



Research Article

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A Highly Validated RP-HPLC Method Development for the Simultaneous Estimation of Dapagliflozin and Saxagliptin in Tablet Dosage Forms

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of Dapagliflozin and Saxagliptin in Tablet dosage form. Chromatogram was run through Standard BDS C8 column (50 × 4.6 mm, 5 μ) The Mobile phase containing Potassium dihydrogen phosphate: Acetonitrile in the ratio 55:45, pH was adjusted to 3.8 with dilute orthophosphoric acid. The solution was pumped through the column at a flow rate of 1 ml/min. The column temperature was maintained at 30°C. Optimized wavelength selected was 210 nm. Retention time of Dapagliflozin and Saxagliptin were found to be 2.266 min and 2.805 min. % RSD of the Dapagliflozin and Saxagliptin were found to be 0.5 and 0.5 respectively. % Recovery was obtained as 98.98% and 98.72% for Dapagliflozin and Saxagliptin respectively. LOD, LOQ values obtained from regression equations of Dapagliflozin and Saxagliptin were 0.20, 0.60 and 0.26, 0.79 respectively. Regression equation of Dapagliflozin is $y = 37377x + 89244$, and $y = 12254x + 3122$ of Saxagliptin. The retention times were decreased so that the run time also decreased. So the method developed was simple and economical that can be applied successfully for simultaneous estimation of both Dapagliflozin and Saxagliptin in bulk and combined tablet formulation.

Keywords: Dapagliflozin, Saxagliptin, RP-HPLC, Validation, Simultaneous estimation.

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INTRODUCTION

Dapagliflozin is a sodium-glucose co-transporter 2 (SGLT2) inhibitor used as an adjunct to diet and

exercise to improve glycemic control in adults with type 2 diabetes mellitus. Dapagliflozin is chemically called as (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-(4-

ethoxybenzyl) phenyl]-6-(hydroxymethyl) tetrahydro-2H pyran-3, 4, 5-triol as shown in (figure 1). Common side effects include yeast infections of the vagina or penis, urinary tract infections, and changes in urination, including urgent need to urinate more often, in larger amounts, or at night. [1-9]

Dapagliflozin inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2), which is responsible for at least 90% of the glucose reabsorption in the kidney. Blocking this transporter causes blood glucose to be eliminated through the urine. The efficacy of this medication class has yet to be determined, but in initial clinical trials, Dapagliflozin lowers HbA_{1c} by 0.90 percentage points when added to metformin. [1-9]

Saxagliptin, is a new oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Saxagliptin is chemically called as (1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile as shown in (figure 2). [1, 10, 11]

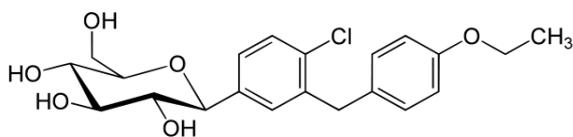


Fig. 1: Structure of Dapagliflozin

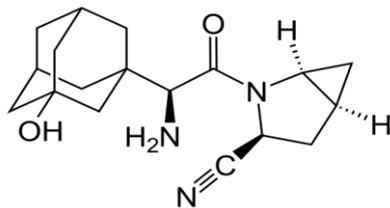


Fig. 2: Structure of Saxagliptin

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. [Bristol-Myers Squibb Press Release] 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. Saxagliptin forms a reversible, histidine-assisted covalent bond between its nitrile group and the S630 hydroxyl oxygen on DPP-4. 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. [1, 10, 11]

The literature review revealed that several analytical methods have been reported for Dapagliflozin and

Saxagliptin in UV-Spectrophotometry, RP-HPLC, individually and in combination. This research work implicates the simultaneous estimation of Dapagliflozin and Saxagliptin by RP-HPLC in tablet dosage forms. This present study reports simultaneous estimation of Dapagliflozin and Saxagliptin by RP-HPLC in tablet dosage form. [12-18]

MATERIALS AND METHODS

Materials

Dapagliflozin and Saxagliptin pure drugs (API), Combination Dapagliflozin and Saxagliptin tablets (QTERN), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents were obtained from Rankem.

Instruments

Electronics Balance-Denver, pH meter-BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10 mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Dapagliflozin and Saxagliptin solutions.

Methods

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard stock solutions: Accurately weighed 10 mg of Dapagliflozin, 5 mg of Saxagliptin and transferred to individual 10 ml volumetric flasks separately. 3/4th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2 (1000µg/ml of Dapagliflozin and 500µg/ml of Saxagliptin).

Preparation of Standard working solutions (100% solution): 1 ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent (100µg/ml Dapagliflozin of and 50µg/ml of Saxagliptin).

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 5 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000µg/ml of Dapagliflozin and 500µg/ml of Saxagliptin).

Preparation of Sample working solutions (100% solution): 1 ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent (100µg/ml of Dapagliflozin and 50µg/ml of Saxagliptin).

Preparation of buffer

0.01N KH₂PO₄ Buffer: Accurately weighed 1.36 g of Potassium dihydrogen phosphate in a 1000 ml of

Volumetric flask add about 900 ml of milli-Q water added and degassed to sonicate and finally make up the volume with water then 1 ml of Triethylamine was

added and then pH was adjusted to 3.8 with dil. Orthophosphoric acid solution.

Method development

Method development was done by changing various, mobile phase ratios, buffers etc.

| Parameters | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 | Optimized Method |
|----------------------|---|---|--|---|---|---|
| Mobile phase | Water and Methanol (50:50) | Water: Acetonitrile (50:50) | 0.01N KH ₂ PO ₄ (4.8):Acetonitrile (50:50) | 60% OPA buffer:40% Acetonitrile | 55% 0.1% OPA buffer:45% Acetonitrile | 55% buffer: 45% Acetonitrile |
| Flow rate | 1 ml/min | 1 ml/min | 1 ml/min | 1 ml/min | 1 ml/min | 1 ml/min |
| Column | Discovery C18 (4.6 × 250 mm, 5µm) | Discovery C18 (4.6 × 250 mm, 5µm) | Discovery C18 (4.6 × 250 mm, 5µm) | ODS C18 (4.6 × 250 mm, 5µm) | BDSC18 (4.6 × 150 mm, 5µm) | BDSC8 (4.6 × 150 mm, 5µm) |
| Detector wave length | 210 nm | 210 nm | 210 nm | 210 nm | 210 nm | 210 nm |
| Column Temp | 30°C | 30°C | 30°C | 30°C | 30°C | 30°C |
| Injection volume | 10µL | 10µL | 10µL | 10µL | 10µL | 10µL |
| Run time | 8.0 min | 14 min | 10 min | 10 min | 10 min | 6 min |
| Diluent | Water and Acetonitrile in the ratio 50:50 | Water and Acetonitrile in the ratio 50:50 | Water and Acetonitrile in the ratio 50:50 | Water and Acetonitrile in the ratio 50:50 | Water and Acetonitrile in the ratio 50:50 | Water and Acetonitrile in the ratio 50:50 |

Optimization of chromatographic conditions

| Trial | Results |
|------------------|--|
| Trial 1 | Dapagliflozin was not eluted, so further trial was carried out. |
| Trial 2 | Both peaks were eluted but peak shapes are not good So, Further trial was carried out. |
| Trial 3 | Dapagliflozin Saxagliptin both peak are eluted but Saxagliptin peak having tailing, Dapagliflozin having lees plate count and fronting so, further trial was carried out. |
| Trial 4 | Dapagliflozin & Saxagliptin both peak are eluted but Dapagliflozin was eluted at void time, peak shapes were not good and base line is not good so, further trial was carried out. |
| Trial 5 | Both peaks were eluted, but both peaks having fronting So further trial was carried out. |
| Optimized method | Both peaks have good resolution, tailing Factor, theoretical plate count and resolution. |

RESULTS AND DISCUSSION

Observation: Dapagliflozin and Saxagliptin were eluted at 2.266 min and 2.805 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated (Fig. 3).

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines [12] (Table 1 and Fig. 4).

Linearity: Six linear concentrations of Dapagliflozin (25-150µg/ml) and Saxagliptin (12.5-75µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Dapagliflozin was $y = 37377x + 89244$ and of Saxagliptin was $y = 12254x + 3122$ Correlation coefficient obtained was 0.999 for the two drugs (Table 2 and Fig. 5 & 6).

Precision: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.6% and 0.2% respectively for Dapagliflozin and Saxagliptin (Table 3).

System Precision: Table 3.

Repeatability: Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.5% respectively for Dapagliflozin and Saxagliptin (Table 4).

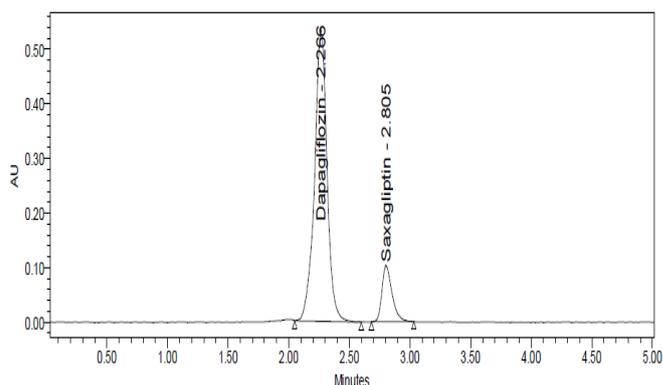


Fig. 3: Optimized chromatogram

Table 1: System suitability parameters for Dapagliflozin and Saxagliptin

| S. No | Dapagliflozin | | | Saxagliptin | | | | |
|-------|---------------|---------|-----------------|-------------|---------|-----------------|---------|------------|
| | Inj | RT(min) | USP Plate Count | Tailing | RT(min) | USP Plate Count | Tailing | Resolution |
| 1 | | 2.257 | 2619 | 0.98 | 2.802 | 5618 | 1.33 | 1.6 |
| 2 | | 2.261 | 2727 | 0.97 | 2.803 | 5730 | 1.34 | 1.6 |
| 3 | | 2.266 | 2751 | 0.97 | 2.803 | 5286 | 1.39 | 1.7 |
| 4 | | 2.270 | 2598 | 0.96 | 2.803 | 4994 | 1.41 | 1.7 |
| 5 | | 2.272 | 2561 | 1.03 | 2.804 | 5144 | 1.31 | 1.6 |
| 6 | | 2.275 | 2530 | 1.01 | 2.805 | 5772 | 1.37 | 1.6 |

Table 2: Linearity table for Dapagliflozin and Saxagliptin

| Dapagliflozin | | Saxagliptin | |
|---------------|-----------|--------------|-----------|
| Conc (µg/mL) | Peak area | Conc (µg/mL) | Peak area |
| 0 | 0 | 0 | 0 |
| 25 | 1051797 | 12.5 | 154760 |
| 50 | 2031439 | 25 | 306194 |
| 75 | 2942756 | 37.5 | 473244 |
| 100 | 3811209 | 50 | 618538 |
| 125 | 4743235 | 62.5 | 771469 |
| 150 | 5666991 | 75 | 914419 |

Table 3: System precision table of Dapagliflozin and Saxagliptin

| S. No | Area of Dapagliflozin | Area of Saxagliptin |
|-------|-----------------------|---------------------|
| 1. | 3824237 | 616187 |
| 2. | 3867517 | 614797 |
| 3. | 3842883 | 614559 |
| 4. | 3871852 | 615571 |
| 5. | 3809148 | 617990 |
| 6. | 3837088 | 615398 |
| Mean | 3842121 | 615750 |
| S.D | 24338.7 | 1240.3 |
| %RSD | 0.6 | 0.2 |

Table 4: Repeatability table of Dapagliflozin and Saxagliptin

| S. No | Area of Dapagliflozin | Area of Saxagliptin |
|-------|-----------------------|---------------------|
| 1. | 3809246 | 611715 |
| 2. | 3827051 | 613026 |
| 3. | 3855433 | 615976 |
| 4. | 3823260 | 616734 |
| 5. | 3812089 | 618397 |
| 6. | 3855001 | 611720 |
| Mean | 3830347 | 614595 |
| S.D | 20380.5 | 2827.0 |
| %RSD | 0.5 | 0.5 |

Table 5: Intermediate precision table of Dapagliflozin and Saxagliptin

| S. No | Area of Dapagliflozin | Area of Saxagliptin |
|-------|-----------------------|---------------------|
| 1. | 3929409 | 1088672 |
| 2. | 3904203 | 1095698 |
| 3. | 3925132 | 1099574 |
| 4. | 3914937 | 1095548 |
| 5. | 3926559 | 1097245 |
| 6. | 3926559 | 1101211 |
| Mean | 3921133 | 1096325 |
| S.D | 9678.7 | 4355.2 |
| %RSD | 0.2 | 0.4 |

Intermediate precision: Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.2% and 0.4% respectively for Dapagliflozin and Saxagliptin (Table 5).

Accuracy: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 98.98% and 98.72% for Dapagliflozin and Saxagliptin respectively (Table 6 & 7, Fig. 7, 8 & 9).

Sensitivity: Table 8.

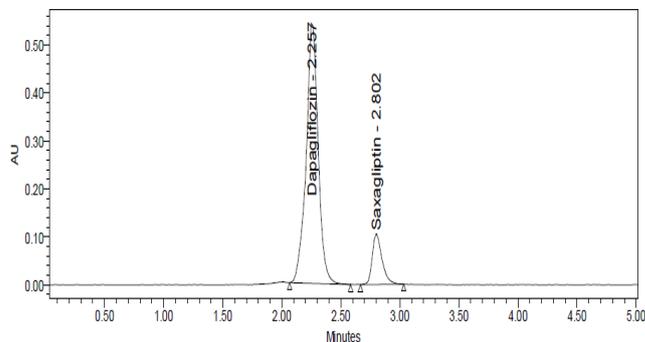


Fig. 4: System suitability chromatogram

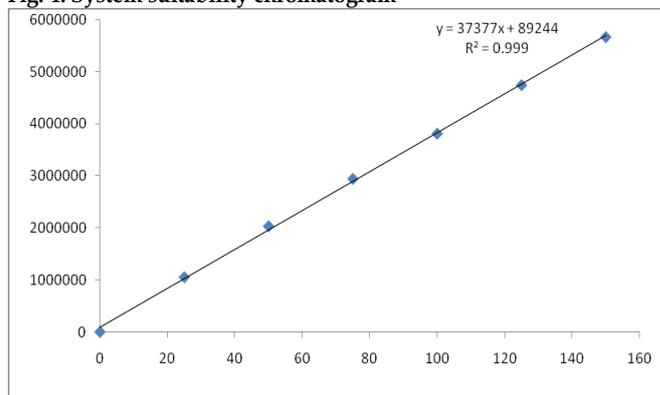


Fig. 5: Calibration curve of Dapagliflozin

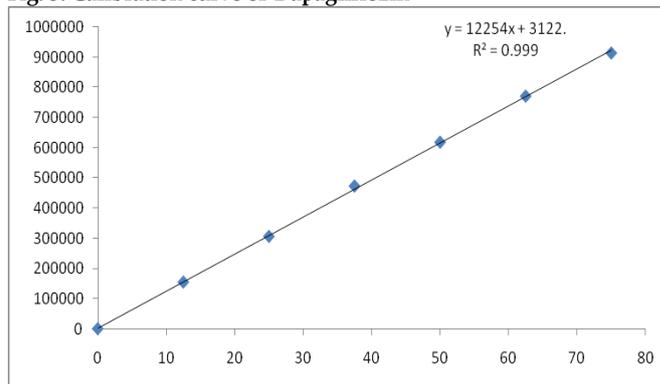


Fig. 6: Calibration curve of Saxagliptin

Table 6: Accuracy table of Dapagliflozin

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---------|-----------------------|--------------------------|------------|----------------|
| 50% | 50 | 49.4969366 | 98.99 | 98.98% |
| | 50 | 49.1189769 | 98.24 | |
| | 50 | 49.2521337 | 98.50 | |
| 100% | 100 | 99.2575648 | 99.26 | |
| | 100 | 99.6980228 | 99.70 | |
| | 100 | 99.8285844 | 99.83 | |
| 150% | 150 | 148.790138 | 99.19 | |
| | 150 | 148.622308 | 99.08 | |
| | 150 | 147.029296 | 98.02 | |

Table 7: Accuracy table of Saxagliptin

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---------|-----------------------|--------------------------|------------|----------------|
| 50% | 25 | 24.6866329 | 98.75 | 98.72% |
| | 25 | 24.7202546 | 98.88 | |
| | 25 | 24.5829933 | 98.33 | |
| 100% | 50 | 49.0874816 | 98.17 | |
| | 50 | 49.1721071 | 98.34 | |
| | 50 | 49.61433 | 99.23 | |
| 150% | 75 | 73.9643382 | 98.62 | |
| | 75 | 74.185164 | 98.91 | |
| | 75 | 74.4015016 | 99.20 | |

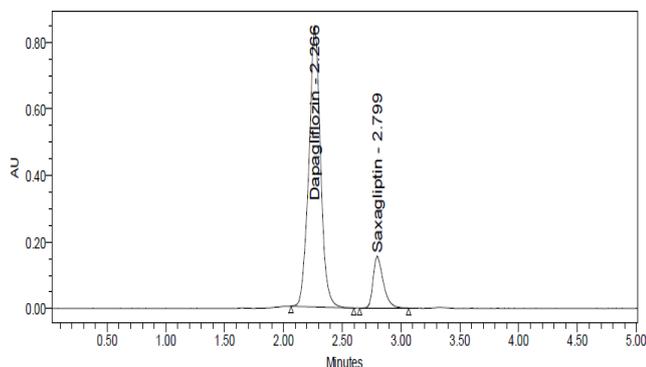


Fig. 7: Accuracy 50% chromatogram of Dapagliflozin and Saxagliptin

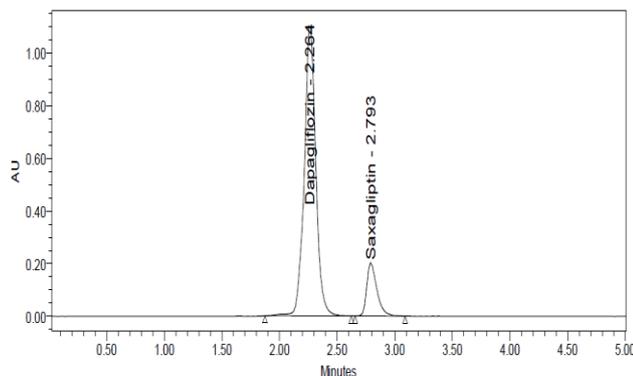


Fig. 8: Accuracy 100% chromatogram of Dapagliflozin and Saxagliptin

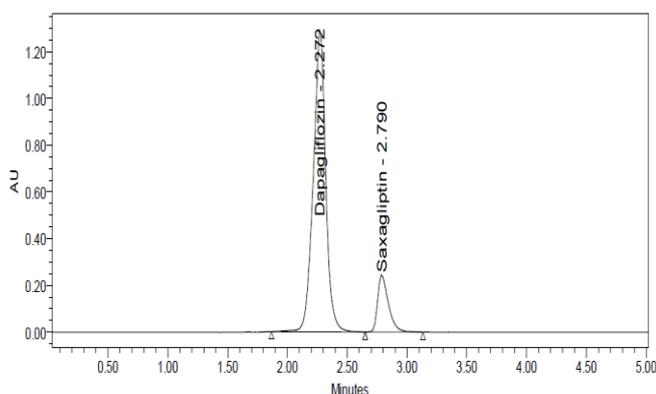


Fig. 9: Accuracy 150% chromatogram of Dapagliflozin and Saxagliptin

Table 8: Sensitivity table of Dapagliflozin and Saxagliptin

| Molecule | LOD | LOQ |
|---------------|------|------|
| Dapagliflozin | 0.20 | 0.60 |
| Saxagliptin | 0.26 | 0.79 |

Table 9: Robustness data for Dapagliflozin and Saxagliptin

| S. No | Condition | %RSD of Dapagliflozin | %RSD of Saxagliptin |
|-------|--------------------------|-----------------------|---------------------|
| 1 | Flow rate (-) 0.9 ml/min | 1.4 | 0.5 |
| 2 | Flow rate (+) 1.1 ml/min | 1.6 | 1.6 |

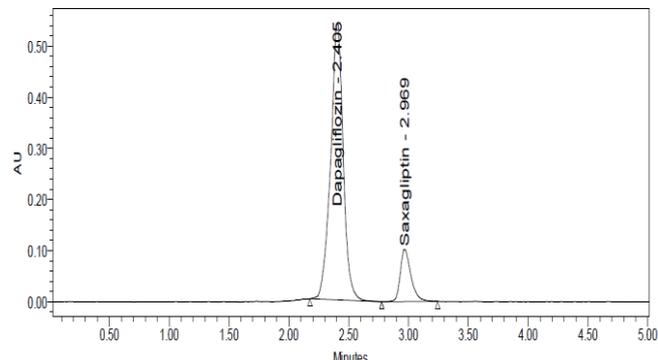


Fig. 10: Flow minus chromatogram of Dapagliflozin and Saxagliptin

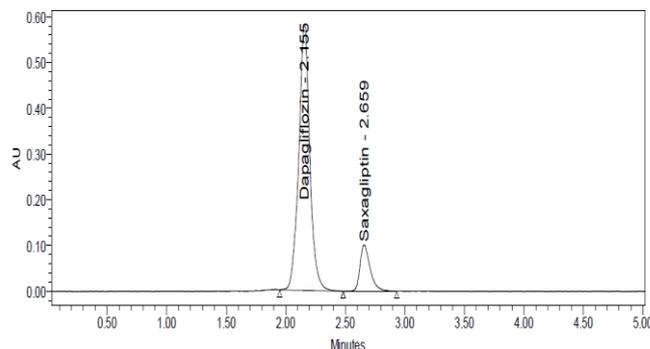


Fig. 11: Flow plus chromatogram of Dapagliflozin and Saxagliptin

Table 10: Assay data of Dapagliflozin

| S. No | Standard Area | Sample area | % Assay |
|--------|---------------|-------------|---------|
| 1 | 3824237 | 3809246 | 99.05 |
| 2 | 3867517 | 3827051 | 99.51 |
| 3 | 3842883 | 3855433 | 100.25 |
| 4 | 3871852 | 3823260 | 99.41 |
| 5 | 3809148 | 3812089 | 99.12 |
| 6 | 3837088 | 3855001 | 100.23 |
| Avg | 3842121 | 3830347 | 99.59 |
| St dev | 24338.7 | 20380.5 | 0.5299 |
| %RSD | 0.6 | 0.5 | 0.5 |

Table 11: Assay data of Saxagliptin

| S. No | Standard Area | Sample area | % Assay |
|--------|---------------|-------------|---------|
| 1 | 616187 | 611715 | 99.25 |
| 2 | 614797 | 613026 | 99.46 |
| 3 | 614559 | 615976 | 99.94 |
| 4 | 615571 | 616734 | 100.06 |
| 5 | 617990 | 618397 | 100.33 |
| 6 | 615398 | 611720 | 99.25 |
| Avg | 615750 | 614595 | 99.71 |
| St dev | 1240.3 | 2827.0 | 0.46 |
| %RSD | 0.2 | 0.5 | 0.46 |

Robustness: Robustness conditions like Flow minus (0.9 ml/min), Flow plus (1.1 ml/min), was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table 9, Fig. 10 & 11).

Assay: AstraZeneca pharmaceuticals (Qtern), bearing the label claim Dapagliflozin 10mg, Saxagliptin 5mg. Assay was performed with the above formulation. Average % Assay for Dapagliflozin and Saxagliptin obtained was 99.59% and 99.71% respectively (Table 10 & 11).

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. Regarding the pH adjustment in mobile phase for the acid and base degradation studies have movement in retention time of drugs. But due to neutralized acid sample with 2N Base solution and base sample with 2N Acid solution there will be no change in retention time (Table 12 & 13, Fig. 12, 13, 14 & 15).

Table 12: Degradation data of Dapagliflozin

| S. No | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|-------|-----------------------|-----------------|--------------|------------------|
| 1 | Acid | 4.71 | 0.110 | 0.295 |
| 2 | Alkali | 2.63 | 0.110 | 0.295 |
| 3 | Oxidation | 1.91 | 0.286 | 0.619 |
| 4 | Thermal | 0.87 | 0.286 | 0.315 |

Table 13: Degradation data of Saxagliptin

| S. No | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|-------|-----------------------|-----------------|--------------|------------------|
| 1 | Acid | 4.98 | 0.259 | 0.971 |
| 2 | Alkali | 2.69 | 0.060 | 0.939 |
| 3 | Oxidation | 1.85 | 0.619 | 0.747 |
| 4 | Thermal | 0.54 | 0.619 | 0.747 |

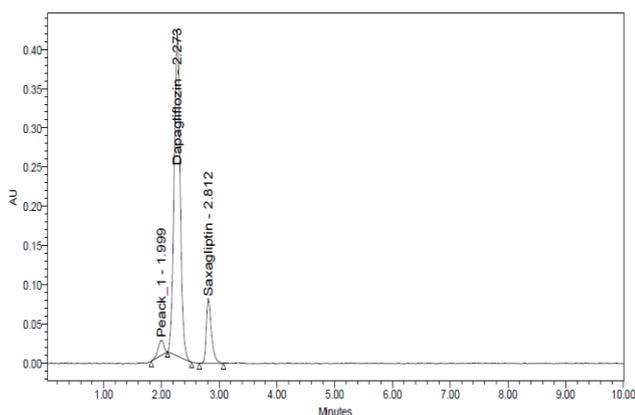


Fig. 12: Acid chromatogram of Dapagliflozin and Saxagliptin

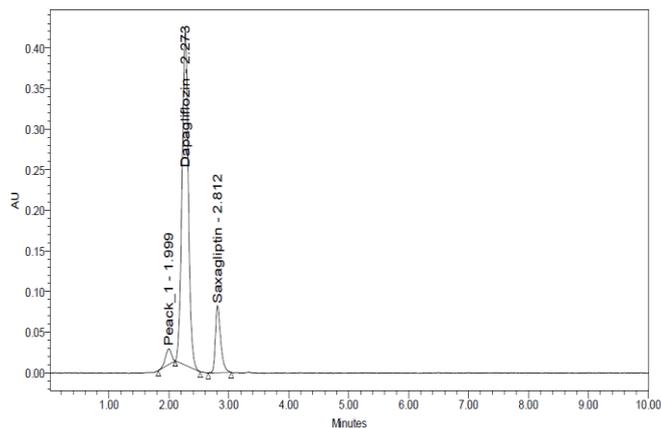


Fig. 13: Base chromatogram of Dapagliflozin and Saxagliptin

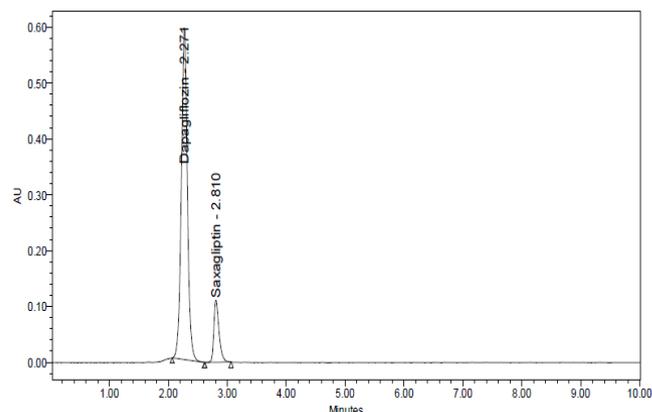


Fig. 14: Peroxide chromatogram of Dapagliflozin and Saxagliptin

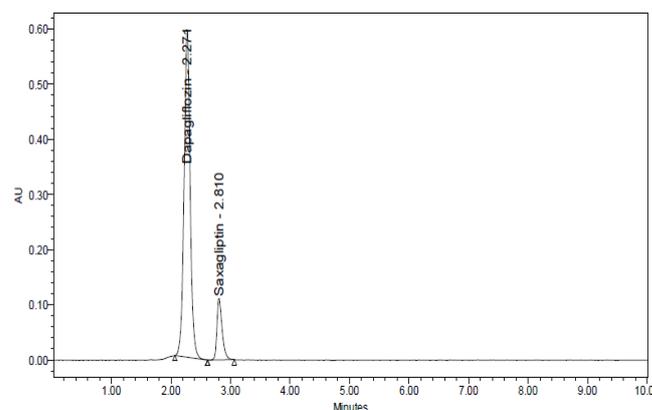


Fig. 15: Thermal chromatogram of Dapagliflozin and Saxagliptin

A simple, Accurate, precise method was developed for the simultaneous estimation of the Dapagliflozin and Saxagliptin in Tablet dosage form. The RP-HPLC method developed and validated allows a simple and rapid quantitative determination of Dapagliflozin and Saxagliptin in tablet dosage forms. All the validation parameters were found to be within the limits according to ICH guidelines. The proposed method was found to be simple, accurate and specific for the drugs of interest irrespective of the excipients present and the short retention times allows the analyst to analyze number of samples in a short period. The method developed was found to be simple, accurate, precise, rugged, robust and stable under forced degradation conditions. So the established method can be successfully applied for the routine analysis for marketed formulations.

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