56444 | 316347 | 56133 |
| Injection-4 | 315150 | 56445 | 319509 | 56124 |
| Injection-5 | 320979 | 56203 | 319175 | 56948 |
| Injection-6 | 316258 | 56139 | 317693 | 56919 |
| Average | 318150.7 | 56281.3 | 317963.5 | 56490.0 |
| Std Dev | 2457.0 | 172.6 | 1359.9 | 419.8 |
| %RSD | 0.8 | 0.3 | 0.4 | 0.7 |

**Table 7:**Accuracy (recovery) data for Dapagliflozin

| %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 161058.3 | 10 | 10.04 | 100.39 | 100.64% |
| 100% | 323719.3 | 20 | 20.18 | 100.89 | |
| 150% | 484374.0 | 30 | 30.19 | 100.64 | |

*Average of three determinations

**Table 8:** Accuracy (recovery) data for Saxagliptin

| %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 28244.7 | 5 | 5.01 | 100.26 | 100.36% |
| 100% | 56457.3 | 10 | 10.02 | 100.20 | |
| 150% | 85035.3 | 15 | 15.09 | 100.61 | |

**Table 9:** Results of LOD & LOQ

| Parameter | Drug name | Baseline noise (µV) | Signal obtained (µV) | S/N ratio |
|---|---|---|---|---|
| LOD | Dapagliflozin | 43 | 130 | 3.02 |
| | Saxagliptin | 43 | 129 | 3.00 |
| LOQ | Dapagliflozin | 43 | 433 | 10.07 |
| | Saxagliptin | 43 | 431 | 10.02 |

**Table 10:** Results for variation in flow for Saxagliptin and Dapagliflozin

| Drug | Flow Rate (ml/min) | System Suitability Results | | USP Resolution |
|---|---|---|---|---|
| | | USP Plate Count | USP Tailing | |
| Saxagliptin | 0.9 | 3067.03 | 1.29 | 12.44 |
| Dapagliflozin | | 5361.12 | 1.16 | |
| Saxagliptin | 1.0 | 2589.12 | 1.44 | 11.46 |
| Dapagliflozin | | 4825.77 | 1.19 | |
| Saxagliptin | 1.1 | 2526.15 | 1.27 | 11.48 |
| Dapagliflozin | | 4766.36 | 1.13 | |

**Table 11:** Results for variation in mobile phase composition for Saxagliptin and Dapagliflozin

| Drug | Change in Organic Composition in the Mobile Phase | System Suitability Results | | USP Resolution |
|---|---|---|---|---|
| | | USP Plate Count | USP Tailing | |
| Saxagliptin | 10% less | 3078.29 | 1.19 | 12.97 |
| Dapagliflozin | | 12.97 | 4573.25 | |
| Saxagliptin | *Actual | 2589.12 | 1.44 | 11.46 |
| Dapagliflozin | | 11.46 | 4825.77 | |
| Saxagliptin | 10% more | 2521.39 | 1.19 | 9.37 |
| Dapagliflozin | | 9.37 | 4756.36 | |

*****Results for actual Mobile phase composition have been consideredfrom Accuracy standard.

**Table 12:** Results for Stability of Dapagliflozin and Saxagliptin

| Sample Name | Dapagliflozin | | Saxagliptin | |
|---|---|---|---|---|
| | Area | % Degraded | Area | % Degraded |
| **Standard** | 320211.3 | | 56232.7 | |
| **Acid** | 295636 | 7.67 | 54275 | 3.48 |
| **Base** | 302783 | 5.44 | 52453 | 6.72 |
| **Peroxide** | 289767 | 9.51 | 53967 | 4.03 |
| **Thermal** | 316254 | 1.24 | 51867 | 7.76 |
| **Photo** | 286735 | 10.45 | 50162 | 10.80 |

## 4. Conclusions

The simultaneous estimation of Dapagliflozin and Saxagliptin was done by RP-HPLC.The assay of Dapagliflozin and Saxagliptin was performed with tablets and the % assay was found to be 100.53 and 100.10 which shows that the method is useful for routine analysis. The linearity was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The method show precision 0.8 and 0.3 and intermediate precision 0.4 and 0.7 for Dapagliflozin and Saxagliptin which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.64% and 100.36% for Dapagliflozin and Saxagliptin. The validation of developed

method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criterion for LOD and LOQ is 3 and 10.The LOD and LOQ for Dapagliflozin was found to be 3.02 and 10.07 and LOD and LOQ for Saxagliptin was found to be 3.00 and 10.02. The robustness limit for mobile phase variation and flow rate variation are well within the limit, the % degradation results are in limits which shows that the method is having good system suitability and precision under given set of conditions.

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