A REVIEW ON RP-HPLC METHODS FOR QUANTITATIVE ESTIMATION OF VILDAGLIPTIN AND DAPAGLIFLOZIN PROPANEDIOL MONOHYDRATE IN PHARMACUETICAL DOSAGE FORM

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Abstract: Vildagliptin and Dapagliflozin propanediol monohydrate is a dominant and particular inhibitor of dipeptidyl peptidase-4 (DPP-4), orally active, that management of glycemic control in patients with type 2 diabetes (T2DM) firstly by increasing pancreatic islet function. Precise, rapid, and accurate RP-HPLC method for evaluation of Vildagliptin and Dapagliflozin propanediol monohydrate from its tablet dosage form was developed and validated as per ICH guidelines. Substantial literature search on vildagliptin high points majorly high-performance liquid chromatography (HPLC), UV spectroscopy, LC-MS, and HPTLC as analytical methods for analysis from solid and pharmaceutical dosage form. In similar way, detailed literature review discloses HPLC, UV, LC-MS, and HPTLC method for determination of dapagliflozin propanediol monohydrate in bulk and pharmaceutical dosage form. The RP-HPLC method was developed for the resolution of Vildagliptin and Dapagliflozin propanediol mnohydrate in tablet dosage form. The method was validated and establish to be simple, accurate, sensitive, and precise.

Keywords: Diabetes mellitus, Vildagliptin, Dapagliflozin propanediol monohydrate, RP-HPLC, Method developments.

INTRODUCTION:
Diabetes mellitus is nowadays affects more than 400 million people globally, and by 2040 that number is expected to rise to 700 million. Type 2 diabetes mellitus (T2DM) is generally found in adults due to a decrease in the production and activity of insulin. Many anti-diabetic medications have been developed to lower elevated blood glucose levels, but T2DM still causes considerable morbidity and mortality; with more than 6 million deaths in 2020 by diabetes [1]. In addition to vision loss and limb amputation owing to retinopathy and foot sores, the elevated blood glucose level also results in some cardiovascular and kidney problems. As a result, numerous anti-diabetic combinations with various mechanisms of action are created to lower blood glucose levels (HbA1c). [2]. Dipeptidyl peptidase-4 (DPP-4) inhibitors together with vildagliptin, sitagliptin, linagliptin, and others have been broadly used to control diabetes over the past ten decades [3]. Yet only using insulin-dependent anti-diabetic drugs won’t be adequate to lower glycated hemoglobin (HbA1c) levels. Therefore, for better glycemic control, currently created sodium-glucose cotransporter-2 (SGLT-2) inhibitors [4] are given in addition to DDP-4 inhibitors [5], [6]. The fixed-dose combination of vildagliptin and dapagliflozin had a superior cardio-protective effect along with decreased HbA1c levels [7].

Vildagliptin inhibits dipeptidyl peptidase-4 (DPP-4). This in turn inhibits the deactivation of GLP-1 by DPP-4, allowing GLP-1 to potentiate the production of insulin in the beta cells. Dipeptidyl peptidase-4 role in blood glucose control is thought to be through degradation of GIP and the degradation of GLP-1 [8,9]. On contrast, dapagliflozin propanediol monohydrate (DAPA), is chemically an oxane derivative with triol moiety and having hypoglycemic action through suppression of sodium-glucose cotransporter-2 system, which is increase available in combination of other oral hypoglycemic agents operating on different principle for better glycemic control [10].
Extensive literature search on vildagliptin high point majorly UV spectroscopy, high performance liquid chromatography (HPLC), LC-MS, and HPTLC as analytical methods for evaluation from solid and pharmaceutical dosage form [11,12]. In similar way, detailed literature review disclose UV, HPLC, LC-MS, and HPTLC method for resolution of DAPA in bulk and pharmaceutical dosage form [13,14]. The literature survey disclosed only one reverse phase high performance liquid chromatography (RP-HPLC) method for quantification of both the components which has serious limitation. Based on above-mentioned reality, it was decided to develop and validate a new, simple, precise and accurate, stability designating RP-HPLC method for the resolution of Vildagliptin and DAPA in synthetic mixture.

Table 1: Chemical and Physical properties of Vildagliptine and Dapagliflozin propanediol monohydrate (DAPA) [14-20]

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Vildagliptin</th>
<th>Dapagliflozin propanediol monohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUPAC Name</td>
<td>(2S)-1-{2-[3-hydroxyadamantan-1-yl]amino}acetyl]pyrrolidine-2-carbonitrile</td>
<td>(2S)-propane-1,2-diol—(1S)-1,5-anhydro-1-[4-chloro-3-(4-ethoxybenzyl)phenyl]-D-glucitol—water (1/1/1)</td>
</tr>
<tr>
<td>Chemical Formula</td>
<td>C17H25N3O2</td>
<td>C24H35ClO9</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>303.3993 g/mole</td>
<td>502.98 g/mol</td>
</tr>
<tr>
<td>Melting point</td>
<td>153-155°C</td>
<td>74-80°C</td>
</tr>
<tr>
<td>Physical state</td>
<td>Solid</td>
<td>Solid</td>
</tr>
<tr>
<td>solubility</td>
<td>Soluble in Water and Methanol</td>
<td>Soluble in ethanol, methanol, dimethylsulfoxide, and dimethylformamide</td>
</tr>
<tr>
<td>pKa</td>
<td>14.71 &amp; 9.03 Strongest acidic and basic respectively</td>
<td>12.57 source chemaxon</td>
</tr>
<tr>
<td>Therapeutic Use</td>
<td>Used to control hyperglycemia in type 2 diabetes mellitus.</td>
<td>Used to control hyperglycemia in type 2 diabetes mellitus.</td>
</tr>
</tbody>
</table>

EXPERIMENTAL METHODS:
Determination of melting point:
Melting point was determined using digital melting point apparatus. The reference melting point of vildagliptin is 153-155 °C and Dapagliflozin propanediol monohydrate is 74-80°C.

Chemicals and materials:
All used reagents were HPLC grade as; “Methanol, acetonitrile, Tetra butyl ammonium hydroxide, Bio phosphoricacid, Potassium hydrogen phthalate,” purchased. All other chemicals were of analytical reagent grade unless specified. All the glass were washed with detergent, rinsed thoroughly with distilled water, and dried prior to use [21].
Dissolve 0.435 gm (gram) of dipotassium hydrogen phosphate in 100 ml (milliliter) of HPLC water. Filter the solution through 0.45 µ nylon filter. Adjust the pH-6.5 with dilute orthophosphoric acid and degas before use. Preparation of mobile phase: Prepare a mixture of buffer (dipotassium hydrogen phosphate) and acetonitrile in the ratio of 60:40. Adjust the pH 6.5 with diluted ortho phosphoric acid (OPA). This solution was sonicated for 5 min (minute) for degassing and filtered through 0.45 µ millipore filter. Dissolve 0.435 gm (gram) of dipotassium hydrogen phosphate in 100 ml (milliliter) of HPLC water. Filter the solution through 0.45 µ nylon filter. Adjust the pH-6.5 with dilute orthophosphoric acid and degas before use.

Preparation of mobile phase:
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Mobile phase and solutions [23-29]
Mixed a mixture of acetonitrile buffer, and methanol in the ratio of 480:450: 70 and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration. The mobile phase was made by mixing acetonitrile and 0.1% triethylamine (pH-5.0) in ratio 50:50% v/v. The prepared mobile phase was sonicated and filtered through 0.45µm membrane filter. 50:50% v/v. The prepared mobile phase was sonicated and filtered through 0.45μm membrane filter.

Preparation of sample solutions:
Solution state analysis: An correctly weighed 10.0mg of standard DAPA and quantity of tablet powder equivalent to about 10.0mg of DAPA was shifted to series of 10.0mL volumetric flasks. To each flask 10.0mL of reagent (0.1N HCl/ 0.1N NaOH/ 3% H2O2/ Distill water) was added and kept at 600C for a period of 180 min. The sample solutions were withdrawn at 30min interval upto 180 min for all stress conditions. The 1.0 mL stressed samples were diluted to 10.0mL with mobile phase (Conc. 100µg/mL). The content of each flasks were sonicated for 15min and samples were filtered separately. A 6.0mL portion of the above sample solutions were further diluted to 10.0mL with mobile phase (Conc. 60µg/mL). The acidic and alkaline stressed samples were neutralized prior to dilution with mobile phase. A 20µL volume of each finally diluted stressed solution was injected separately and the results of % degradation in alkaline, acid, oxidative and neutral hydrolysis.

Figure 3: Solution state degradation of DAPA

Solid state analysis: Standard DAPA and tablet powder was spread on petri dish kept in the oven at 60°C, humidity chamber at 40 ºC, 75% RH and UV chamber at expect the use of UV radiation 254nm separately. After 48 hours, an accurately measured quantity of 10.0mg of Std. DAPA and weight of tablet powder equivalent to 10.0mg of DAPA was withdrawn and transferred to 10.0mL volumetric flask and volume was made up to the mark with mobile phase separately. The content of the flasks was filtered through 0.45µm nylon filter paper. A 1.0mL portion of the
filtrate was further diluted to 10.0mL with mobile phase. A 6.0 mL portion further diluted to 10.0mL with mobile phase (60µg/mL). A 20µL volume of each final diluted stressed solution was injected separately and the results of % degradation for solid state analysis.

Figure 4: Solid state degradation peak of DAPA

6ml standard reserve solution was pipetted out and diluted up to 10 ml to obtain resultant solution of 60µg/ml. The consequential solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):
LOD and LOQ were determined from the following formulae,
LOD = 3.3 x SD/ S
LOQ = 10 x SD/ S
Where,
SD is standard deviation
S is slope of the calibration curve
LOD is defined as lowest concentration of analytic anticipates to be consistently illustrious from blank and at which recognition is practicable and inside limits. LOD was found to be 0.98 and 2.1 (Table 3). Results suggested that the concentration of Vildagliptin below up to 0.98µg/ml could be successfully detected by the use of current method. LOQ is defined as lowest concentration at which analyte cannot only be found but at which some predefined goals for bias and imprecision are met. The LOQ may be equivalent to LOD or it could be at much higher concentration. LOQ was found to be 2.98 and 6.39 (Table 3). Results showed that the concentration of Vildagliptin 2.98 µg/ml and DAPA in 2.83 µg/ml and higher could be effectively quantified with proposed method.

Table 3: Results obtained for LOD and LOQ.

<table>
<thead>
<tr>
<th>Drug</th>
<th>LOD (µg/ml)</th>
<th>LOQ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vildagliptin</td>
<td>0.98</td>
<td>2.98</td>
</tr>
<tr>
<td>DAPA</td>
<td>2.1</td>
<td>6.39</td>
</tr>
</tbody>
</table>

Validation of Proposed HPLC Method [23-29]:
Validation of the advanced method was carried out as per ICH and USP guidelines.

Accuracy:
Accuracy of the advanced method was ascertained on the basis of recovery studies carry out by standard addition method. Weighed the pre-analyzed tablet powder equivalent to 2.5mg; a known amounts of standard drug was added at different levels 50-150%. The resultant solutions were then re-analyzed by the developed method. At each concentration, each sample was analyzed thrice at each level to check repeatability and from the data it was analyzed that the methods were found to accurate.

Precision:
Precision of any analytical method was indicates as SD and % RSD of series of calculations. Precision of estimation of vildagliptine and DAPA by proposed method was ascertained by replicate analysis of homogeneous samples of tablets.

Ruggedness:
Ruggedness of proposed methods was carry out to examine effect of non procedure related factors such as apparatus and analysts. For this study vildagliptin and Dapagliflozin was analyzed by proposed methods using two different analyst restraining similar operational and environmental conditions.
It is the capacity of the method to remain unaltered by small but deliberate changes in method parameters. The analysis was performed by slightly changing the pH, mobile phase composition, and detection wavelength and flow rate.

**Linearity and Range:**
Accurately weighed tablet powder equivalent to 80, 90, 100, 110 and 120% of label claim was taken and dilutions were made as described under marketed formulation. Then each solution was injected and chromatograms were recorded. A graph of concentration Vs. Area under curve was plotted for the drug.

![Figure 5: Linearity and range plot of Vildagliptin](image)

![Figure 6: Linearity and range plot of DAPA](image)

**Precision:**
From the calibration range found in aforementioned section 6.2.3.1, three quality control (QC) standards were decided viz. 35, 55 and 75µg/ml as LQC, MQC and NQC respectively. The solutions for QC standards were prepared by diluting stock solution (100µg/ml) of 1.5, 3.5 and 5.5ml solutions up to 10 ml. Area of each QC standard was recorded for intra-day and inter-day precision in six replicates as per ICH guidelines Q2R1. Results were noted to compute mean, standard deviation (SD) and percent relative standard deviation (%RSD) [30].

% Accuracy from precision data: Percent accuracy was determined using observations of precision study using following formula. Limit for % accuracy is NMT 5% RSD.

\[
\% \text{ Accuracy} = \frac{\text{Mean Measured concentration} - \text{Nominal Concentration}}{\text{Nominal Concentration}}
\]

**CONCLUSION:**
The RP-HPLC method was evolved for the determination of Vildagliptin and Dapagliflozin propanediol monohydrate in tablet dosage form. The method was validated and found to be simple, accurate, sensitive, and precise. Hence, it can be used successfully for routine analysis of Vildagliptin and Dapagliflozin propanediol monohydrate from its tablets.

The proposed composition was point to develop a precise, sensitive, and accurate rp-hplc method for the estimation of Vildagliptin and Dapagliflozin propanediol monohydrate and in bulk drug and in pharmaceutical tablet dosage form. To make it convenient the analysis of the constituent peaks of Vildagliptin and Dapagliflozin propanediol monohydrate, composition of methanol with water in mixed amalgamation were tried as mobile phase on a c18 stationary phase.
REFERENCES: