# ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN AND LINAGLIPTIN TABLETS BY RP-HPLC

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## ABSTRACT

**Objectives:** The objective of this study was to developed and validated for the routine simultaneous analysis of Dapagliflozin and Linagliptin in Bulk Form and Marketed Pharmaceutical dosage forms.

**Methods:** The estimation of Dapagliflozin and Linagliptin was accomplished with X-Bridge $C_{18}$, 250 mm x 4.6 mm, 5µm with PDA detector. The mobile phase consists of Mobile Phase A Containing Phosphate Buffer: Acetonitrile (900:100v/v) and Mobile Phase B containing Phosphate Buffer: Acetonitrile (300:700 v/v) at flow rate of 1.0mL/min. The detection wavelength was 230nm, respectively.

**Results:** In the developed method, the retention time of Dapagliflozin and Linagliptin was found to be 15.639min and 5.853min respectively. The reverse phase high performance liquid chromatography method produces linear response for Dapagliflozin and Linagliptin that was found in the ranges of 12–74µg/mL and 6–28 µg/mL respectively. The % recovery studies were found to be within the limits. The Theoretical plates, tailing factor and resolution were also found be to be within the range as per ICH Guidelines. Calculated information acquired for both the preliminaries roughly coordinates with the information given by design expert programming which shows the genuineness of the chromatographic condition.

**Conclusion:** The developed method was validated according to the ICH guidelines. The linearity, precision, range, and robustness were within the limits as specified by the ICH guidelines. Hence, the method was found to be simple, accurate, precise, economic, and reproducible.

**Key Words:** Dapagliflozin and Linagliptin, ICH guidelines, Accuracy, Precision, RP-HPLC.

# INTRODUCTION

Dapagliflozin is a selective sodium-glucose co-transporter subtype 2 (SGLT2) inhibitor with antihyperglycemic activity. Dapagliflozin[1] selectively and potently inhibits SGLT2 compared to SGLT1, which is the cotransporter of glucose in the gut. Dapagliflozin[2] is a C-glycosyl comprising beta-D-glucose in which the anomeric hydroxy group is replaced by a 4-chloro-3-(4-ethoxybenzyl) phenyl group. Used (in the form of its propanediol monohydrate) to improve glycemic control, along with diet and exercise, in adults with type 2 diabetes. It has a role as a hypoglycemic agent and a sodium-glucose transport protein subtype 2 inhibitor. It is a C-glycosyl compound, an organochlorine compound and aromatic ether. Dapagliflozin[3] is a sodium-glucose cotransporter 2 inhibitor indicated for managing diabetes mellitus type 2. When combined with diet and exercise in adults, Dapagliflozin helps to improve glycemic control by inhibiting glucose resorption in the proximal tubule of the nephron and causing glycosuria. Dapagliflozin was approved by the FDA on Jan 08, 2014. The IUPAC Name of Dapagliflozin is (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-[(4-ethoxy phenyl) methyl] phenyl]-6-(hydroxy methyl) oxane-3, 4, 5-triol. The Chemical Structure of Dapagliflozin is as following

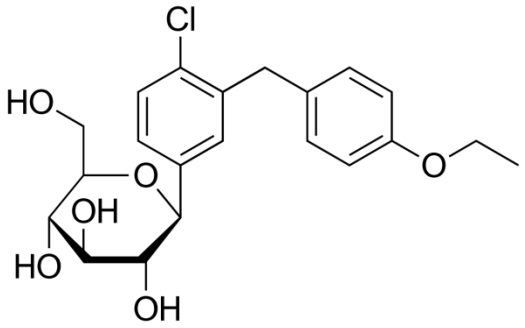


**Fig-1: Chemical Structure of Dapagliflozin**

Linagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor which is used in combination with diet and exercise in the therapy of type 2 diabetes, either alone or in combination with other oral hypoglycemic agents. Linagliptin has been linked to rare instances of clinically apparent liver injury. Linagliptin[4] is a potent, orally bioavailable dihydropurinedione-based inhibitor of dipeptidyl peptidase 4 (DPP-4), with hypoglycemic activity. The inhibition of DPP-4 by Linagliptin appears to be longer lasting than that by some other DPP-4 inhibitors tested. Linagliptin[5] is a xanthine that is 7H-xanthine bearing (4-methylquinazolin-2-yl)methyl, methyl, but-2-yn-1-yl and 3-aminopiperidin-1-yl substituents at positions 1, 3, 7 and 8 respectively (the R-enantiomer). Used for treatment of type II diabetes. It has a role as an EC 3.4.14.5 (dipeptidyl-peptidase IV) inhibitor and a hypoglycemic agent. It is a member of quinazolines and an aminopiperidine. It is functionally related to a 7H-xanthine. The IUPAC Name of Linagliptin[6] is 8-[(3R)-3-amino piperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methyl quinazolin-2-yl) methyl] purine-2, 6-dione. The Chemical Structure of Linagliptin as shown in fig-1.

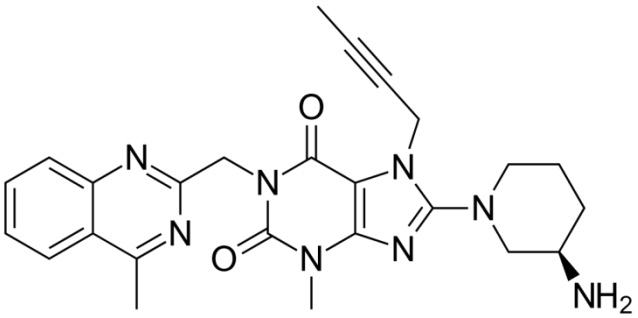

**Fig-2: Chemical Structure of Linagliptin**

As per the past literature review[30-63], Dapagliflozin and Linagliptin was estimated by different chromatographic methods such as liquid chromatography–mass spectrometry, normal phase high-performance liquid chromatography (HPLC), and reversed-phase HPLC (RP-HPLC) for simultaneous determination and by different spectroscopic methods[7] such as ultraviolet (UV), UV first derivative spectroscopy, and moreover several methods[8] were there for the determination of Dapagliflozin and Linagliptin in bulk and its pharmaceutical dosage forms[9]. Hence, the present paper reports that to develop and validate an accurate, simple, precise, economical, rapid method for the simultaneous estimation of Dapagliflozin and Linagliptin by RP-HPLC method[10] and its forced degradation studies[11].

# MATERIALS AND METHODS

## MATERIALS

### Drug Samples (Raw material)

Dapagliflozin and Linagliptin were obtained sample from Micro labs Limited, Bangalore, India.

### Formulation Used

Tablets containing Dapagliflozin 10mg and Linagliptin 5mg.

Chemical and Solvent Used

- Water (Milli Q),
- Methanol (HPLC Grade),
- Acetonitrile (HPLC Grade),
- Ortho phosphoric acid (EMPARTA),
- Ammonium dihydrogen phosphate (EMPARTA) was purchased from Merck.

### Instruments Used

### HPLC Specification

- Model: Agilent-1260 infinity
- Software: Empower
- Syringe Filter: 0.45µm-33mm Nylon filter
- Column: Waters X-bridge C-18 _ 5µm x 4.6 x 250 mm
- Temperature: Ambient

### Others

- Top load balance: Sartorius [Secura]
- Analytical balance: Mettler Toledo

- pH Meter: Metrohm 900
- Centrifuge apparatus: Neuation ifuge L400P
- Ultrasonicator

**METHOD**

In the present work an attempt was made to develop and validate simple, precise and accurate HPLC method for the estimation of Dapagliflozin and Linagliptin in bulk and in tablet dosage form.

**HPLC Method**

**Selection of Mobile Phase**

The method development[12] and validation of Dapagliflozin and Linagliptin required greater resolution[13] .Hence different solvent were tried.

**Selection of Column**

Experiments with different columns[14] were conducted to achieve the best separation of analyte, blank.

**Selection of Flow Rate**

Flow rate selection[15] was done depending on retention time, peak symmetry, separation of impurities[16].

**System Suitability**

System suitability parameter[17] had to be selected based on the tailing factor resolution, plate count % RSD. In general % RSD for peak should not be more than 2.

**Preparation of buffer solution**

Accurately weigh and transfer 8.6g of ammonium dihydrogen phosphate in 1000mL milli-Q water and mix well. Adjust pH to 3.0± 0.05 with diluted ortho phosphoric acid. Filter[18] the buffer through 0.45µ nylon membrane filters.

**Preparation of Mobile Phase-A**

Mixture of Buffer and Acetonitrile in the ratio of 900:100v/v respectively, mixes well and sonicated to degas[19].

**Preparation of Mobile Phase-B**

Mixture of Buffer and Acetonitrile in the ratio of 300:700v/v respectively, mixes well and sonicated to degas.

**Preparation of Diluent 1**

Use methanol as diluent-1

**Preparation of Diluent-2/ Blank**

Prepare a mixture of Buffer and Acetonitrile in the ratio of 500:500v/v respectively, mixes well to sonicate to degas.

**Preparation of Dapagliflozin standard stock solution**

Accurately weigh and transferred 30.6mg of Dapagliflozin propanediol monohydrate [equivalent to 25.0mg of Dapagliflozin] workings standard[20] into a 50mL volumetric flask add 30mL diluent-1 and sonicate to dissolve the content. Dilute upto the mark with diluent-1 and mix well.

**Preparation of Linagliptin Standard Stock Solution**

Accurately weighed and transferred 25.0mg of Linagliptin working standard into 100mL volumetric flask add 70mL of diluents-1 and sonicate to dissolve the content. Dilute upto the mark with diluent and mix well.

**Preparation of standard solution**

Further pipette and transfer 5.0mL of above Dapagliflozin standard stock solution[21] and 5.0mL of above Linagliptin standard stock solution into a 50mL volumetric flask and dilute upto the mark with diluents-2 and mix well.

**Preparation of sample solution**

Weigh 20 tablets to determine the average weight of the tablet. Accurately weight[22] and transfer 5 intact tablets into a 250mL volumetric flask. Add about 10mL of water to disperse the tablets completely. Add about 150mL of diluents-1 and sonicate for about 20minutes with intermediate shaking to dissolve the contents. Keep the solution to cool at room temperature. Dilute upto the mark with diluent- 1 and mix well. Centrifuge the solution at 4000rpm for 15minutes. Pipette and transfer 5.0mL of above supernatant clear solution into a 20mL of volumetric flask make upto the volume with diluent-2 and mix well. Filter the solution through 0.45µm PVDF syringe filter by discarding first 5mL of filtrate.

**Validation of Developed Method**

**Linearity**

A calibration curve[23] was plotted between concentration and Peak area. Dapagliflozin was linear[24] with the concentration range[25] of 12-74µg/mL and Linagliptin was linear with concentration range of 6-38µg/mL at 230nm.

**Precision**

The repeatability of the method[26] was confirmed by the analysis of formulation was repeated for six times with the same concentration. The amount of drug present in the tablet formulation[27] was calculated. The percentage RSD[28] and confidence interval were calculated.

**Accuracy**

Accuracy[29] of the method was confirmed by recovery studies[30]. To the pre-analyzed formulation, a known quantity of raw material was added and the procedure was followed as per the analysis of formulation[31]. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD and confidence interval were calculated.

**Specificity**

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected that may be expected to be present in the sample matrix. It is a measure of the degree of interference from such things as other active ingredients, excipients, impurities and degradation products, ensuring that a peak response is due to only to a single component; that is; that no co-elution exists. Specificity[32] is measured and documented in a separation by the resolution, plate count, and tailing factor[33].

**Robustness**

Robustness[34] of the method was confirmed by deliberate change in the flow rate, wavelength[35] and the mobile phase composition were made to evaluate the impact on proposed method. The sample were injected in six replicates and % RSD was calculated.

**System Suitability**

The system suitability parameters[36-40] were evaluated from standard chromatograms by calculating the Tailing factor, resolution, % RSD of retention time and peak areas from six replicate injections.

**Forced degradation studies**

The stress degradation study[41-46] was carried out on the sample preparations of tablet. The degradation was evaluated by calculating the peak purity of the analyte peak.

**Filter interference study**

The sum of standard solution[47] was filtered through 0.45µ-33mm Nylon filter, .45µ- 33mm PVDF filter, and 45µ-33 mm PTFE filter. The sum of sample solution was centrifuged and filtered through 0.45µ-33mm Nylon filter, 45µ-33mm PVDF filter, 45µ-33mm PTFE filter. All the solution injected into the chromatographic system[48-50] and the peak response was measured for Dapagliflozin and Linagliptin peak. Comparison the %difference in between filtered and unfiltered solution was calculated.

## RESULTS AND DISCUSSION

**Method Development**

A precise reverse phase chromatography[51] for the stimulation estimation of Dapagliflozin and Linagliptin was developed. The Dapagliflozin and Linagliptin was prepared and scanned in the UV-region from 200-400nm. Both the drugs showed the marked absorbance at 230nm. For getting optimized chromatographic condition[52] various trials have been performed by changing the mobile phase[53] and the composition of the mobile phase.

**Table-1: Optimized Chromatographic Condition**

| Parameters | Conditions |
|---|---|
| Column | X-Bridge C18, 250x4.6mm, 5µm |
| Mobile phase A | Phosphate Buffer: Acetonitrile (900:100v/v) |
| Mobile phase B | Phosphate Buffer: Acetonitrile (300:700 v/v) |
| Diluent-1 | Methanol |
| Diluent-2 | Phosphate Buffer: Acetonitrile (500:500 v/v) |
| pH | 3.0 |
| Flow rate | 1.0mL/min |
| Wavelength | 230nm |
| Injection Volume | 25µL |
| Column oven Temp | 30 °C |
| Sample Temp. | 25°C |
| Run time | 25minutes |

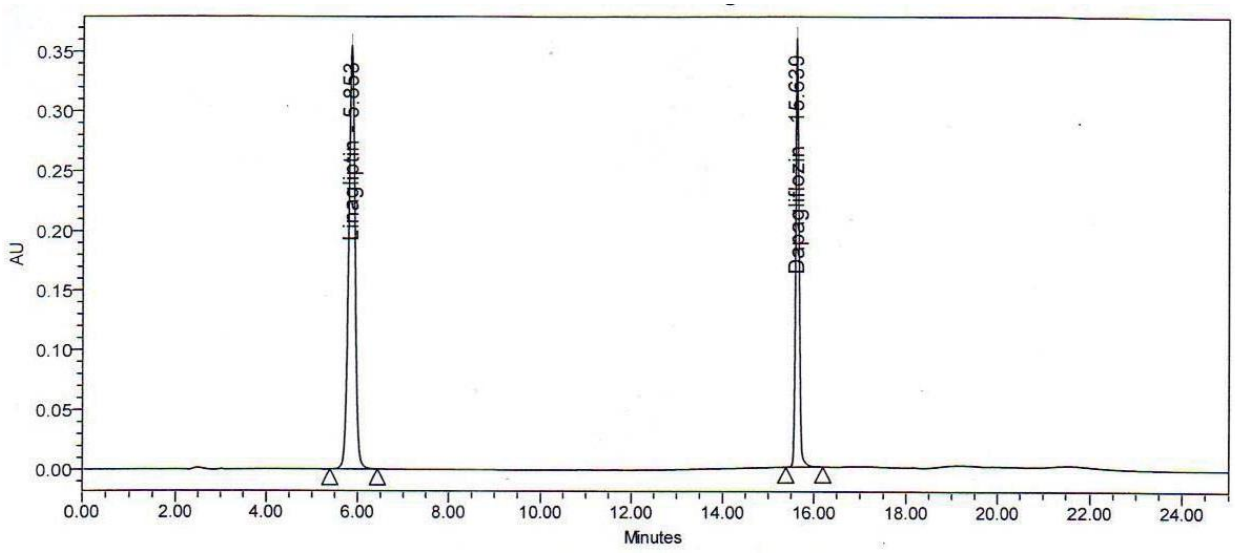


**Fig-3: Optimized Chromatographic Condition**

By using the optimized chromatographic conditions a stock solution of Dapagliflozin and Linagliptin was prepared by using the buffer and pH adjusted to 3 by adding diluted orthophosphoric acid with the concentration of 0.1-0.5ml for 50µg/ml from the stock solution concentration range of 12-74ug/ml for Dapagliflozin and 0.1-0.5ml for 6- 38µg/ml for Linagliptin was prepared and injected to the chromatographic system. The slope intercept and correlation coefficient[54] were calculated. The slope, intercept, correlation coefficient of Dapagliflozin was found to be 59360.1302, 39677.2662, 0.9999 and Linagliptin was found to be 168442.9091, 62986.3592, 1.0000. Calibration curve was plotted by taking concentration Vs Area. The calibration shows that linear response[55] was obtained over range of Concentration used and shown in figure 4 & 5 and the tables are shown in 2 & 3. The range demonstration that the method is linear outside the limit of expected use.

Tablets were (10mg Dapagliflozin and 5mg Linagliptin) selected for analysis. The nominal concentration of Dapagliflozin and Linagliptin from the linearity was prepared and the absorbance of the solution was measured at the selected wavelengths. The percentage label claim in tablet formulation was found to be 98.6 % for Dapagliflozin and 104.5 % for Linagliptin. The amount present in tablet formulation was good concord with the label claim and the Percentage Relative Standard Deviation[56] (%RSD) was also found to be 0.2 for Dapagliflozin and 0.1 for Linagliptin. The %RSD value indicates that the method as good precision.

From the standard stock solution concentration solution was prepared and injected into the chromatographic system in six replicate injections. System suitability parameters such as Theoretical plates, Tailing factor and % RSD were calculated. Theoretical plates and Tailing Factor, Retention Time, Asymmetry Factor and %RSD of Dapagliflozin was found to be 93897, 1.1, 15.720 and Linagliptin was found to be 4927, 1.1, 5.683 respectively and Resolution[57] for Dapagliflozin were found to be 37.3. The result indicates the good precision of the developed method. The System Suitability parameters were presented in table 5.

Specificity[58] was evaluated by injecting the Blank solution, Standard and Sample solution. Blank shows no peak, standard, sample solution of Dapagliflozin and Linagliptin shows Retention time at 15.806min Dapagliflozin of and 5.880min 0f Linagliptin respectively.

Peak purity test of Dapagliflozin and Linagliptin was passes the test. The specificity parameters and chromatograms and results were shown in table 10 & 11.

The accuracy of the method is confirmed by recovery analysis. To the analyzed formulation, known quantities of the standard drugs were added at the concentration of 25%, 50%, 100% and 150% concentration. The area of the solutions was measured and percentage recovery[59] was calculated. The percentage recovery of Dapagliflozin and Linagliptin was found to be in range of 98.40 to 99.9 and 98.7 to 100.7 respectively. The %RSD value was found to be less than 2. The %RSD indicates that there is no interference of excipients during the analysis. The data recovery analysis is given in table 9.

Forced degradation study was evaluated by calculating the peak purity of the analyte peak. Stress degradation studies[60] like acid stress, alkali stress, peroxide stress, photolytic control, photolytic stress and thermal stress was carried out for Dapagliflozin and Linagliptin sample. The peak purity[61] of the Dapagliflozin and Linagliptin sample was passes the test. Forced degradation study peak purity chromatograms[62] and results were shown in table 9 & 10.

Filter interference study[63] was evaluated by standard and sample solution was filtered through 0.45µ-Nylon filter, 0.45µ-PVDF filter, 0.45µ-PTFE filter and some portion was centrifuged. Compared the %difference in between filtered and unfiltered solution was calculated for standard and sample solution. The %difference was shown in table 14-15.

**Method Validation**

**Linearity and Range:**

**Table-2: Linearity Results of Dapagliflozin**

| Parameters | Values* |
|---|---|
| Detection wavelength (nm) | 230nm |
| Beer's law limit (µg/mL) | 12-74 (µg/mL) |
| Correlation coefficient (r) | 0.9999 |
| Regression equation(y=mx+c) | Y = 59702x + 21068 |
| Slope (m) | 59702 |
| Intercept (c) | 21068 |

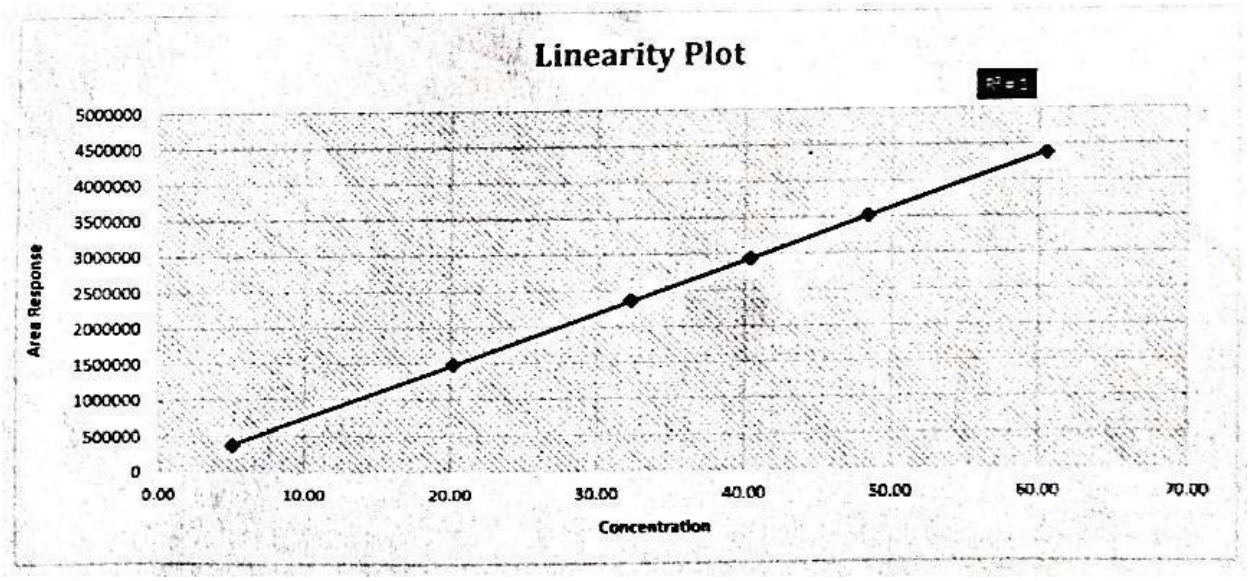


**Fig-4: Calibration Curve of Dapagliflozin**

**Table-3: Linearity Results of Linagliptin**

| Parameters | Values* |
|---|---|
| Detection wavelength (nm) | 230nm |
| Beer's law limit (µg/mL) | 6-28 (µg/mL) |
| Correlation coefficient (r) | 0.9999 |
| Regression equation(y=mx+c) | Y=16954x + 33208 |
| Slope (m) | 16954 |
| Intercept (c) | 33208 |

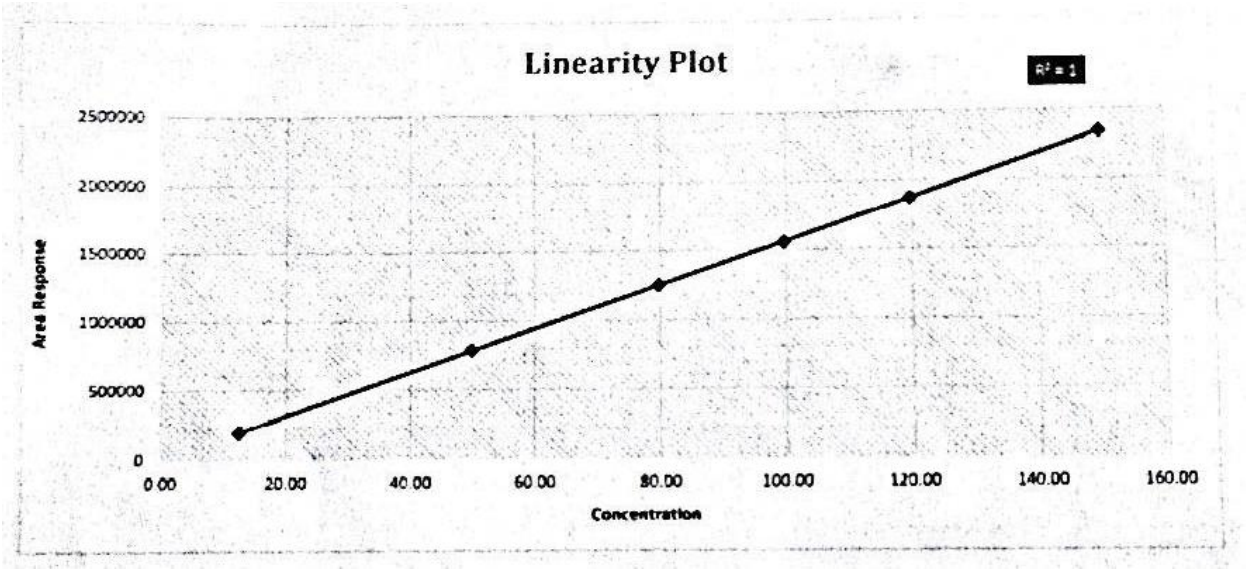


**Fig-5: Calibration Curve of Linagliptin**

**Table-4: Linearity of Dapagliflozin and Linagliptin**

| Linearity | Dapagliflozin | | Linagliptin | |
|---|---|---|---|---|
| | ncentration (µg/mL) | Average Area response | ncentration (µg/mL) | Average Area response |
| **25%** | 12.2367 | 752363 | 6.2995 | 1100207 |
| **50%** | 24.4734 | 1490236 | 12.5990 | 2195112 |
| **85%** | 39.1574 | 2379489 | 20.1583 | 3472254 |
| **100%** | 48.9467 | 2952072 | 25.1979 | 4322223 |
| **120%** | 58.7361 | 3540363 | 30.2375 | 5165970 |
| **150%** | 73.4201 | 4374637 | 37.7969 | 6405313 |

| | | |
|---|---|---|
| **Correlation Coefficient** | **0.9999** | **1.0000** |
| **Slope** | **59360.130** | **168442.9091** |
| **ercept** | **39677.266** | **62986.359** |

**System Suitability:**

**Table-5: System Suitability Parameters for Dapagliflozin and Linagliptin**

| S.No. | Parameters | Dapagliflozin | Linagliptin |
|---|---|---|---|
| 1 | Theoretical plate | 93987 | 4927 |
| 2 | Tailing factor | 1.24 | 1.06 |
| 3 | Retention time | 15.639 | 5.853 |
| 4 | Asymmetric factor | | |
| 5 | %RSD | 0.2 | 0.3 |
| 6 | Resolution | 49.5 | |

**System Precision:**

**Table-6: System Precision**

| | Dapagliflozin 10mg and Linagliptin 5mg Tablets | |
|---|---|---|
| **Injection Number** | **Dapagliflozin** | **Linagliptin** |
| **1** | 2890180 | 4124653 |
| **2** | 2891383 | 4130312 |
| **3** | 2879173 | 4132870 |
| **4** | 2892094 | 4144064 |
| **5** | 2880528 | 4153526 |
| **Average** | 2886672 | 4137085 |
| **Std.dev** | 6282.54 | 115788.87 |
| **%RSD** | 0.2 | 0.3 |

**Method Precision:**

**Table-7: Method Precision for Dapagliflozin**

| Product Name | Dapagliflozin 10mg and Linagliptin 5mg Tablets | |
|---|---|---|
| **Sr. No** | **Assay (mg)** | **Assay % of LC** |
| **1** | 9.83 | 98.3 |
| **2** | 9.85 | 98.5 |
| **3** | 9.84 | 98.4 |
| **4** | 9.86 | 98.6 |
| **5** | 9.88 | 98.8 |
| **6** | 9.88 | 98.8 |
| **Average** | **9.86** | **98.6** |
| **Std.dev** | **0.021** | **0.21** |
| **%RSD** | **0.2** | **0.2** |

**Table-8: Method Precision for Linagliptin**

| Product Name | Dapagliflozin 10mg and Linagliptin 5mg Tablets | |
|---|---|---|
| **Sr. No** | **Assay (mg)** | **Assay % of LC** |
| **1** | 5.23 | 104.6 |
| **2** | 5.22 | 104.4 |
| **3** | 5.22 | 104.5 |
| **4** | 5.23 | 104.6 |
| **5** | 5.22 | 104.5 |
| **6** | 5.23 | 104.6 |
| **Average** | **5.23** | **104.5** |
| **Std.dev** | **0.005** | **0.08** |
| **%RSD** | **0.1** | **0.1** |

**Accuracy:**

**Table-9: Accuracy of Dapagliflozin and Linagliptin**

| ecovery Level | Dapagliflozin | | | Linagliptin | | |
|---|---|---|---|---|---|---|
| | Amount Spiked (mg) | Amount Recovered (mg) | % Recovery | Amount Spiked (mg | Amount Recovered (mg) | % Recover y |
| 25% | 12.6560 | 12.6017 | 99.9 | 6.3162 | 6.3592 | 100.7 |
| 50% | 26.0042 | 25.5953 | 98.4 | 12.8409 | 12.6753 | 98.7 |
| 100% | 50.9812 | 50.8180 | 99.7 | 25.8440 | 25.9581 | 100.4 |
| 150% | 76.0865 | 75.2516 | 98.9 | 38.1597 | 38.0477 | 99.7 |
| Overall % Recovery | | | 99.2 | Overall % Recovery | | 99.9 |
| Overall %RSD for % Recovery | | | 0.7 | Overall %RSD for % Recovery | | 0.9 |

**Specificity:**

**Table-10: Peak Purity of Dapagliflozin and Linagliptin Sample**

| Standard Solution | Dapagliflozin | | | Linagliptin | | |
|---|---|---|---|---|---|---|
| Product Name | Purity Angle | Purity Threshold | Results (Peak Purity) | Purity Angle | Purity Threshold | Results (Peak Purity) |
| Dapagliflozin10mg and Linagliptin 5mg | 3.630 | 10.794 | Passes | 8.552 | 24.447 | Passes |

**Table-11: Placebo Interference Study**

| Observation | Placebo Preparation |
|---|---|
| % Interference | No interference |

**Forced Degradation Studies:**

**Table-12: Forced Degradation Study Peak Purity Results of Dapagliflozin**

| Stress Condition | Purity Angle | Purity Threshold | Peak Purity |
|---|---|---|---|
| **Acid Stress** | 1.436 | 2.263 | Pass |
| **Alkali Stress** | 1.488 | 2.264 | Pass |
| **Peroxide Stress** | 1.463 | 2.268 | Pass |
| **Photolytic Control** | 2.681 | 5.905 | Pass |
| **Photolytic Stress** | 2.839 | 7.424 | Pass |
| **Thermal stress** | 2.827 | 6.555 | Pass |

**Table-13: Forced Degradation Study Peak Purity Results of Linagliptin**

| Stress Condition | Purity Angle | Purity Threshold | Peak Purity |
|---|---|---|---|
| **Acid Stress** | 0.128 | 2.336 | Pass |
| **Alkali Stress** | 0.127 | 2.333 | Pass |
| **Peroxide Stress** | 0.129 | 2.356 | Pass |
| **Photolytic Control** | 5.581 | 12.625 | Pass |
| **Photolytic Stress** | 6.064 | 16.240 | Pass |
| **Thermal stress** | 5.282 | 14.078 | Pass |

**Filter Interference Study:**

**Table-14: Filter Interference Data for Standard Solution**

| | Dapagliflozin | | Linagliptin | |
|---|---|---|---|---|
| **Prep. Type** | **Area** | **%Diff** | **Area** | **%Diff** |
| Unfiltered | 2890782 | NA | 4127483 | NA |
| Nylon filter | 2891185 | 0.0 | 4131941 | -0.1 |
| PVDF filter | 2892739 | -0.1 | 4148530 | -0.5 |
| PTFE filter | 2890571 | 0.0 | 4130422 | -0.1 |

**Table-15: Filter Interference Data for Sample Solution**

| Prep. Type | Dapagliflozin | | Linagliptin | |
|---|---|---|---|---|
| | Area | %Diff | Area | %Diff |
| Centrifuged | 2952855 | NA | 4270531 | -0.1 |
| Nylon filter | 2952128 | 0.0 | 4274730 | 0.2 |
| PVDF filter | 2955025 | -0.1 | 4262062 | 0.2 |
| PTFE filter | 2951684 | 0.0 | 4263881 | 0.2 |

## SUMMARY AND CONCLUSION

A simple, rapid, precise and accurate HPLC method was developed and validated for estimation of Dapagliflozin and Linagliptin in pure form and in tablet dosage form.
The methods employed for the analysis of Dapagliflozin and Linagliptin was HPLC method.

**HPLC Method**

A simple and rapid HPLC method was developed for the estimation Dapagliflozin and Linagliptin in bulk and in Tablet dosage form. The mobile phase consisting of buffer and acetonitrile. In the ratio of 90:10%v/v and 30:70%v/v was selected for the analysis. 230nm was selected as the detection wavelength. With the above optimized conditions, the linearity range was fixed as 12 to 74µg/ml for Dapagliflozin and 6 to 38µg/ml for Linagliptin. The correlation coefficient was found to be 0.9999and 1.0000 for Dapagliflozin and Linagliptin, respectively.
Tablet containing 10mg of Dapagliflozin and 5mg Linagliptin was selected for analysis. The percentage label claim in the tablet formulation was found to be 98.6% for Dapagliflozin and 104.5% for Linagliptin. The precision of the method was confirmed by the repeated analysis of formulation. The %RSD was found to be 0.2 and 0.1 and for Dapagliflozin and Linagliptin, respectively.
A simple, precise, rapid and accurate HPLC method was developed for the determination of Dapagliflozin and Linagliptin in bulk and in combination tablet formation.

## REFERENCES


1. Anonymous chemspider.com/Chemical-Structure.8063384.html
2. Anonymous go. drugbank.com/drug/DB06292 (05.03.2022).
3. Anonymous pubchem.ncbi.nlm.nih.gov/compound/Dapagliflozin
4. Anonymous go.drugbank.com/drugs/DB08882 (05.03.2022)
5. Anonymous pubchem.ncbi.nlm.nih.gov/compound/Linagliptin
6. https://en.wikipedia.org/wiki/Linagliptin
7. Afzal, S. J. S., Asif, M., & Khan, P. M. A. A. (2018). Validation of stability indicating high performance liquid chromatographic method for simultaneous determination of assay of Linagliptin and Metformin drugs in the pharmaceuticals tablet formulations using bupropion as a common internal standard. European Journal of Biomedical, 5(2), 1111-1119.
8. Ameeduzzafar, El-Bagory, I., Alruwaili, N. K., Imam, S. S., Alomar, F. A., Elkomy, M. H., & Elmowafy, M. (2020). Quality by design (QbD) based development and validation of bioanalytical RP-HPLC method for Dapagliflozin: Forced degradation and preclinical pharmacokinetic study. Journal of Liquid Chromatography & Related Technologies, 43(1-2), 53-65.
9. Attimarad, M., Nagaraja, S. H., Aldhubaib, B. E., Nair, A., & KN, V. (2014). Simultaneous determination of metformin and three gliptins in pharmaceutical formulations using RP HPLC: application to stability studies on Linagliptin tablet formulation. Diabetes, 1, 4.
10. Aubry, A. F., Gu, H., Magnier, R., Morgan, L., Xu, X., Tirmenstein, M., & ArnoldM. (2010). Validated LC–MS/MS methods for the determination of Dapagliflozin, a sodium- glucose co-transporter 2 inhibitor in normal and ZDF rat plasma. Bioanalysis, 2(12), 2001-2009.
11. Ayoub, B. M. (2015). UPLC simultaneous determination of Empagliflozin, Linagliptin and metformin. RSC advances, 5(116), 95703-95709.
12. Badugu, L. R. (2012). A Validated RP-HPLC method for the determination of Linagliptin. Am. J. PharmTech Res, 2(4), 463-470.
13. Banik, S., Karmakar, P., & Miah, M. A. H. (2015). Development and validation of a UV-spectrophotometric method for determination of Vildagliptin and Linagliptin in bulk and pharmaceutical dosage forms. Bangladesh Pharmaceutical Journal, 18(2), 163-168.
14. Bhole, R. P., Wankhede, S. B., & Pandey, M. (2017). Stability indicating HPTLC method for simultaneous estimation of Empagliflozin and Linagliptin in pharmaceutical formulation. Analytical Chemistry Letters, 7(1), 76-85.
15. Chatwal R. G, Anand K. S, High performance liquid chromatography, Instrumental methods of chemical analysis, 5th Ed., Himalaya Publishers, Mumbai, 2010. pp 2.570- 2.629.
16. Debata, J., Kumar, S., Jha, S. K., & Khan, A. (2017). A New RP-HPLC method development and validation of Dapagliflozin in bulk and tablet dosage form. Int J Drug Dev Res, 9(2), 48-51.
17. Deepan, T., & Dhanaraju, M. D. (2018). Stability indicating HPLC method for the simultaneous determination of Dapagliflozin and Saxagliptin in bulk and tablet dosage form. Current Issues in Pharmacy and Medical Sciences, 31(1), 39-43.

18. Deepan, T., Rao, M. B., & Dhanaraju, M. D. (2017). Development of validated stability indicating assay method for simultaneous estimation of metformin and Dapagliflozin by RP-HPLC. European Journal of Applied Sciences, 9(4), 189-199.
19. Dias, B. C. L., Fachi, M. M., de Campos, M. L., Degaut, F. L. D., Peccinini, R. G., & Pontarolo, R. (2019). A new HPLC–MS/MS method for the simultaneous quantification of SGLT2 inhibitors and metformin in plasma and its application to a pharmacokinetic study in healthy volunteers. Biomedical Chromatography, 33(11), e4663.
20. El-Bagary, R. I., Elkady, E. F., & Ayoub, B. M. (2013). Spectrophotometric methods for the determination of Linagliptin in binary mixture with metformin hydrochloride and simultaneous determination of Linagliptin and metformin hydrochloride using high performance liquid chromatography. International journal of biomedical science: IJBS, 9(1), 41.
21. El-Desouky, E. A., Abdel-Raoof, A. M., Abdel-Fattah, A., Abdel-Zaher, A., Osman, A. O., Abdel-Monem, A. H., & Morshedy, S. (2021), Determination of Linagliptin and Empagliflozin by UPLC and HPTLC techniques aided by lean six sigma approach, Biomedical Chromatography, 35(7), e5102.
22. El-Zaher, A. A., Hashem, H. A., Elkady, E. F., & Allam, M. A. (2019). A validated LC-MS/MS bioanalytical method for the simultaneous determination of Dapagliflozin or Saxagliptin with metformin in human plasma. Micro chemical Journal, 149, 104017.
23. Ganorkar, A., Askarkar, S., Gupta, K., & Umekar, M. (2020). Validated stability indicating and assay method development of Linagliptin in formulation by RP-HPLC using quality by design. Orbital: The Electronic Journal of Chemistry, 12(2), 48-61.
24. Ganorkar, S. B., Sharma, S. S., Patil, M. R., Bobade, P. S., Dhote, A. M., & Shirkhedkar, A. A. (2021). Pharmaceutical Analytical Profile for Novel SGL-2 Inhibitor: Dapagliflozin. Critical Reviews in Analytical Chemistry, 51(8), 835-847.
25. Gundala, A., Prasad, K. V. S. R. G., & Koganti, B. (2019). Application of quality by design approach in RP-HPLC method development for simultaneous estimation of Saxagliptin and Dapagliflozin in tablet dosage form. Brazilian Journal of Pharmaceutical Sciences, 55.
26. Gurrala, S., Raj, S., Subrahmanyam, C. V. S., & Anumolu, P. D. (2021). Multivariate optimization of liquid chromatographic conditions for determination of Dapagliflozin and Saxagliptin, application to an in vitro dissolution and stability studies. Future Journal of Pharmaceutical Sciences, 7(1), 1-11.
27. Hassib, S. T., Taha, E. A., Elkady, E. F., & Barakat, G. H. (2019). Validated liquid chromatographic method for the determination of (Canagliflozin, Dapagliflozin or Empagliflozin) and metformin in the presence of (1-cyanoguanidine). Journal of Chromatographic Science, 57(8), 697-707.
28. Hayashi, T., Fukui, T., Nakanishi, N., Yamamoto, S., Tomoyasu, M., Osamura, A., ... & Hirano, T. (2017). Dapagliflozin decreases small dense low-density lipoprotein- cholesterol and increases high-density lipoprotein 2-cholesterol in patients with type 2 diabetes: comparison with Sitagliptin. Cardiovascular diabetology, 16(1), 1-13.
29. Jadhav, S. B., Reddy, P. S., Narayanan, K. L., & Bhosale, P. N. (2017). Development of RP-HPLC, stability indicating method for degradation products of Linagliptin in presence of

metformin HCl by applying 2 level factorial design; and identification of impurity- VII, VIII and IX and synthesis of impurity-VII. Scientia Pharmaceutica, 85(3), 25.

30. Jani, B. R., Shah, K. V., & Kapupara, P. P. (2015). Development and validation of UV spectroscopic first derivative method for simultaneous estimation of Dapagliflozin and metformin hydrochloride in synthetic mixture. J Bioequiv, 1(1), 102.
31. Kazi, M., Alqahtani, A., Ahmad, A., Noman, O. M., Aldughaim, M. S., Alqahtani, A. S., & Alanazi, F. K. (2021). Development and optimization of Sitagliptin and Dapagliflozin loaded oral self-nano emulsifying formulation against type 2 diabetes mellitus. Drug Delivery, 28(1), 100-114.
32. Khomitskaya, Y., Tikhonova, N., Gudkov, K., Erofeeva, S., Holmes, V., Dayton, B., & Tang, W. (2018). Bioequivalence of Dapagliflozin/Metformin extended-release fixed- combination drug product and single-component Dapagliflozin and metformin extended- release tablets in healthy Russian subjects. Clinical Therapeutics, 40(4), 550-561.
33. Kim, C. H., Lee, S. H., & Kim, K. S. (2020). Development of a Novel Dapagliflozin orally disintegrating tablets. Yakhak Hoeji, 64(4), 334-342.
34. Kommineni, V., Chowdary, K. P. R., & Prasad, S. V. U. M. (2018). Development and validation of a new HPLC method for the simultaneous estimation of Saxagliptin and Dapagliflozin and its application in pharmacokinetic studies. Int Res J Pharm Med Sci, 1, 16-24.
35. Konari, S. N., Jacob, J. T., & Prakash, V. (2017). Stability indicating UV spectrophotometric method for Linagliptin and Metformin in pharmaceutical dosage form. Pharmaceutical Methods, 8(2).
36. Lloyd R. Snyder, Joseph J. Kirkland, Joseph L. Glajch. Practical HPLC method development, 2nd ed.; Wiley India Pvt. Ltd.: New Delhi, India 2011.
37. Madhusudhan, P., Reddy, M. R., & Devanna, N. (2015). RPHPLC method development and validation for simultaneous determination of Linagliptin and Empagliflozin in tablet dosage form. International advanced research journal in science, engineering and technology, 2(2), 95-99.
38. Maher, H. M., Abdelrahman, A. E., Alzoman, N. Z., & Aljohar, H. I. (2019). Stability-indicating capillary electrophoresis method for the simultaneous determination of metformin hydrochloride, Saxagliptin hydrochloride, and Dapagliflozin in pharmaceutical tablets. Journal of Liquid Chromatography & Related Technologies, 42(5-6), 161-171.
39. Mante, G. V., Gupta, K. R., & Hemke, A. T. (2017). Estimation of Dapagliflozin from its tablet formulation by UV-spectrophotometry. Pharmaceutical Methods, 8(2), 102- 107.
40. Mante, G. V., Hemke, A. T., & Umekar, M. J. (2018). RP-HPLC Method for Estimation of Dapagliflozin from its Tablet. International Journal of ChemTech Research, 11(01), 242-248.
41. Michael W. Dong. Modern HPLC for Practicing Scientists, 1st ed.; John Wiley & Sons Inc.: Hoboken, New Jersey, USA 2006.
42. Moncy, S., Rohini Reddy, G., Sunil Kumar, P., Chaitanya, G., Priyanka, E., & Bindu, H. (2014), Simultaneous determination of Metformin hydrochloride and Linagliptin by RP-HPLC in bulk and pharmaceutical formulations. Indo American Journal of Pharmaceutical Research, 4, 4047-4053.

43. Mourad, S. S., El-Kimary, E. I., Hamdy, D. A., & Barary, M. A. (2016). Stability- indicating HPLC-DAD method for the determination of Linagliptin in tablet dosage form: application to degradation kinetics. Journal of chromatographic science, 54(9), 1560- 1566.
44. Moussa, B. A., Mahrouse, M. A., & Fawzy, M. G. (2019). A validated LC-MS/MS method for simultaneous determination of Linagliptin and Metformin in spiked human plasma coupled with solid phase extraction: Application to a pharmacokinetic study in healthy volunteers. Journal of pharmaceutical and biomedical analysis, 163, 153-161.
45. Naazneen, S., & Sridevi, A. (2016). Development and validation of stability indicating RP-HPLC method for simultaneous estimation of Empagliflozin and Linagliptin in tablet formulation. Der Pharmacia Lettre, 8(17), 57-65.
46. Nasser, S., Salama, I., Mostafa, S. M., & Elgawish, M. S. (2018). Comparative high-performance liquid chromatographic and high-performance thin-layer chromatographic study for the simultaneous determination of Dapagliflozin and Metformin hydrochloride in bulk and pharmaceutical formulation. JPC-Journal of Planar Chromatography- Modern TLC, 31(6), 469-476.
47. P.D. Sethi. HPLC: Quantitative Analysis of Pharmaceutical Formulations, 1st ed.; CBS Publishers & Distributors: New Delhi, India 2008.
48. Pandya, R. H., Rathod, R., & Maheswari, D. G. (2014). Bioanalytical method development and validation for simultaneous determination of Linagliptin and Metformin drugs in human plasma by RP-HPLC method. Pharmacophore, 5(2), 202- 218.
49. Rizk, M., Attia, A. K., Mohamed, H. Y., & Elshahed, M. (2021). Stability indicating HPLC-Fluorescence detection method for the simultaneous determination of Linagliptin and Empagliflozin in their combined pharmaceutical preparation. European Journal of Chemistry, 12(2), 168-178.
50. Rizk, M., Attia, A. K., Mohamed, H. Y., & Elshahed, M. S. (2020). Thermo analytical study and purity determination of Anti-diabetic drugs Linagliptin and Empagliflozin in drug substances. Chem. Res. J., 5, 6-20.
51. Sanagapati, M., Lakshmi, D. K., Reddy, N. G., & Sreenivasa, S. (2014). Development and validation of stability-indicating RP-HPLC method for determination of Dapagliflozin. Journal of Advanced Pharmacy Education & Research, 4(3).
52. Sen, G., Babu, K. R., Annapurna, N., Vekariya, N. A., & Jagadeesh, V. Validation of Stability-Indicating Reverse Phase HPLC Method for the Determination of Related Substances in Dapagliflozin Drug Substance.
53. Shah, P. A., Shrivastav, P. S., Shah, J. V., & George, A. (2019). Simultaneous quantitation of metformin and Dapagliflozin in human plasma by LC–MS/MS: application to a pharmacokinetic study. Biomedical Chromatography, 33(4), e4453.
54. Sharma B. K. High performance liquid chromatography, Instrumental methods of chemical analysis, 24th Ed., Goel Publishing House, Meerut, 2005. pp 295- 300.
55. Shirisha, S., Haque, M. A., Sireesha, D., Bakshi, V., & Harshini, S. (2014). Development and validation of RP-HPLC method for simultaneous estimation of Metformin and Linagliptin in combined pharmaceutical dosage form. Int J Pharm Res Sci, 2, 491-495.
56. Singh, N., Bansal, P., Maithani, M., & Chauhan, Y. (2018). Development and validation of a stability-indicating RP-HPLC method for simultaneous determination of Dapagliflozin and Saxagliptin in fixed-dose combination. New Journal of chemistry, 42(4), 2459-2466.

57. Srivani, J., Uma Mahesh, B., & Veeresham, C. (2016). Development and validation of stability indicating HPTLC method for simultaneous determination of Linagliptin and metformin. Int. J. Pharm. Pharm. Sci, 8(1), 112-115.
58. Suma, B. V., & Deveswaran, R. (2019). An overview on analytical methods for Dapagliflozin-an antidiabetic drug. Int J Pharm Sci Res, 10(6), 2688-92.
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