

RP-HPLC Method for Dapagliflozin and Metformin HCL in Bulk and Combined Formulation

Khagga Bhavyasri¹, Thaneeru Surekha¹, Samreen Begum¹, Mogili Sumakanth¹

¹Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Affiliated to Osmania university, Barkatpura, Hyderabad, Telangana-500027, India.

Abstract

The given RP-HPLC technique was found to be clear-cut, specific, exact, and economical for estimation of Dapagliflozin and Metformin HCl in bulk and tablet dosage form. Stationary phase Phenomenex C18 250mm x 4.6 mm were utilized as chromatographic conditions. Water: Methanol in the ratio of 50:50 was used as fluid phase. The flow rate was maintained at 5 1.0ml/min, wave length of detection was 230 nm and diluent was water all maintained at a 30°C column temperature. As an optimized procedure, the conditions were finalized. By injecting the standard six times, apt system factors were studied and the outcomes were considerably below the acceptance criteria. Metformin HCl linearity was found to be 2-7 ppm and Dapagliflozin linearity was found to be 60-210 ppm with $r^2=0.999$. For Dapagliflozin and Metformin HCl, precision value was found to be 0.8214% and 0.6342% respectively. For Dapagliflozin, LOD and LOQ values were found to be 345000 ppb and 415000 ppb respectively and for Metformin HCl LOD and LOQ values were found to be 263000 and 324000 ppb respectively. Assessment of the formulated market was carried out by using above method and percentage of Dapagliflozin and Metformin HCl was found to be 99.73 % and 99.85 % respectively.

Keywords: Dapagliflozin, Metformin, Liquid chromatography, Combined dosage forms, Simultaneous estimation

INTRODUCTION

The many forms of drugs used to treat diabetes are known as antidiabetic medicines. All of these medications lower blood glucose levels to a safe level (normoglycemia) and relieve diabetes symptoms like increased urination, thirst and ketoacidosis. Long-term consequences of diabetes, such as nephropathy (kidney disease), neuropathy (nerve damage), and retinopathy (damage to the retina of the eye), are also prevented or slowed by antidiabetic medications. A condition known as Type I diabetes causes the body to produce insulin. Therefore, for the effective treatment of type 1 diabetes, insulin is the only medication. Injected insulin decreases blood glucose levels by acting like natural insulin. Insulin resistance is a situation in which the cells of the body do not respond to insulin in the same manner that they do in those without diabetes [1-5].

Glycosuria - SGLT-2 inhibitors prevent glucose from being reabsorbed in the renal tubules, causing glucose to be lost in the urine. This results in slight weight loss and a drop in blood sugar levels, putting you at risk of hypoglycemia. Oral preparations can be used alone or in combination with other drugs. They are preferred as a second or third drug for type 2 diabetics who are controlled with Metformin Hydrochloride, in addition to GLP-1 agonists [6-11]. For diabetic patients with cardiovascular illness, these are considered as first-line treatments, particularly heart failure,

because they lower the risk of hospitalization. Dapagliflozin, Canagliflozin, and Empagliflozin are some of examples.

Biguanides - Biguanides decrease hepatic glucose production while increasing glucose absorption in the peripheral tissues, such as skeletal muscle [12-15]. Metformin Hydrochloride, a biguanide, is the most often used medication for type 2 diabetes in adolescents and teenagers, albeit in patients with poor liver or kidney function it should be used with caution. Metformin Hydrochloride is the only commonly prescribed diabetic medicine that does not cause weight gain [16-20].

Description of Dapagliflozin

In patients with type 2 diabetes, this drug is used to control

Address for correspondence: Khagga Bhavyasri, Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Affiliated to Osmania university, Barkatpura, Hyderabad, Telangana-500027, India. bhavya.khagga@gmail.com

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high blood sugar. Dapagliflozin controls high blood sugar and helps to prevent loss of limbs, kidney damage and blindness. Sexual function and nerve problem are also prevented. To reduce the risk of possible heart failure, this medication is also used in people with type 2 diabetes and heart disease [21-26]. Structure of Dapagliflozin is shown in **Figure 1**.

Structure:

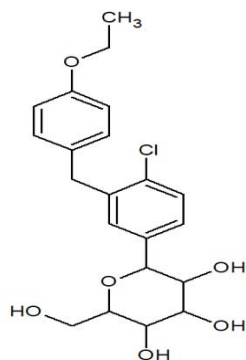


Figure 1. Dapagliflozin structure

Molecular Weight: 408.873

Molecular Formula: C₂₁H₂₅ClO₆

Solubility: Methanol, Water, Acetonitrile [27-29]

Mechanism of action: Sodium-glucose co transporter2 (SGLT2) is inhibited by Dapagliflozin, this co transporter is primarily located in the proximal tubule of the nephron. Because SGLT2 helps in the 90% resorption of glucose in the kidneys, inhibiting it results in the excretion of glucose to be eliminated in the urine [30-32]. In type 2 diabetes patients, this excretion not only allows for better glycemic management but also possible loss of weight.

pKa: 12.6

Uses: Regulating high blood sugar levels in persons with type 2 diabetes helps to avoid renal damage and reduces the risk of heart attack or stroke.

Description of Metformin Hydrochloride

It's a drug that's used to treat type 2 diabetes. Metformin is a biguanide that is used to treat hyperglycemia. It reduces the liver's glucose synthesis while increasing the insulin sensitivity of bodily tissues [33]. Structure of Metformin HCl is shown in **Figure 2**.

Structure:

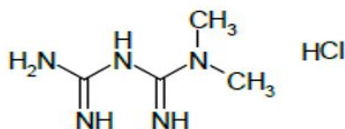


Figure 2. Metformin HCl Structure

Molecular Weight: 129.1639

Molecular Formula: C₄H₁₁N₅

Solubility: Water

Mechanism of action: Metformin lowers blood glucose levels through lowering hepatic glucose synthesis, as well as lowering glucose absorption and use in the intestine.

pKa: 12.4

Uses: It controls the blood sugar, prevents the damage of kidneys, blindness and nerve problems.

Significance of Combined Dosage Form

In people with type 2 diabetes, a combination is recommended to improve glycemic control. They are available in the form of extended-release tablets. Dapagliflozin prevents the absorption of glucose in the kidneys. This helps to lower the blood glucose levels. Metformin Hydrochloride act by lowering sugar absorption from the stomach and lowering sugar release from the liver. Patients with type 2 diabetes and heart disease are given this medicine to reduce their risk of heart failure [34].

Available brands are: Xigduo, Oxramet

MATERIALS AND METHODS

Dapagliflozin & Metformin Hydrochloride pure drug was obtained as gift sample from Gland Pharma Hyderabad, India. Dapagliflozin & Metformin HCl (OXRAMET) formulation was purchased from local drug store [11]. HPLC grade Methanol, HPLC grade Water. Analytical grade sodium hydroxide (NaOH), hydrochloric acid (HCl), hydrogen peroxide (H₂O₂) were put to work. Marketed formulation of Dapagliflozin and Metformin HCl is shown in **Figure 3**.

Diluent: Water was chosen based on drug solubility.



Figure 3. Formulations of Dapagliflozin and Metformin HCl

Dapagliflozin Standard Stock Solution Preparation

Weigh 10mg of Dapagliflozin accurately and transfer to 10 ml and volumetric flasks, then add 3/4th of the diluent and sonicate for 10 minutes. The flask was filled up to the mark with diluent and labelled as Standard stock solution (1000 g/ml of Dapagliflozin).

Metformin Standard Stock Solution Preparation

Weigh 10mg of Dapagliflozin accurately and transfer to 10 ml and volumetric flasks, then add 3/4th of the diluent and sonicate for 10 minutes. The flask was filled up to the mark with diluent and labelled as Standard stock solution (1000 g/ml of Metformin).

Standard Working Solution Preparation (Dapagliflozin)

Using a micropipette, 0.02ml of Dapagliflozin from stock solution was extracted into a 10ml volumetric flask and made up with diluent (0.2 µg/ml of Dapagliflozin).

Standard Working Solutions Preparation (Metformin)

Pipette out 0.1ml of Metformin from stock solution into a 10ml volumetric flask and fill with diluent (10µg/ml of Metformin).

Preparation of Combined Standard Working Solutions

1 ml 0.2 ppm of Dapagliflozin and 1 ml of 10 ppm Metformin HCl was pipetted into 10 ml volumetric flask and three quarters of diluent was added and sonicated for 10 minutes and made up to the mark with diluent to get mixed standard solution containing 0.2 ppm of Dapagliflozin and 10ppm of Metformin, so that the drugs Dapagliflozin and Metformin were in the ratio equal to that of the marketed formulation.

Preparations of Serial Dilutions for Calibration Curve

For Metformin HCl: Pipette 0.1 ml from the stock into a 100 ml volumetric flask, add the diluent, shake vigorously, and make up the solution with water to reach a concentration of 100 ppm. Pipette out 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 ml of the 100 ppm solution and transfer to separate 10 ml volumetric flasks, making up the final volume to 10 ml with diluent to obtain 2, 3, 4, 5, 6, 7 ppm solutions respectively.

For Dapagliflozin: Pipette 0.6, 0.9, 1.0, 1.2, 1.5, 1.8, 1.8, 2.1 ml from 1000 ppm solution into separate 10 ml volumetric flasks and dilute to 10 ml with diluent to give 60, 90, 120, 150, 180, 210 ppm solutions, respectively

Preparations of Serial Dilutions for Accuracy

For Metformin HCl: To the sample solution of 0.2+10 ppm of Dapagliflozin and Metformin HCl, 2 ppm of Metformin standard is spiked at 50% accuracy level. 4 ppm of Metformin standard is spiked at 100% accuracy level. 6 ppm of Metformin standard is spiked at 150% accuracy level.

For Dapagliflozin: To the sample solution of 0.2+10 ppm of Dapagliflozin and Metformin HCl, 60 ppm of Dapagliflozin standard is spiked at 50% accuracy level. 120 ppm of Dapagliflozin standard is spiked at 100% accuracy level.

180 ppm of Dapagliflozin standard is spiked at 150% accuracy level.

RESULTS AND DISCUSSION

Validation of the Method

This was done as per system specificity, exactness, precision, limit of detection (LOD), and quantitation, suitability and linearity the method was validated.

System Suitability

Working standard was produced as per procedure from a Dapagliflozin and Metformin standard solution and injected five times into the HPLC system. The determination of the system suitability factors was performed by using standard Chromatograms, which were acquired by computing the percent RSD of retention time, theoretical plates, tailing factor and peak areas from five replicate injections. Standard deviation and Relative standard deviation (%RSD) were all within the bounds. R_t of Dapagliflozin and Metformin HCL was found to be 3.338 and 2.178 respectively.

Specificity

No peaks were observed when blank was injected.

Precision

Repeatability: Six combined working standard solutions of Dapagliflozin 0.2 ppm and Metformin 10 ppm are injected and followed by calculation of the percentage amount and %RSD was found to be 0.8214 and 0.6342 for Dapagliflozin and Metformin HCl respectively.

Linearity

Linearity of assay method is demonstrated by injecting 6 standard solutions about 2 ppm to 7 ppm of Metformin and 60 ppm to 210 ppm Dapagliflozin. The Graph for Metformin HCl was plotted by taking concentrations against peak area which is shown in **Figure 3**. The Graph for Metformin HCl was plotted by taking concentrations against peak area which is shown in **Figure 3**. Linearity for Metformin HCl at 25%,50% ,75%,100%,125% and 150% was found to be 2.172,2.174,2.160,2.170,2.171 and 2.173 respectively. Linearity for dapagliflozin at 25%,50%, 75%, 100%, 125% and 150% was found to be 3.336, 3.338, 3.311, 3.330, 3.329 and 3.331 respectively

Accuracy

In triplicate manner three Concentrations of 50%, 100%, 150% are injected. For metformin, mean %recovery was found to be 99.83 %, 99.79 % and 99.75 % for 50%, 100% and 150% respectively. For Dapagliflozin, mean %recovery was found to be 99.45 %, 99.79 % and 99.84 % for 50%, 100% and 150% respectively.

Limit of Detection (LOD)

In this method LOD of Dapagliflozin and metformin was found to be 345000 ppb and 263000 ppb respectively.

Limit of Quantitation (LOQ)

In this method LOQ of Dapagliflozin and metformin was found to be 415000ppb and 324000 ppb respectively.

Robustness

Flow rate -0.1ml, flow rate +0.1ml are part of the small deliberate changes made in this method. %RSD OF DAPA and MET for Flow rate, -0.1ml/min was found to be 0.6214 and 0.7369 respectively. %RSD OF DAPA and MET for Flow rate, +0.1ml/min was found to be 0.8624 and 0.5159 respectively.

Assay of Marketed Formulation

Sample and standard solutions were individually injected into the system facilitating the recording of chromatograms and calculation of the present drug in sample. Assay Chromatogram of Standard is shown in **Figure 4**. Assay Chromatogram of Sample is shown in **Figure 5**. **Table 1** shows the %Assay results of Dapagliflozin and Metformin HCl. **Table 2**: Summary of HPLC Validation Parameters.

Calculation

$$\begin{aligned}
 \text{ASSAY} = & \frac{\text{Sample peak area}}{\text{Standard peak area}} \\
 & \times \frac{\text{Standard dilution factor}}{\text{Sample dilution factor}} \\
 & \times \frac{\text{Average weight of tablets}}{\text{Label claim}} \\
 & \times \text{Potency of standard}
 \end{aligned} \quad (1)$$

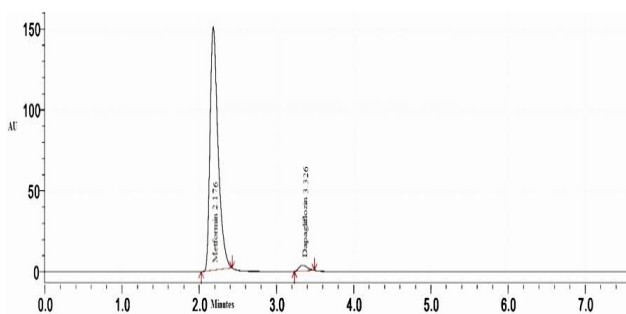


Figure 4. Assay Chromatogram of Standard

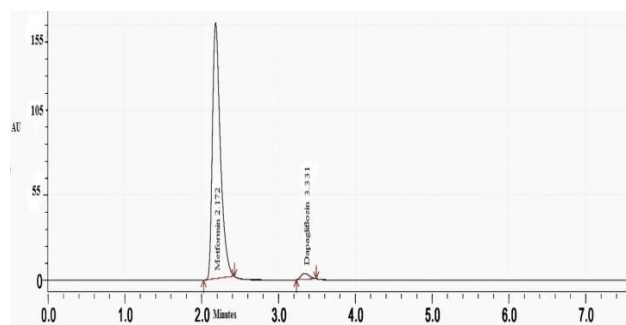


Figure 5. Assay Chromatogram of Sample

Table 1. %Assay results

Name of the Drug	Label claim	% Assay
Dapagliflozin	10 mg	98.41
Metformin	500 mg	99.58

Table 2. Summary of Validation Parameters (HPLC)

Parameters	Dapagliflozin	Metformin
Calibration range (µg/ml)	60 - 210 ppm	2-7 ppm
Optimized wave length	230 nm	230 nm
Retention time	2.178 min	3.338 min
Correlation coefficient (r ²)	0.999	0.999
Precision (%RSD)	0.8214	0.6342
% Recovery	99.79%	99.78 %
Limit of Detection (ppb)	345000	263000
Limit of Quantitation (ppb)	415000	324000

CONCLUSION

The given RP-HPLC technique was found to be clear-cut, specific, exact, and economical for estimation of Dapagliflozin and Metformin HCl in bulk and tablet dosage form. Stationary phase Phenomenex C₁₈ 250mm x 4.6 mm, 5µ. The Mobile phase comprising of Water: Methanol in the ratio of 50:50 and flow rate was maintained at 1.0ml/min, detection wave length was 230 nm and maintaining of the temperature at 30°C are the chromatographic conditions used. Water is used as a diluent. As an optimized procedure, the conditions were finalized. By injecting the standard six times, system suitability parameters were studied, and the results were considerably below the acceptance criteria. Linearity study for Metformin HCl was found to be 2-7 ppm and for Dapagliflozin it was found to be 60-210 ppm, with r²=0.999. For Dapagliflozin and Metformin HCl, precision value was found to be 0.8214% and 0.6342% respectively. LOD and LOQ are 345000 ppb and 415000 ppb respectively for Dapagliflozin. LOD and LOQ are 263000 and 324000 ppb respectively for Metformin. Assessment of the formulated market was carried out by using above method and percentage of Dapagliflozin and Metformin HCl was found to 99.73 % and 99.85 % of Dapagliflozin and Metformin HCl respectively.

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