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# METHOD DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN BY RP-HPLC

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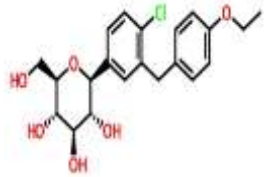
**ABSTRACT**

*For the coincident evaluation of Dapagliflozin and Saxagliptin in bulk form; Chromatography was run through Intersil-ODS $C_{18}$ column (250mm× 4.6mm, 5micron) Mobile phase containing Methanol: Water was pumped through the column in the ratio of 45: 55. The flow rate was 1ml/min. The temperature help was ambient i.e., upto30$^0$c. The optimized selected wavelength was 210nm. The retention time of Dapagliflozin and Saxagliptin was found to be 4.707min and6.68 min respectively. The %RSD of Dapagliflozin and Saxagliptin was found to be 0.031 and 0.036 respectively. The values of LOD and LOQ obtained from Dapagliflozin and Saxagliptin was 0.56, 1.69 and 0.57, 1.74 respectively. The retention time was decreased and the run time also decreased, so the method development was simple and economical that can be applied successfully for simultaneous estimation of combination of two anti- diabetic drugs; Dapagliflozin and Saxagliptin.*

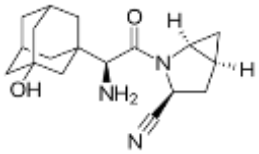
**KEY WORDS**: *Dapagliflozin and Saxagliptin, RP-HPLC.*

## INTRODUCTION

Method development and validation of Anti-diabetic drugs.i.e., Dapagliflozin and saxagliptin by Reverse phase-HPLC method by using Methanol and Acetonitrile as solvents. Dapagliflozin is an oral diabetics medicine that helps to control blood sugar levels, it helps the kidneys get rid of glucose from your bloodstream. It helps to treat type-2 diabetes along with diet and exercise. Saxagliptin is used as monotherapy or in combination to treat type-2 diabetes.




**Dapagliflozin**




**Saxagliptin**

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## MATERIALS AND METHODS

**Instruments-Instruments**

- HPLC –Waters Model NO.2690/5 series Compact System Consisting of Inertsil-C18 ODS column.
- Electronic balance (SARTORIOUS)
- Sonicator( FAST CLEAN)

**Substances containing chemicals**

- Methanol HPLC Grade.
- Buffer(KH2PO4)Hplc Grade.

**Raw Equipment(Unprocessed Materials)**

Dapagliflozin and Saxagliptin are working standards.

## RESULTS AND DISCUSSION.

**Method development:** Method development was done by changing various, mobile phase ratios, buffers et

**ADVANCED METHOD (OPTIMIZED METHOD)**

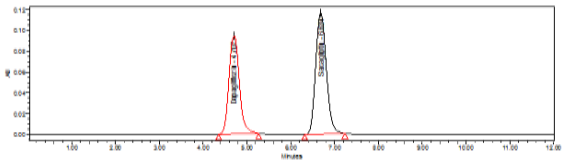
**Mobile Phase:** Degassed Methanol and Buffer in the ratio of 45:55 V/V.

**Preparation of pH 3.4 Phosphate buffer**: 2.7218g of KH2PO4 was weighed and transferred into a 1000ml beaker, later it was dissolved and diluted to 1000ml with HPLC water and the pH was adjusted to 3.4 with orthophosphoric acid.

**Chromatographic conditions that have been optimized**

| Parameters | Method |
|---|---|
| Stationary phase (column) | Inertsil -ODS $C_{18}$(250 x 4.6 mm, 5 µ) |
| Mobile Phase | Methanol : Buffer (45:55) |
| Flow rate (ml/min) | 1.0 ml/min |
| Run time (minutes) | 12 min |
| Column temperature (°C) | Ambient |
| Volume of injection loop (µl) | 20 |
| Detection wavelength (nm) | 210nm |
| Drug RT (min) | 4.707min for Dapagliflozin and 6.684 for Saxagliptin. |

**Standard chromatogram**



**Standard chromatogram**

**Inference: At RTs of 4.707 minutes for Dapagliflozin and 6.684 minutes for Saxagliptin, a chromatogram was obtained.**

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| S.NO | Name of the peak | Retention time(min) |
|---|---|---|
| 1 | **Dapagliflozin** | 4.707 |
| 2 | **Saxagliptin** | 6.684 |

**INFORMATION OF HIGH VALUE (VALIDATION DATA)**
**PRODUCTS FOR THE SYSTEM (SYSTEM SUITABILITY)**

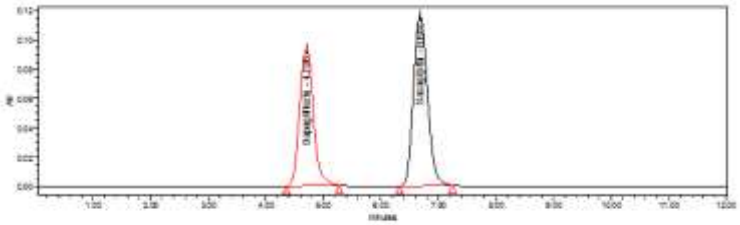
**TABLE- 1(a): Data on Dapagliflozin System Suitability**

| Injection | RT | Peak Area | USP Plate count | USP Tailing |
|---|---|---|---|---|
| 1 | 4.706 | 674753 | 10953.609752 | 1.153539 |
| 2 | 4.707 | 674261 | 10951.014286 | 1.155271 |
| Mean | 4.7078 | 678433.8 | 10768.34 | 1.155774 |
| SD | 0.001483 | 6031.135 | ------- | ------- |
| % RSD | 0.031506 | 0.888979 | ------- | ------- |

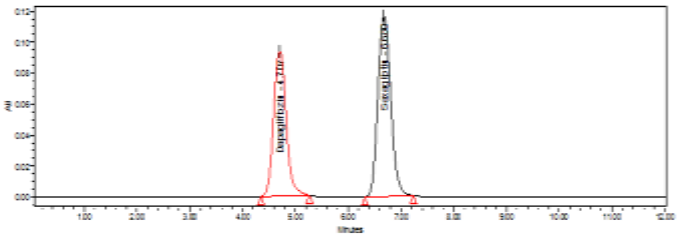
**TABLE-1(b): Data on Saxagliptin System Suitability**

| Injection | RT | Peak Area | USP Plate count | USP Tailing |
|---|---|---|---|---|
| 1 | 6.681 | 1218805 | 9478.317159 | 0.899633 |
| 2 | 6.680 | 1214014 | 9452.196217 | 0.893423 |
| Mean | 6.6826 | 1228593 | 9573.997 | 0.892407 |
| SD | 0.002408 | 122124.07 | ------- | ------- |
| % RSD | 0.036039 | 1.800764 | ------- | ------- |

**System suitability chromatograms (standards)**



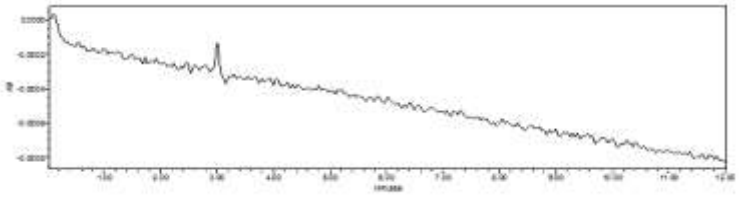
**Inference: Standard Chromatogram-1 System Suitability**



**Inference: Norm Chromatogram-2 device appropriateness**

**DESCRIPTION(SPECIFICITY):**

**Blank Chromatograph**



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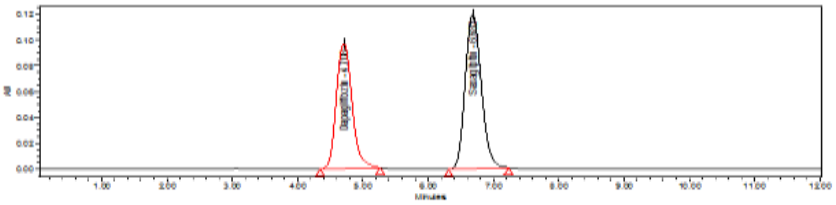
**Chromatogram Standard**



**Inference: For Dapagliflozin, a Rt of 4.708min was obtained, while for Saxagliptin, a Rt of 6.682min was obtained.**

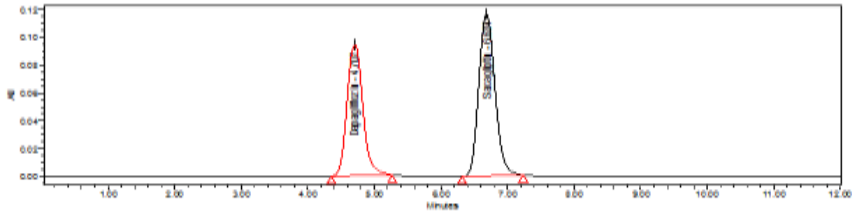
**TABLE-2(i): Data of Repeatability (System precision) forDapagliflozin**

| **Concentration 100ppm** | **Injection** | **Peak Areas of Dapagliflozin** | **%Assay** |
|---|---|---|---|
| | 1 | 674753 | 98.66 |
| | 2 | 674261 | 99.30 |
| Statistical**Analysis** | **Mean** | 678433.8 | 100.00 |
| | **SD** | 6031.135 | 1.107678 |
| | **% RSD** | 0.888979 | 1.10 |

**TABLE-2(ii): Information on Saxagliptin Reliability (System Precise)**

| **Concentration 100ppm** | **Injection** | **Peak Areas of Saxagliptin** | **%Assay** |
|---|---|---|---|
| | 1 | 1218805 | 99.95 |
| | 2 | 1214014 | 100.24 |
| *Statistical**Analysis*** | **Mean** | 1228593 | 99.91 |
| | **SD** | 22124.07 | 0.35819 |
| | **% RSD** | 1.800764 | 0.35 |

**Detailed chromatograms of systems**



**(standard-1)**

**(b)Method precision**

**TABLE-3(i): Data of Repeatability (Method precision) for Dapagliflozin**

| **Concentration 100ppm** | **Injection** | **Peak Areas of Dapagliflozin** | **%Assay** |
|---|---|---|---|
| | 1 | 633495 | 98.55 |
| | 2 | 635992 | 98.88 |
| *Statistical* **Analysis** | **Mean** | 637312 | 99.278 |
| | **SD** | 5988.879 | 0.827236 |
| | **% RSD** | 0.0891 | 0.83 |

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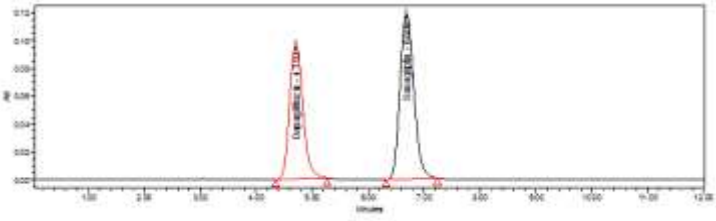
**TABLE-3(ii): Data of Repeatability (Method precision) for Saxagliptin**

| Concentration 100ppm | Injection | Peak Areas of Saxagliptin | %Assay |
|---|---|---|---|
| | 1 | 1202110 | 98.6 |
| | 2 | 1203700 | 99.02 |
| *Statistical* Analysis | Mean | 1202687.6 | 98.48 |
| | SD | 771.5483 | 0.352647 |
| | % RSD | 0.1358 | 0.35 |

**Repeatability chromosomes (Repeatable Chromatograms)**

**Inference: Chromatograph with high repeatability**

**(Standard-1)**



**Intermediate precision**

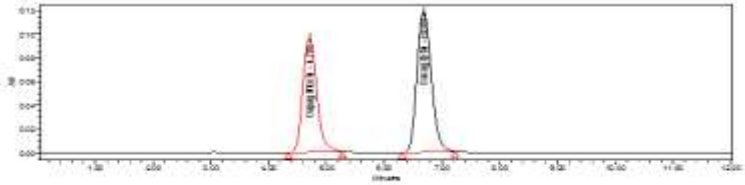
**Table4: Data of Intermediate precision (Analyst 2) for Dapagliflozin**

| Concentration 100ppm | Injection | Peak Areas of Dapagliflozin | %Assay |
|---|---|---|---|
| | 1 | 636792 | 99.99 |
| | 2 | 634360 | 99.66 |
| *Statistical* Analysis | Mean | 644607.8 | 100.37 |
| | SD | 6392.59 | 0.753536 |
| | % RSD | 1.183 | 0.75 |

**(ii)Specifications for Saxagliptin Intermediate (Analyst 2)**

| Concentration 100ppm | Injection | Peak Areas of Saxagliptin | %Assay |
|---|---|---|---|
| | 1 | 1205267 | 99.78 |
| | 2 | 1205625 | 99.95 |
| | 3 | 1205840 | 100.00 |
| *Statistical* Analysis | Mean | 1206333.5 | 100.19 |
| | SD | 12572.599 | 1.100898 |
| | % RSD | 1.24 | 1.09 |

**Chromatograms of Intermediate Precision:Inference: 1**



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**Resilience (ACCURACY)**

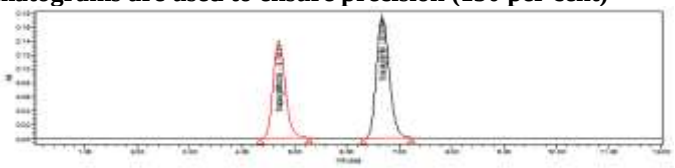
**(i)Dapagliflozin data with accuracy**

| Concentration % of spiked level | Amount added (ppm) | Amount found (ppm) | % Recovery | Statistical Analysis of % Recovery | |
|---|---|---|---|---|---|
| 150% Injection 1 | 60 | 60.12 | 100.21 | **MEAN** | 99.97333 |
| 150% Injection 2 | 60 | 59.76 | 99.61 | | |
| 150% Injection 3 | 60 | 60.06 | 100.10 | **%RSD** | 0.31 |

**(ii)Saxagliptin data with accuracy**

| Concentration % of spiked level | Amount added (ppm) | Amount found (ppm) | % Recovery | Statistical Analysis of % Recovery | |
|---|---|---|---|---|---|
| 150% Injection 1 | 60 | 60.08 | 100.14 | **MEAN** | 100.02 |
| 150% Injection 2 | 60 | 59.97 | 99.96 | | |
| 150% Injection 3 | 60 | 59.98 | 99.98 | **%RSD** | 0.09 |

**Chromatograms are used to ensure precision (150 per cent)**



**Inference: Standard 1 chromatogram**

**Variability (LINEARITY):**

**TABAL 6:Data of Linearity (Dapagliflozin)**

| Concentration (ppm) | Average Area | Statistical Analysis | |
|---|---|---|---|
| 0 | 0 | Slope | 18600 |
| 20 | 632546 | y-Intercept | 276.2 |
| 30 | 658296 | Correlation Coefficient | 1 |
| 40 | 694400 | | |
| 50 | 730308 | | |
| 60 | 916282 | | |
| 70 | 9402046 | | |

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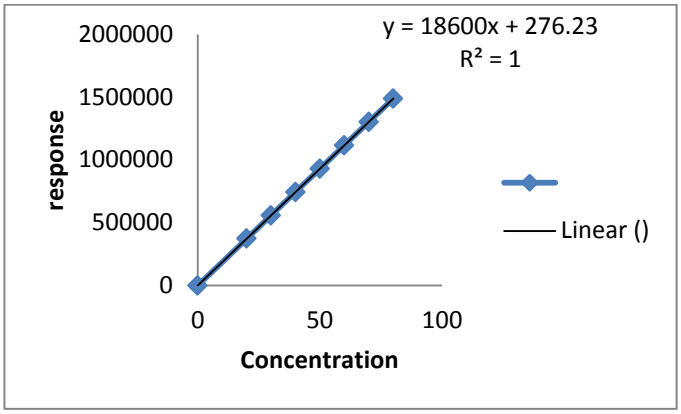
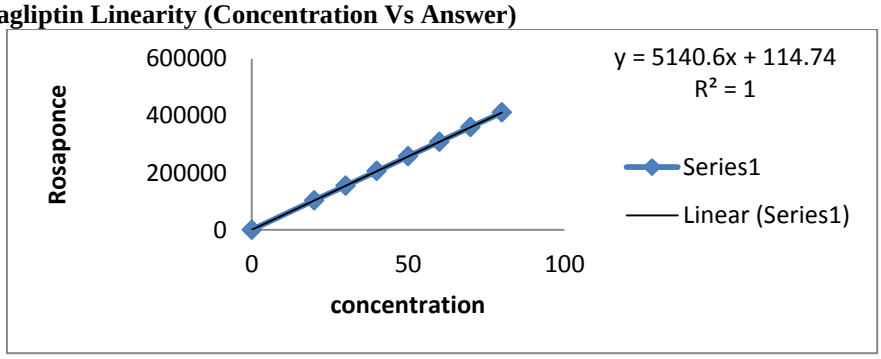
**(a) Dapagliflozin's Linearity Plot (concentration vs response)**



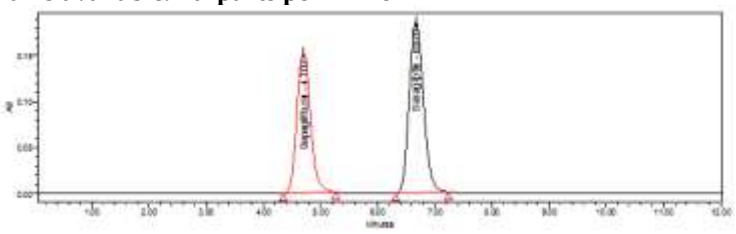

**Ii) Details on linearity (Saxagliptin),**

| Concentration (ppm) | Average Area | Statistical Analysis | |
|---|---|---|---|
| 0 | 0 | Slope | 5140 |
| 20 | 1202965 | y-Intercept | 114.7 |
| 30 | 1254371 | Correlation Coefficient | 1 |
| 40 | 1295856 | | |
| 50 | 1297167 | | |
| 60 | 1308577 | | |
| 70 | 1359903 | | |

**(b)Plot of Saxagliptin Linearity (Concentration Vs Answer)**




**There are chromatograms available. 70 parts per million**



**Inference: The standard chromatogram of 70 ppm**

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**TABLEMENT: 7**

**(i) Data on System Variability (Dapagliflozin)**

**(ii) System-2**

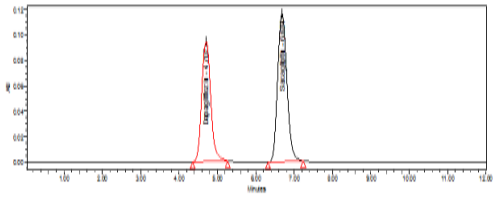
| S.NO: | Peak area | Assay % of Dapagliflozin |
|---|---|---|
| 1 | 634360 | 98.65 |
| 2 | 634098 | 98.63 |
| **Mean** | 634180.8 | 98.64 |
| **%RSD** | 0.019 | 0.12 |

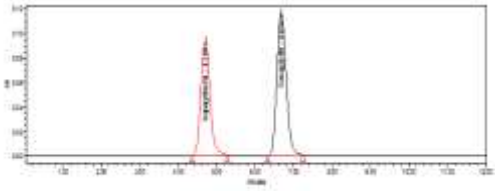
**Data on device variations (Saxagliptin)**

**System-2**

| S.NO: | Peak area | Assay % of Saxagliptin |
|---|---|---|
| 1 | 1203625 | 99.98 |
| 2 | 1202225 | 99.30 |
| **Mean** | 1203136.3 | 99.07667 |
| **%RSD** | 1.35 | 0.56 |

**Fig42-47,System to system variability chromatograms**





**Inference: std- 1** **Inference: std- 2**

**chromatogram showing system-to-system variability**

**Resiliency (Robustness):**

**TABLE: 8(i) There's proof that flux rate variability has an impact (Dapagliflozin):**

| Flow 0.8 ml | Std Area | Tailing factor | Flow 1.0 ml | Std Area | Tailing factor | Flow 1.2 ml | Std Area | Tailing factor |
|---|---|---|---|---|---|---|---|---|
| | 620286 | 1.322089 | | 634322 | 1.604878 | | 602077 | 1.285372 |
| | 619282 | 1.331920 | | 635792 | 1.584354 | | 601854 | 1.319385 |
| | 621337 | 1.296438 | | 634360 | 1.543805 | | 602403 | 1.292055 |
| | 620456 | 1.315454 | | 635696 | 1.568590 | | 603421 | 1.304561 |
| | 620765 | 1.326551 | | 633147 | 1.559986 | | 602465 | 1.294621 |
| **Avg** | 620425 | 1.31849 | **Avg** | 634663.4 | 1.572323 | **Avg** | 602444 | 1.299199 |
| **SD** | 754.0018 | 0.013728 | **SD** | 1100.917 | 0.023367 | **SD** | 599.8833 | 0.013223 |
| **%RSD** | 0.086 | 1.04 | **%RSD** | 0.184 | 1.48 | **%RSD** | 0.09 | 1.01 |

**TABLE: 8(ii) Flow rate shift impact data (Saxagliptin)**

| Flow 0.8 ml | Std Area | Tailing factor | Flow 1.0 ml | Std Area | Tailing factor | Flow 1.2 ml | Std Area | Tailing factor |
|---|---|---|---|---|---|---|---|---|
| | 1273707 | 1.362089 | | 1206349 | 1.280574 | | 1266195 | 1.285372 |
| | 1273211 | 1.352617 | | 1205267 | 1.279932 | | 1265885 | 1.299385 |
| | 1273948 | 1.376926 | | 1205625 | 1.261721 | | 1266303 | 1.308063 |
| | 1273465 | 1.345752 | | 1205840 | 1.276089 | | 1267243 | 1.274662 |
| | 1273862 | 1.374925 | | 1205735 | 1.250640 | | 1265762 | 1.267630 |
| **Avg** | 1273638.6 | 1.362462 | **Avg** | 1205763.2 | 1.269791 | **Avg** | 166277.6 | 1.287022 |
| **SD** | 3301.369 | 0.013609 | **SD** | 392.1635 | 0.01314 | **SD** | 582.9758 | 0.016786 |
| **%RSD** | 1.041 | 0.99 | **%RSD** | 0.19 | 1.03 | **%RSD** | 0.35 | 1.3 |

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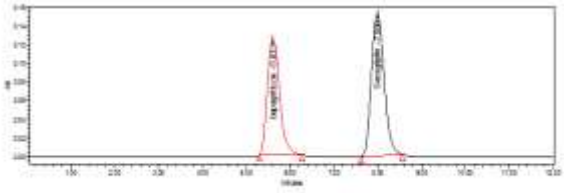
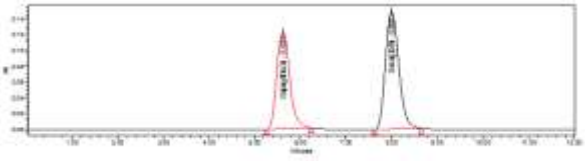
**Fig48-49, Robustness chromatograms**

**a) Variation in flow rate (for 0.8 ml/min flow) has an effect.**



**b) Inference: Standard for robustness chromatogram - 1**



**Inference: Standard for robustness chromatogram - 2**

## SUMMARY AND CONCLUSION

**Summary Table**

| **Parameters** | **Dapagliflozin** | Saxagliptin | **LIMIT** |
|---|---|---|---|
| Regressioncoefficient | 0.999 | 0.999 | R<1 |
| Slope(m) | 18599.8434 | 5140 | |
| Intercept(c) | 276.2281 | 114.73 | |
| Regression equation (Y=mx+c) | y =18599.8434x + 276.2281 | y = 5140x + 114.73 | |
| **Assay(% mean assay)** | 98.64% | 99.07% | 90-110% |
| **Specificity** | Specific | Specific | No interference of any peak |
| **System precision %RSD** | 0.889 | 1.800 | NMT 2.0% |
| **Method precision %RSD** | 0.0891 | 0.1358 | NMT 2.0% |
| **Accuracy % recovery** | 99.78% | 99.80% | 98-102% |
| **LOD** | 0.56 | 1.69 | NMT 3 |
| **LOQ** | 0.57 | 1.74 | NMT 10 |

## CONCLUSION

Chromatography was run through Intersil-ODS $C_{18}$ column (250mm× 4.6mm, 5micron) Mobile phase containing Methanol: Water was pumped through the column in the ratio of 45: 55. The flow rate was 1ml/min. The temperature help was ambient i.e., upto$30^0$c. The optimized selected wavelength was 210nm. The retention time of Dapagliflozin and Saxagliptin was found to be 4.707min and6.68 min respectively. The %RSD of Dapagliflozin and Saxagliptin was found to be 0.031 and 0.036 respectively. The values of LOD and LOQ obtained from Dapagliflozin and Saxagliptin was 0.56, 1.69 and 0.57, 1.74 respectively. The retention time was decreased and the run time also decreased, so the method development was simple and economical that can be applied successfully for simultaneous estimation of combination of two anti- diabetic drugs; Dapagliflozin and Saxagliptin.

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