

# Development and Validation of RP-HPLC Method for Estimation of Dapagliflozin in Raw and Bulk Dosage Form

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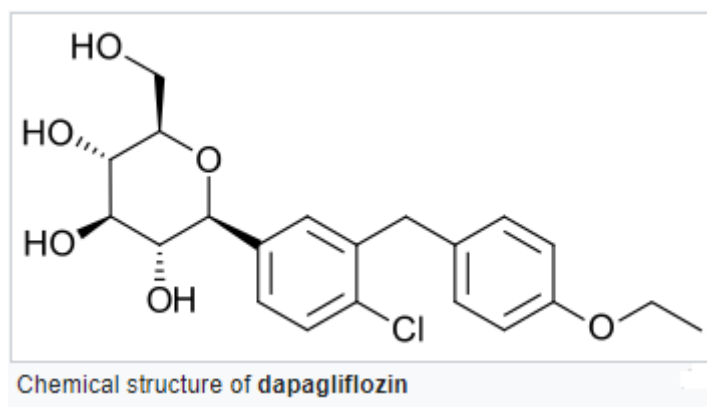
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**Abstract-** Dapagliflozin is used as Antidiabetic drug that works on kidney, by reabsorption of glucose in kidney by sodium glucose co-transporter. [1] Drug is used alone or in combination with drugs such as Saxagliptin, Metformin, Glimepiride. Various analytical methods are used for the estimation of API Dapagliflozin, such as RP-HPLC, UPLC and UV Spectroscopy. This review mainly focuses on the gathering information about mobile phases and other chemicals used in the separation and estimation of Dapagliflozin in single or in combination by RP-HPLC. The following study depicts the review on analytical methods which include estimating the Antidiabetic drug.

**Keywords-** Dapagliflozin, Diabetes Mellitus, RP-HPLC

## INTRODUCTION

Type 2 Diabetes Mellitus (T<sub>2</sub>DM) is a chronic as absolute or relative insulin deficiency [2]. Pancreatic  $\beta$ -cell function is gradually deteriorated in patients of Type 2 Diabetes Mellitus which is reflected into inadequate glycemic control on a long run [4]. Dapagliflozin is chemically known as (1S)-1, 5-anhydro-1-C- [4-chloro-3-[(4-ethoxy phenyl)methyl]-D-glucite with molecular formula C<sub>21</sub>H<sub>25</sub>ClO<sub>6</sub> and molecular weight 408.98 g/mol. [3] Dapagliflozin is used Antidiabetic drug that works on kidney, by reabsorption of glucose in kidney by sodium glucose co-transporter [1]. Dapagliflozin's mechanism of action is complementary to and different from the mechanisms of currently available antidiabetic drugs as it involves the direct and insulin independent estimation of glucose by the kidney [5]. DAPA selectively blocks for the SGLT<sub>2</sub> Over SGLT<sub>1</sub>



Dapagliflozin reduces plasma glucose concentration by elevating the renal glucose excretion [6]. In addition, recent studies have shown relatively fast action of dapagliflozin, with decreases in fasting plasma glucose levels with in one week of treatment [7]. The critical and important physicochemical characterization of dapagliflozin is given in table 1. [8].

Parameter	Discription
CAS Number	461432-26-8
Molecular formula	C <sub>21</sub> H <sub>25</sub> ClO <sub>6</sub>
Molecular Weight	408.98 g/mol
Appearance	Solid
Melting Point	74.78°C
Solubility	Ethanol , Dimethyl formamide
Drug Type	Approved

**Table 1: Physicochemical parameters of Dapagliflozin**

**Mechanism of action:**

Dapagliflozin blocks reabsorption of filtered glucose in the kidney, increasing urinary glucose excretion and reducing blood glucose levels. Its mechanism of action is **independent of pancreatic  $\beta$  cell function and modulation of insulin sensitivity**

**Pharmacokinetics:**

Dapagliflozin metabolism **occurs predominantly in the liver and kidneys by uridine diphosphate-glucuronosyltransferase-1A9 to the major metabolite dapagliflozin 3-O-glucuronide** (this metabolite is not an SGLT2 inhibitor at clinically relevant exposures).

**Pharmacodynamics:**

Dapagliflozin is an orally active, highly selective SGLT2 inhibitor that improves glycemic control in patients with type 2 diabetes mellitus (T2DM) by **reducing renal glucose reabsorption leading to urinary glucose excretion (glucosuria)**.

**Standard solution preparation**

Twenty tablets were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well [9,10]. A quantity of powder of Dapagliflozin equivalent to 100 mg were transferred to clean and dry 100 ml volumetric flask and 70 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade m ethanol. One ml (1 ml) of the prepared stock solution diluted to 100 ml and was filtered through membrane filter (0.45  $\mu$ m) and finally sonicated to degas

**Sample solution preparation**

25 mg of Dapagliflozin working standard was accurately weighed and transferred into a 25 ml clean dry volumetric flask. Add about 20 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution) [11]. Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 10 ppm Dapagliflozin working standard solution. The solution was mixed well and filtered through 0.45  $\mu$ m filter. 12

**Preparation of Standard solution**

Accurately weigh and transfer 10 mg of Metformin and Dapagliflozin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.6 ml of the above Metformin and 0.3ml of the Dapagliflozin stock solutions into a 10ml volumes ic flask and dilute up to the mark with diluent.

**Preparation of Sample solution**

Take average weight and crush in a mortar by using pestle and weight powder 10 mg equivalent weight of Metformin and Dapagliflozin sample into a 10mL clean dry volumetric flask and add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.6 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. 21

**Standard & Sample preparation for analysis:**

25 mg of Dapagliflozin standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.

Further dilution was done by transferring 0.1ml of the above solution into a 10ml volumetric make up to volume with mobile phase.

14

#### **Preparation of Standard Solutions**

Standard stock solution of Dapagliflozin was prepared by dissolving 10 mg of Dapagliflozin in 10 ml of diluent (Buffer: Acetonitrile, 50:50 v/v) in a 10 ml clean dry volumetric flask and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by Sonicator to get the concentration of 1000µg/ml of Dapagliflozin. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Dapagliflozin.

#### **Preparation of Sample Solutions of Dapagliflozin**

Twenty tablets were accurately weighed and powdered and tablet powder equivalent to 262 mg of Dapagliflozin was taken into 10 ml clean dry volumetric flask, diluent was added and sonicated to dissolve completely and volume was made up to volume with the diluent. The above sample solution was filtered and suitably diluted to get a concentration of and 80 µg/ml of Dapagliflozin. 2

Sr No.	Matrix ISSN: 2455-2631	Technique	Mobile Phase	Column	Maximum Absorbance	Flow Rate (ml/min.)	R.T.	Reference
					April 2023 IJSDR   Volume 8 Issue 4			
1	Dapagliflozin & saxagliptin	HPLC	Phosphate Buffer (PH 4) & Acetonitrile (50:50 v/v)	XTerra C18 (150mm*4.6mm, 5µ)	225nm	1ml/min	2.7min	2
2	Dapagliflozin & impurities in tablet dosages form	HPLC	Mobile Phase A- Buffer PH 6.5 Mobile Phase B- Acetonitrile: Water (90:10)	Hypersil BDS C18 (250nm*4.6mm, 5µ)	245nm	1ml/min	2.8 min	6
3	Dapagliflozin & saxagliptin in bulk and tablet dosages form	RP-HPLC	Acetonitrile: Water (60:40)	XTerra RP18 (150mm*4.6mm, 5µ)	248nm	1ml/min	2.09min	9
4	Dapagliflozin in tablet dosages form	RP-HPLC	Buffer: Acetonitrile: Methanol (60:37:03% v/v/v)	Zorbax Eclips XDB C18 (150nm*4.6mm, 5µ)	220nm	1ml/min	5.9 min	10
5	Dapagliflozin API	RP-HPLC	Buffer: Acetonitrile (60:40)	Hypersil BDS C18 (250nm*4.6mm, 5µ)	245nm	1ml/min	2.7 min	11
6	Dapagliflozin in bulk and tablet dosages form	RP-HPLC	Phosphate Buffer: Acetonitrile (60:40 v/v)	Waters C18 25cm*4.6mm,5µ )	237nm	1ml/min	3.4 min	12
7	Dapagliflozin & saxagliptin in formulation	RP-UPLC	0.1% O-phosphoric Acid: Acetonitrile (40:60)	Ethylene bridge Hybrid (BEH) C18 (2.1*100mm,1.7 µ)	254nm	0.3ml/min	1.2 min	13
8	Dapagliflozin in bulk and tablet dosages form	RP-HPLC	Methanol: Acetonitrile: O-phosphoric Acid (75:25:05 v/v/v)	C18(25cm*4.6m m,5µ)	246nm	1ml/min	2.7 min	14
9	Dapagliflozin & Metformin HCl in bulk and tablet dosages form	RP-HPLC	Water: Methanol (50:50)	Phenomenex C18 (250nm*4.6mm, 5µ)	230nm	1ml/min	2.1 min	15
10	Saxagliptin, Dapagliflozin and Metformin HCl	RP-HPLC	Buffer PH4: Acetonitrile (50:50)	LC-20 AT C18(250mm*4.6 mm,2.6µ)	258nm	1ml/min	4.2 min	16
11	Dapagliflozin & Saxagliptin	HPLC	Acetonitrile: Phosphate Buffer (26:74v/v)	SPOLAR C18 (250mm*4.6mm, 5µ)	236nm	0.96ml/min	3.5min	17
12	Dapagliflozin in Raw and in tablet dosage form	RP-HPLC	Methanol: Water (75:25v/v)	C18 Zorbax Eclipse plus (150mm*4.6mm, 25µ)	230nm	1ml/min	3.1 min	18
13	Dapagliflozin in tablet dosage form	HPLC	Acetonitrile: Water acidified with 0.1% of formic acid (42:58v/v)	Synergic fusion C18 (150*4.6mm,20µ )	245nm	1ml/min	10 min	19
14	Saxagliptin, Dapagliflozin in tablet dosage form	RP-HPLC	Acetonitrile: 0.1% O-Phosphoric acid (50:50)	C18 (250mm*4.6mm, 5µ)	210nm	0.98ml/min	3.4 min	20

15	Dapagliflozin & Metformin in bulk and in synthetic mixture	RP-HPLC	Acetonitrile: Water (75:25% v/v)	Phenomenex Luna C18 (250nm*4.6mm I.D,5μ)	285nm	1ml/min	5.4 min	21
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**RP-HPLC method (23)**

Separation by RP – HPLC is similar to the extraction of different compounds from water into an organic solvent, where more hydrophobic (non - polar) compounds extract into the non - polar phase. The column (typically C8 and C18 bonded phase) is less polar than the water - organic phase. Sample molecules partition between the polar mobile phase and non – polar C8 and C18 stationary phase and more hydrophobic (non - polar) compounds are retained more strongly. Polar compounds are less strongly held and elute from the column first and vice versa. In RP – HPLC the retention of a compound is determined by its polarity, experimental conditions, mobile phase, column and temperature. RP – HPLC columns are efficient, stable and reproducible.

**Dosage and Administration:**

**Initial dose:** 5 mg orally once a day may increase to 10 mg orally once a day for additional glycaemic control if lower dose has been tolerated

**Maximum dose:** 10 mg/day to reduce the risk of hospitalization for heart failure: 10 mg orally once a day.

**Applications of HPLC method (22):**

- The various applicability, speed, sensitivity of HPLC is the most popular chromatography technique used for purification and all types of biological molecules.
- The system is widely used in clinical and pharmaceutical work as it possible to apply biological fluids such as serum and urine directly to the column.
- RP-HPLC has biggest impact on the separation of oligo peptides and proteins. Wide range of applications in organic chemistry.
- Chromatography separation of anions can be carried out by using ion exchange ion pair chromatography and ion exclusion chromatography.
- Chromatography separation of cation superficially sulphonated inert polymer resins have been used. Most widely used in Agri chemicals i.e., analysis of pesticides in cleaning water.
- Mainly applied in food analysis Widely applied in forensic science for the separation of morphine and metabolites extracted from blood plasma.
- Modern applications are mainly in pharmaceuticals.

**CONCLUSION:**

The results of the analysis of pharmaceutical dosage form by the developed RP-HPLC method are highly accurate, precise and robust and are in good agreement with the label claim of the drug. A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Dapagliflozin API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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