

# Development And Validation of a Simple and Rapid Rp-Hplc Method for The Simultaneous Estimation of Dapagliflozin and Vildagliptin in Tablet Dosage Form

Palyam Sunil Kumar<sup>1</sup>, G Srinivasa Rao<sup>2</sup>, P. Ramudu<sup>3</sup>

<sup>1</sup>*Department of Pharmaceutical Analysis, Saastra college of pharmaceutical education and research varigonda (v) T.p Gudur (m) spsr Nellore Dt -524311*

<sup>2</sup>*Principal and Professor, Saastra college of pharmaceutical education and research varigonda (v) T.p Gudur (m) spsr Nellore Dt -524311*

<sup>3</sup>*Assistant Professor, SCPER, varigonda (v) T.p Gudur (m) spsr Nellore Dt -524311*

**Abstract**—A simple, rapid, accurate, and precise reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Dapagliflozin and Vildagliptin in tablet dosage forms. Chromatographic separation was achieved on an Agilent C18 (4.6X250mm, 5µm) column. The mobile phase consisted of a mixture of acetonitrile and phosphate buffer KH<sub>2</sub>PO<sub>4</sub>, pH adjusted to 5.4 with 0.1% formic acid) in a 65:35 v/v ratio, pumped isocratically at a flow rate of 0.9mL/min. UV detection was performed at an optimized wavelength of 212nm, with the column temperature maintained at 30°C.

The retention times for Dapagliflozin and Vildagliptin were significantly minimized to 2.163min and 2.684min, respectively, resulting in a highly economical and short runtime. The method demonstrated excellent linearity, with regression equations of  $y = 50048x + 1180.1$  for Dapagliflozin and  $y = 25331x + 3601.2$  for Vildagliptin. Method precision was confirmed by low percentage relative standard deviation (%RSD) values of 0.3% for Dapagliflozin and 1.0% for Vildagliptin. Accuracy was established with high mean recovery rates of 100.45% and 99.33%, respectively. The limits of detection (LOD) and quantification (LOQ) were determined to be 0.006 µg/mL and 0.019 µg/mL for Dapagliflozin, and 0.22 µg/mL and 0.66 µg/mL for Vildagliptin. Due to its speed, accuracy, and cost-effectiveness, this validated method is highly suitable for routine quality control analysis in the pharmaceutical industry.

**Index Terms**—Dapagliflozin, Vildagliptin, RP-HPLC, Simultaneous Estimation, Quality Control.

## I. INTRODUCTION

The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers.<sup>1</sup> Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data<sup>2</sup>. Hence, development of rugged and robust analytical methods is very important for statutory certification of drugs and their formulations with the regulatory authorities.<sup>3</sup>

The quality and safety of a drug is generally assured by monitoring and controlling the assay and impurities effectively.<sup>4</sup> While assay determines the potency of the drug and impurities will determine the safety aspect of the drug. Assay of pharmaceutical products plays an important role in efficacy of the drug in patients.<sup>5,6</sup>

The wide variety of challenges is encountered while developing the methods for different drugs depending on its nature and properties.<sup>7</sup> This along with the importance of achieving the selectivity, speed, cost, simplicity, sensitivity, reproducibility and accuracy of results gives an opportunity for researchers to come out with solution to address the challenges in getting the new methods of analysis to be adopted by

the pharmaceutical industry and chemical laboratories. Different physico-chemical methods<sup>8</sup> are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physico-chemical methods, the most important are optical (refractometry, polarimetry, emission and fluorescence methods of analysis), photometry (photocolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high performance liquid chromatography) methods.<sup>10,11</sup> Methods such as nuclear magnetic resonance (NMR) and paramagnetic resonance (PMR) are becoming more and more popular.<sup>12</sup> The combination of mass spectroscopy (MS) with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acid-base, precipitation and redox reactions.<sup>13</sup> Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis. The number of new drugs is constantly growing. This requires new methods for controlling their quality.<sup>14</sup>

### 1.1 AIM:

The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for simultaneous estimation of Dapagliflozin, Vildagliptin in bulk and tablet dosage form.

### 1.2 OBJECTIVES:

- To develop a new stability indicating HPLC method for simultaneous estimation of Dapagliflozin and Vildagliptin and to develop the validated method according to ICH guidelines.
- To apply the validated method for the simultaneous estimation of Dapagliflozin and Vildagliptin in pharmaceutical formulation

### 1.3 DRUG PROFILE:

#### Dapagliflozin

Description: dapagliflozin 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.<sup>15</sup>

Structure:

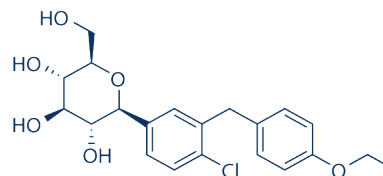


Fig 1.1 Dapagliflozin

#### Vildagliptin

Description:

Vildagliptin is a once-daily dipeptidyl peptidase 4 (DPP-4) inhibitor used in the management of type 2 diabetes mellitus.<sup>16</sup>

Molecular structure:

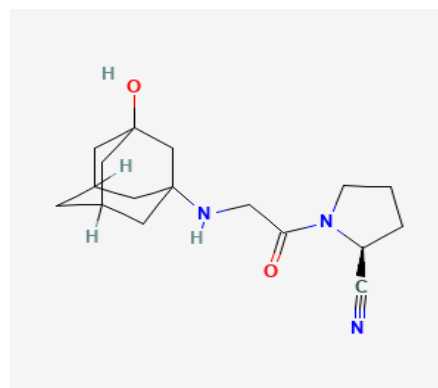


Fig 1.2. Structure Of Vildagliptin

## II. METHODOLOGY

Materials:

- Dapagliflozin and Vildagliptin pure drugs (API) received from spectrum Pharma labs.
- Combination Dapagliflozin and Vildagliptin tablets (Jalra\_Dp) received from local market
- Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are 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 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Dapagliflozin and Vildagliptin solutions.

Methods:

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 70:30

Preparation of Standard stock solutions: Accurately weighed 10mg of Dapagliflozin, 100mg of Vildagliptin was taken and transferred to 100ml volumetric flask separately. 3/4 Th of diluents was added to the flask and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution. (10µg/ml of Dapagliflozin and 100µg/ml of Vildagliptin)

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

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters.(100µg/ml of Dapagliflozin and 1000µg/ml of Vildagliptin)<sup>17</sup>

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

Preparation of buffer:

0.01N Kh<sub>2</sub>po<sub>4</sub> Buffer: Accurately weighed 1.41gm of Potassium dihydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the

volume with water then PH adjusted to 5.4 with dil. Formic acid .

0.1% Formic acid Buffer: 1ml of Conc Formic acid was diluted to 1000ml with water. <sup>18</sup>

Validation:

System suitability parameters:<sup>19</sup>

The system suitability parameters were determined by preparing standard solutions of Dapagliflozin(10ppm) and Vildagliptin(100ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.<sup>20</sup>

Precision:

Preparation of Standard stock solutions: Accurately weighed 10mg of Dapagliflozin, 100mg of Vildagliptin was taken and transferred to 100ml volumetric flask separately. 3/4 Th of diluents was added to the flask and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution. (10µg/ml of Dapagliflozin and 100µg/ml of Vildagliptin)<sup>21</sup>

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (10 µg/ml of Dapagliflozin and 100µg/ml of Vildagliptin) <sup>22</sup>

Preparation of Sample stock solutions: 20 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (100µg/ml of Dapagliflozin and 1000µg/ml of Vildagliptin)<sup>23</sup>

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (10µg/ml of Dapagliflozin and 100µg/ml of Vildagliptin) <sup>24</sup>

Linearity:

Preparation of Standard stock solutions: Accurately weighed 10mg of Dapagliflozin, 100mg of

Vildagliptin was taken and transferred to 100ml volumetric flask separately. 3/4 Th of diluents was added to the flask and sonicated for 10 minutes.

Flasks were made up with diluents and labeled as Standard stock solution. (10µg/ml of Dapagliflozin and 100µg/ml of Vildagliptin)<sup>25</sup>

25% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (2.5µg/ml of Dapagliflozin, and 25µg/ml of Vildagliptin)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (5µg/ml of Dapagliflozin, and 50µg/ml of Vildagliptin)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (7.5µg/ml of Dapagliflozin, and 75µg/ml of Vildagliptin)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (10µg/ml of Dapagliflozin, and 100µg/ml of Vildagliptin)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (12.5µg/ml of Dapagliflozin and 125µg/ml of Vildagliptin)

150% Standard solution: 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (15µg/ml of Dapagliflozin and 150µg/ml of Vildagliptin)

Accuracy:<sup>26</sup>

Preparation of Standard stock solutions: Accurately weighed 10mg of Dapagliflozin, 100mg of Vildagliptin was taken and transferred to 100ml volumetric flask separately. 3/4 Th of diluents was added to the flask and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution. (10µg/ml of Dapagliflozin and 100µg/ml of Vildagliptin)

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters.(100µg/ml of Dapagliflozin and 1000µg/ml of Vildagliptin)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric

flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:<sup>27</sup>

The % Recovery for each level should be between 98.0 to 102

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.8ml/min), Flow plus (1.0ml/min), mobile phase minus, mobile phase plus, temperature minus (27°C) and temperature plus (33°C) 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.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.3ml each of Dapagliflozin, Vildagliptin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents<sup>28</sup>

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.9ml each of Dapagliflozin, Vildagliptin and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.<sup>29</sup>

Degradation studies:

Oxidation:

To 1 ml of stock solution of Dapagliflozin and Vildagliptin, 1 ml of 20% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 10µg/ml&100µg/ml solution

and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.<sup>30</sup>

**Acid Degradation Studies:**

To 1 ml of stock s solution Dapagliflozin and Vildagliptin, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60<sup>0</sup>c the resultant solution was diluted to obtain 10µg/ml&100µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Alkali Degradation Studies:**

To 1 ml of stock solution Dapagliflozin and Vildagliptin, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60<sup>0</sup>c. The resultant solution was diluted to obtain 100µg/ml&10µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Dry Heat Degradation Studies:**

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml&1000µg/ml l solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.<sup>31</sup>

**Photo Stability studies:**

The photochemical stability of the drug was also studied by exposing the 1000µg/ml&100µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200-Watt hours/m<sup>2</sup> in photo stability chamber For HPLC study, the resultant solution was diluted to obtain 10µg/ml&100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.<sup>32</sup>

**Neutral Degradation Studies:**

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60°. For HPLC study, the resultant solution was diluted to 10µg/ml&100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.<sup>33</sup>

**III. RESULTS AND DISCUSSION**

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

Optimized method:

Chromatographic conditions:

Mobile phase:KH<sub>2</sub>PO<sub>4</sub>: Acetonitrile (65:35)

Flow rate: 1 ml/min

Column: Agilent C18 (4.6 x 150mm, 5µm)

Detector wave length :212

Column temperature: 30°C

Injection volume :10µL

Run time :10min

Results : Both peaks have good resolution, tailing factor, Theoretical plate count and resolution. The total runtime for each validation parameter was set to 5mins.

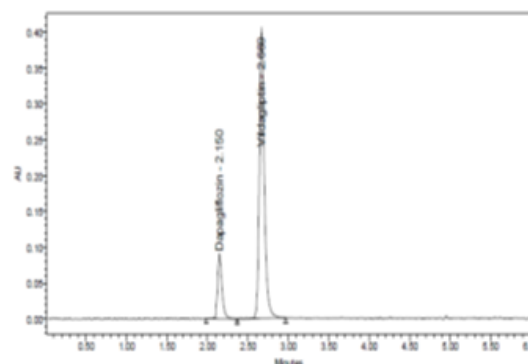


Fig 3.1: Optimized Chromatogram

Observation: Dapagliflozin and Vildagliptin were eluted at 2.150min and 2.669 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Discussion: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Linearity:

Table 3.1: Linearity table for Dapagliflozin and Vildagliptin.

Dapagliflozin		Vildagliptin	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
2.5	130190	25	635948
5	249997	50	1271711

7.5	374482	75	1921264
10	503111	100	2519722
12.5	623372	125	3170373
15	754625	150	3804931

Mean	503462	2548889
S.D	1949.2	28295.4
%RSD	0.4	1.1

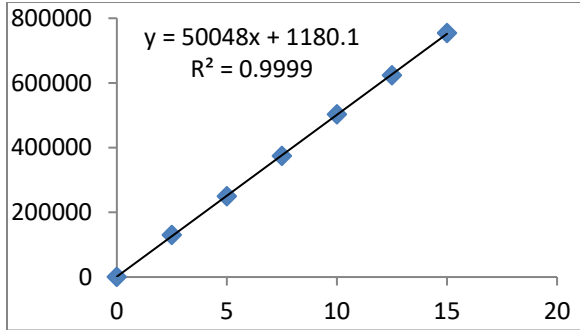


Fig No. 3.3 Calibration curve of Dapagliflozin

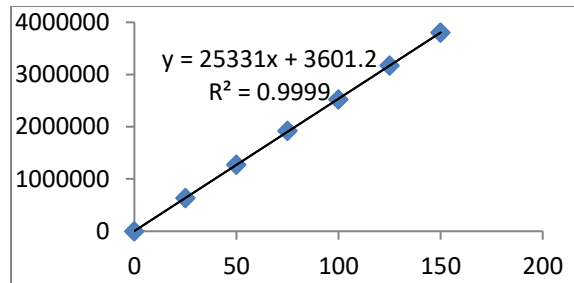


Fig No. 3.4 Calibration curve of Vildagliptin

Discussion: Six linear concentrations of Dapagliflozin (2.5-15µg/ml) and Vildagliptin (25-150µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Dapagliflozin was  $y = 50048x + 1180.1$ . and of Vildagliptin was  $y = 25331x + 3601.2$ . Correlation coefficient obtained was 0.999 for the two drugs.

Precision:

System Precision:

Table 3.2 System precision table of Dapagliflozin and Vildagliptin

S. No	Area of Dapagliflozin	Area of Vildagliptin
1.	503916	2569645
2.	506913	2537089
3.	502195	2524149
4.	501399	2563268
5.	503827	2585739
6.	502520	2513444

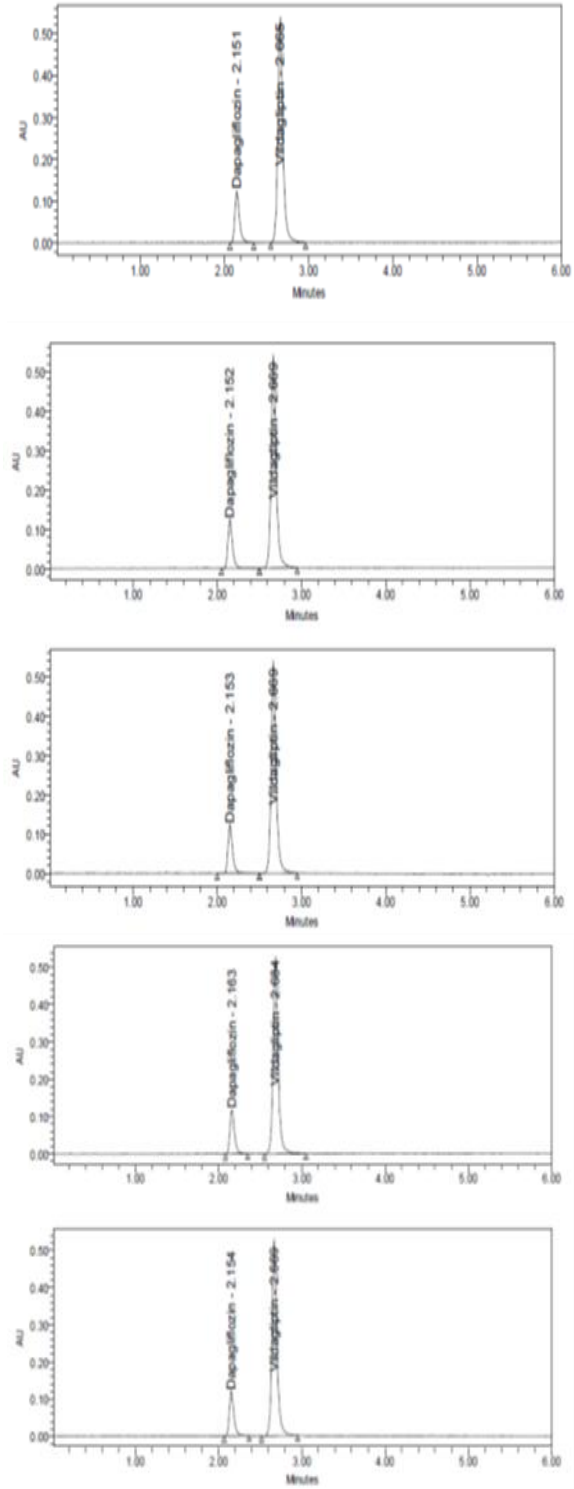


Fig 3.5 System precision chromatogram

Discussion: 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 0.4% and 1.1% respectively for Dapagliflozin and Vildagliptin .As the limit of Precision was less than “2” the system precision was passed in this method.

Accuracy:

Table 3.3 Accuracy table of Dapagliflozin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	5.05	100.92	100.45%
	5	5.00	99.95	
	5	5.07	101.43	
100%	10	10.03	100.28	
	10	10.04	100.43	
	10	10.03	100.33	
150%	15	15.08	100.56	
	15	14.98	99.88	
	15	15.05	100.30	

Table 3.4: Accuracy table of Vildagliptin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	49.74	99.49	99.33%
	50	49.86	99.72	
	50	49.36	98.71	
100%	100	99.20	99.20	
	100	99.61	99.61	
	100	98.73	98.73	
150%	150	147.99	98.66	
	150	150.25	100.17	
	150	149.46	99.64	

Discussion: 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 100.45% and 99.33% for Dapagliflozin and Vildagliptin respectively.

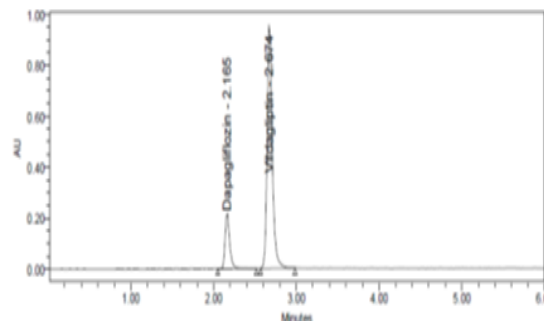


Fig No. 3.6 Accuracy 100% Chromatogram of Dapagliflozin and Vildagliptin

Sensitivity:

Table 3.5 Sensitivity table of Dapagliflozin and Vildagliptin

Molecule	LOD	LOQ
Dapagliflozin	0.006	0.019
Vildagliptin	0.22	0.66

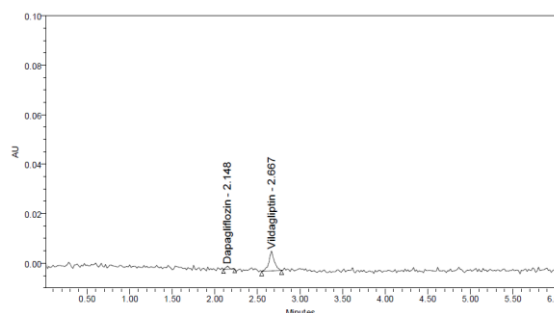


Fig. No. 3.7 LOD Chromatogram of Standard

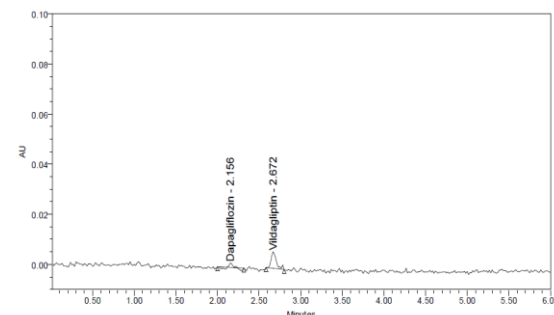


Fig. No.3.8 LOQ Chromatogram of of Standard

Robustness:

Table 3.6 Robustness data for Dapagliflozin and Vildagliptin

S.no	Condition	%RSD of Dapagliflozin	%RSD of Vildagliptin
1	Flow rate (-) 0.8ml/min	0.5	1.5
2	Flow rate (+)	1.2	0.5

	1.0ml/min		
3	Mobile phase (-) 65B:35A	1.1	0.3
4	Mobile phase (+) 75B:25A	3.1	3.7
5	Temperature (-) 27°C	0.9	0.3
6	Temperature (+) 33°C	0.2	0.6

Discussion: Robustness conditions like Flow minus (0.8ml/min), Flow plus (1.0ml/min), mobile phase minus (65B:35A), mobile phase plus (75B:25A), temperature minus (27°C) and temperature plus(33°C) 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.

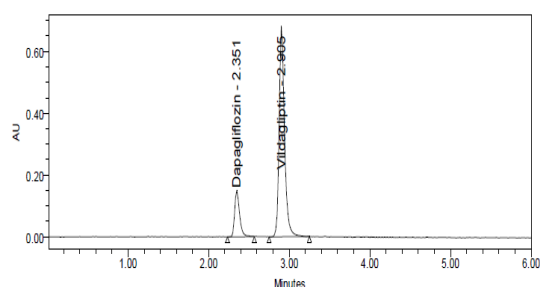


Fig No:3.10 Flow minus Chromatogram of Dapagliflozin and Vildagliptin.

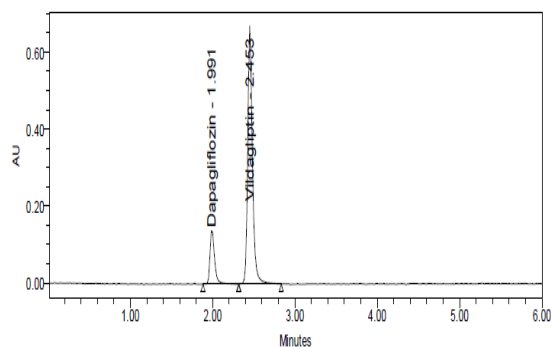


Fig No. 3.11 Flow plus Chromatogram of Dapagliflozin and Vildagliptin.

Assay: Jalra-DP, bearing the label claim Dapagliflozin 100mg, Vildagliptin 10mg. Assay was performed with the above formulation. Average % Assay for Dapagliflozin and Vildagliptin obtained was 99.55% and 99.04% respectively.

Table 3.7 Assay Data of Dapagliflozin

S.no	Standard Area	Sample area	% Assay
1	503916	508165	100.53
2	506913	504150	99.74
3	502195	497178	98.36
4	501399	505161	99.94
5	503827	496987	98.32
6	502520	507706	100.44
Avg	503462	503225	99.55
Stdev	1949.2	4991.3	0.99
%RSD	0.4	1.0	1.0

Table 3.8 Assay Data of Vildagliptin

S. no	Standard Area	Sample area	% Assay
1	2569645	2528584	99.00
2	2537089	2521090	98.71
3	2524149	2522071	98.75
4	2563268	2536474	99.31
5	2585739	2545230	99.66
6	2513444	2524016	98.83
Avg	2548889	2529578	99.04
Stdev	28295.4	9517.0	0.373
%RSD	1.1	0.4	0.4

3.9 Summary Table

Parameters	Dapa-gliflozin	Vilda-gliptin	LIMIT
Linearity Range (µg/ml)	2.5-15 µg/ml	25-150 µg/ml	R < 1
Regression coefficient	0.999	0.999	
Slope(m)	50048x	25331	
Intercept(c)	1180.1	3601.2	
Regression equation (Y=mx+c)	y = 50048x + 1180.1	y = 25331x + 3601.2.	
Assay (% mean assay)	99.55%	99.04%	90-110%
Specificity	Specific	Specific	No interference of any peak
System precision %RSD	0.3%	1.1	NMT 2.0%
Method precision %RSD	0.4%	1.0	NMT 2.0%
Accuracy %recovery	100.45%	99.33%	98-102%

LOD	0.006	0.22	NMT 3
LOQ	0.019	0.66	NMT 10
Robustness	FM	0.5	1.5
	FP	1.2	0.5
	MM	1.1	0.3
	MP	3.1	3.7
	TM	0.9	0.3
	TP	0.2	0.6
			%RSD NMT 2.0

#### IV. CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Dapagliflozin and Vildagliptin in tablet dosage form. Retention time of Dapagliflozin and Vildagliptin were found to be 2.163 min and 2.684 min. %RSD of the Dapagliflozin and Vildagliptin were and found to be 0.3 and 1.0 respectively. %Recovery was obtained as 100.45% and 99.33% for Dapagliflozin and Vildagliptin respectively. LOD, LOQ values obtained from regression equations of Dapagliflozin and Vildagliptin were 0.006, 0.019 and 0.22, 0.66 respectively. Regression equation of Dapagliflozin is  $y = 50048x + 1180.1$ . And  $y = 25331x + 3601.2$  of Vildagliptin. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

#### Disclosure statement

The authors declare no potential conflicts of interest.

#### REFERENCE

- [1] B.k Sharma, Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd Edition Goel publication, Meerut, (2007)
- [2] Lindholm.J, Development and Validation of HPLC Method for Analytical and Preparative purpose. Acta Universitatis Upsaliensis, pg. 13-14, (2004).
- [3] Rashmin, An introduction to analytical Method Development for Pharmaceutical formulations. Indoglobal Journal of Pharmaceutical Sciences, Vol.2, Issue 2, Pg 191-196 (2012).
- [4] Malvia R, Bansal V, Pal O.P and Sharma P.K. A Review of High-Performance Liquid Chromatography. Journal of Global Pharma technology (2010)
- [5] Douglas A Skoog, F. James Holler, Timothy A. Niemen, Principles of Instrumental Analysis Pg 725-760.
- [6] Dr.S. Ravi Shankar, Text book of pharmaceutical analysis, Fourth edition, Pg 13.1-13.2
- [7] David G.Watson. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed., Pg 221-232.
- [8] Remington's The Sciences and Practise of Pharmacy, 20th Edition (2000)
- [9] Connors Ka. A Textbook of Pharmaceutical Analysis, Wiley intersciences Inc; Delhi, 3rd Ed, Pg 373-421, (1994)
- [10] Gurdeep R.Chatwal , Sham K. Anand, Instrumental Methods of Chemical Analysis, Pg 2.566-2.638 (2007)
- [11] David G. Watson Pharmaceutical Analysis, A text book for pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed.,Pg- 267-311
- [12] Ashok Kumar, Lalith Kishore, navpreet Kaur, Anroop Nair. Method Development and Validation for Pharmaceutical Analysis. International Pharmaceutica Scientia, Vol 2, Issue 3, Jul-Sep (2012)
- [13] Kaushal.C, Srivatsava.B, A Process of Method Development: A Chromatographic Approach. J Chem Pharm Res, Vol.2, Issue 2, 519-545, (2010)
- [14] Vibha Gupta, Ajay Deep Kumar Jain, N.S.Gill, Kapil, Development and Validation of HPLC method. International Research Journal of Pharmaceutival and Applied Sciences, Vol 2, Issue 4, Jul-Aug (2012)
- [15] Hokanson GC. A life cycle approach to the validation of analytical methods during Pharmaceutical Product Development. Part 1: The Initial Validation Process. Pharm Tech (1994) 92-100
- [16] IUPAC. Compendium of Chemical Terminology, 2nd edn. (The Gold Book). PAC69, 1137 (1997). Glossary of terms used in computational drug design (IUPAC Recommendations).

- [17] K. D. Tripathi, *Essentials of Medical Pharmacology*, 6th Edition, Jaypee brother's medical publishers (P) LTD, p-254-255.
- [18] B. T. Sadhana, S. K. Mohite, M. Snehal, and R. Sucheta, Development and validation of UV Spectrophotometric methods for simultaneous estimation of Voglibose and Dapagliflozin hydrochloride in bulk and tablet dosage form, *Indo Ame.J.Pharm.Res.3* (2013)7018-7024.
- [19] P.M.Pallavi, R.D.Sonali, C.D.Praveen, Development and validation of UV derivative spectrophotometric methods for the determination OF Glimepiride, Dapagliflozin hydrochloride and Pioglitazone hydrochloride in bulk and marketed formulation, *J. Pharmaceut. Sci. Innov.* 1 (2012) 58-62.
- [20] K. Mousumi and P. K. Choudary, HPLC method for estimation of Dapagliflozin hydrochloride in formulated microspheres and tablet dosage form, *Indian J.Pharmaceut. Sci.* 71(2009) 318-320.
- [21] M. A. Saeed, S. Najma and M. Z. Hashim, Development and validation of RP HPLC method for the Analysis of Dapagliflozin, *Pak. J. Pharm. Sci.*, 19 (2006) 231-235 231.
- [22] K.Fatema, Md. Z. Rahman, H. Tasnuva, Md. A. K. Azad, and Md. R. Selim, Development and validation of a simple method for simultaneous estimation of Dapagliflozin hydrochloride and Gliclazide in tablets by using RP-HPLC, *J.Pharm. Sci.* 9 (2010) 83-89.
- [23] S.Dhirender sing, S.C. Dwivedi, K.Ashok, Development and Validation of a RPHPLC method for simultaneous estimation of Pioglitazone and Dapagliflozin in bulk and tablet dosage form. *Int J. Biomed. Adv. Res.* 3 (2012) 197-201.
- [24] M. A. Saeed, S. Najma, M. H. Zuberi, F. A Siddiqui, and U.Haroon, Simultaneous determination of Dapagliflozin, Captopril, Lisinopril, and Enalapril by RP-HPLC: its application in dosage formulations and in human serum, *Med. Chem. Res.* 22 (2013) 5717-5722.
- [25] G.S.Satya, S.K.Ashutosh, J.Saravanan, M.Debnath, V. Greeshma, N.S.Krishna, A new RPHPLC method development for simultaneous estimation of Dapagliflozin and Alogliptin in bulk as well as in pharmaceutical formulation by using PDA detector, *World J. Pharm. Pharm. Sci.* 2(2013)6720-6743.
- [26] P.Vinay, S.P.Roopa, K.Devi, G.Singh, S.Narayana, and S. Suresh, Development and validation of the liquid chromatographic method for the simultaneous estimation of Dapagliflozin, Pioglitazone, and Glimepiride in pharmaceutical dosage forms, *Pharm. Methods.* 3 (2012) 9-13.
- [27] C.R.Harish, P.Ramalingam, P.V.Vamshi, N.Ramesh, and B.Sreeram, Simultaneous determination of Dapagliflozin hydrochloride, Atorvastatin and Glimepiride in tablet dosage forms by RP-HPLC, *Am. J. Pharm. Tech. Res.* 2(2012) 991-998.
- [28] Murthy TGK, Geethanjali J (2014) Development of a Validated RP-HPLC Method for Simultaneous Estimation of Dapagliflozin Hydrochloride and Rosuvastatin Calcium in Bulk and In-House Formulation. *J Chromatogr Sep Tech* 5:252. (2014) doi:10.4172/2157-7064.1000252.
- [29] M. Prathyusha, M. Sandhya, V.U.M Rao, Method Development and Validation for the simultaneous estimation of Dapagliflozin and Fenofibrate by Rp-Hplc Method in marketed formulation, *Int J. Pharm.* 4 (2014) 219-225.
- [30] Panigrahy U. P, Reddy A. S. K. A novel validated RP-HPLC-DAD method for the simultaneous estimation of Dapagliflozin Hydrochloride and Canagliflozin in bulk and pharmaceutical tablet dosage form with forced degradation studies. *Orient J Chem* (2015); 31 (3).
- [31] N. Padmaja and G. Veerabhadram, Development and validation of analytical method for Simultaneous estimation of Vildagliptin and Linagliptin in bulk drugs and combined dosage forms using UV-visible spectroscopy *Der Pharmacia Lettre*, 2015, 7 (12):306-312.
- [32] Shaik Mahammad Noorulla, Sadath Ali, RP-HPLC Method development and validation for the Simultaneous Estimation of Dapagliflozin and Vildagliptin in Tablet Dosage Form. *IJETS*, (2015);11(2).
- [33] P. Madhusudhan, M. Radhakrishna Reddy and N. Devanna RP HPLC Method Development and Validation for Simultaneous Determination of Linagliptin and Vildagliptin in Tablet Dosage Form. *IARJSET*, (2015); 2 (2).