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REVIEW ARTICLE
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"A REVIEW ON ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN: A COMPREHENSIVE REVIEW"

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Abstract

Dapagliflozin, a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor, is widely prescribed for the management of type 2 diabetes mellitus due to its novel mechanism of promoting renal glucose excretion and improving glycemic control. With its growing clinical use and the availability of multiple fixed-dose combinations, the need for reliable, validated analytical methods to support drug development, formulation, quality control, and regulatory compliance has increased significantly. This comprehensive review summarizes the various analytical techniques reported for dapagliflozin, including UV-spectrophotometry, RP-HPLC, UPLC, LC-MS/MS, and stability-indicating assays.

The article discusses the application of these methods in bulk drug and dosage form assay, simultaneous estimation in combination formulations, forced-degradation and stability studies, impurity profiling, bioanalytical quantification in plasma for pharmacokinetic studies, and dissolution testing. Validation parameters such as linearity, precision, accuracy, sensitivity (LOD/LOQ), robustness, and specificity, as outlined by ICH guidelines, are highlighted across reported methods. Special emphasis is given to stability-indicating methods that separate dapagliflozin from its degradants under acid, base, oxidative, photolytic, and thermal conditions.

Despite the effectiveness of conventional methods, challenges such as the use of phosphate buffers and large volumes of toxic organic solvents raise concerns about instrument damage, operator safety, cost, and environmental sustainability. To overcome these limitations, emerging trends focus on developing greener, eco-friendly, and high-throughput analytical approaches.

In conclusion, the review provides an updated and detailed insight into existing analytical strategies for dapagliflozin, while also emphasizing the future need for simple, robust, cost-effective, and environmentally sustainable methods. These advancements will play a crucial role in ensuring the safe and efficient use of dapagliflozin in pharmaceutical development, routine quality control, and clinical research.

Keywords:- Dapagliflozin, Sodium–glucose cotransporter-2 (SGLT2) inhibitors, Antidiabetic drug, Analytical method development, Method validation, International Council for Harmonisation (ICH) guidelines, Pharmaceutical analysis.

Introduction

Diabetes mellitus is a long-term metabolic disorder that poses a major public health challenge worldwide. It not only reduces life expectancy and quality of life but also creates a substantial economic and social burden. Globally, it is recognized as one of the ten leading causes of death in adults, accounting for an estimated four million deaths in 2017 alone [1]. The constant search for better treatment strategies has led to the development of novel drug classes, among which sodium-glucose co-transporter-2 (SGLT2) inhibitors represent a remarkable breakthrough. Dapagliflozin, the first agent approved in this category, has become a cornerstone in modern diabetes therapy [2].

In the United States, almost 40% of patients diagnosed with type 2 diabetes mellitus (T2DM) also live with chronic kidney disease (CKD), and T2DM continues to be the most common underlying cause of end-stage renal disease [3]. Beyond glucose lowering, dapagliflozin has demonstrated additional benefits, particularly in slowing CKD progression and improving outcomes in heart failure [4]. Its pharmacological action differs from that of traditional antidiabetic medications, as it lowers plasma glucose by inhibiting renal SGLT2 transporters, thereby enhancing urinary glucose elimination in an insulin-independent manner [4].

Nevertheless, achieving comprehensive control of diabetes remains a persistent challenge. Less than half of patients with T2DM are able to maintain recommended targets for blood glucose, blood pressure, or lipid levels, and fewer than one in five successfully achieve all three simultaneously [5]. In this context, dapagliflozin offers an effective therapeutic option that addresses both glycemic control and comorbid complications. It was first approved in the United States on 8 January 2014 and had already gained authorization in 38 countries, including across Europe, under the trade name *Forxiga* (Bristol-Myers Squibb Company, Middlesex, UK) [6].

Physicochemical Properties

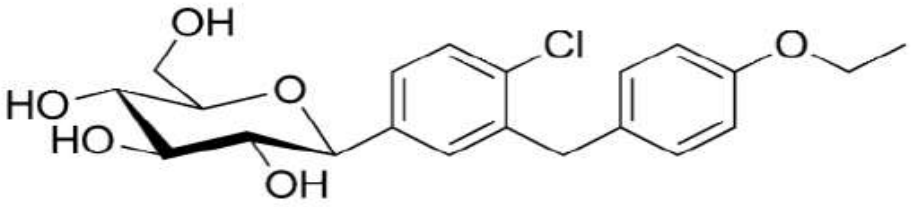

Figure 1: Chemical structure of Dapagliflozin.

- **IUPAC Name:** (2S,3R,4R,5S,6R)-2-[4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol.
- **Molecular Formula:** $C_{21}H_{25}ClO_6$.
- **Molecular Weight:** 408.88 g/mol.
- **Chemical Class:** Sodium-glucose co-transporter-2 (SGLT2) inhibitor (C-aryl glucoside derivative).
- **Appearance:** White to off-white crystalline solid powder.
- **Melting Point:** 78–82 °C (approx.)
- **pKa:** ~12.6 (very weakly acidic)
- **Log P (Partition coefficient):** ~1.8 (moderately lipophilic)
- **Solubility Profile:**
  - Practically insoluble in water (<1 mg/mL at room temperature).
  - Soluble in methanol, ethanol, acetonitrile, and DMSO.
  - Aqueous solubility improves under acidic conditions but remains limited.
- **Stability:**
  - Stable under normal light and temperature conditions.
  - Degrades under strong acidic or basic hydrolytic conditions.
  - Sensitive to oxidative stress.
- **Structural Characteristics:**
  - Belongs to *C-aryl glucoside* class (non-hydrolyzable C–C bond between glucose and aryl ring).

o Has both **hydrophilic** (sugar moiety) and **lipophilic** (aromatic ring with chlorine and ethoxy substituents) regions → contributes to amphiphilic behavior.
o The **chlorophenyl group** increases lipophilicity, aiding passive membrane permeability.[7-10]

Pharmacokinetics

Dapagliflozin is rapidly absorbed following oral administration, with an oral bioavailability of about 78% and peak plasma concentrations (Tmax) achieved within 1–2 hours. Its absorption is not significantly influenced by food intake, allowing flexible administration with or without meals. After absorption, the drug displays a relatively large apparent volume of distribution (~118 L) and is extensively bound to plasma proteins (~91%), mainly albumin. This high binding capacity contributes to its wide distribution throughout the body [11-12].

Metabolically, dapagliflozin undergoes extensive hepatic and renal biotransformation, predominantly via the enzyme UGT1A9, producing dapagliflozin 3-O-glucuronide as its major inactive metabolite. The involvement of cytochrome P450 enzymes is minimal, which reduces the likelihood of drug–drug interactions. Excretion occurs mainly through the renal route (~75% in urine, primarily as metabolites, with less than 2% excreted unchanged), while about 21% is eliminated via feces. The terminal half-life is approximately 12–13 hours, supporting once-daily dosing. Pharmacokinetics remain linear across the therapeutic range (2.5–25 mg). However, drug exposure increases in renal impairment, where efficacy decreases due to reduced glucose excretion, whereas mild to moderate hepatic impairment only slightly alters pharmacokinetics [13-14].

Mechanism of action

Dapagliflozin is a highly selective inhibitor of sodium–glucose co-transporter-2 (SGLT2), which is predominantly expressed in the proximal renal tubule and is responsible for the reabsorption of nearly 90% of filtered glucose (15). By blocking this transporter, dapagliflozin prevents glucose reuptake, thereby enhancing urinary glucose excretion and reducing plasma glucose levels (17). Unlike many conventional anti-diabetic therapies, its mechanism is independent of insulin secretion or pancreatic β-cell function, making it effective even in later stages of type 2 diabetes when β-cell function declines(16).

In addition to glycaemic control and HbA1c reduction, dapagliflozin exerts several pleiotropic benefits. The drug promotes weight loss through caloric elimination, reduces intraglomerular pressure, and lowers cardiac preload and afterload by promoting natriuresis. These effects contribute to improved cardiovascular and renal outcomes, including a reduced risk of heart failure hospitalization and slowing of chronic kidney disease progression. Furthermore, its safety profile allows for use both as monotherapy and in combination with other anti-diabetic agents such as metformin, sulfonylureas, and thiazolidinediones, thereby offering a versatile therapeutic option in diabetes management (18-29).

Analytical Techniques Reported For Dapagliflozin

Official / Reported Methods for Dapagliflozin

Sr. No.	Drug	Method	Method Description	Ref. No.
1	Dapagliflozin-API	Simple UV Spectrophotometric method	Mobile Phase: Distilled water 224 nm 5 Linearity: 5-40 µg/mL r2:0.985	30
2	Dapagliflozin	RP-HPLC	Mobile phase: Phosphate buffer: Acetonitrile (60:40 v/v) Linearity: 10-60 µg/ml LOD: 0.02µg/ml LOQ: 0.06µg/ml	31

Sr. No.	Drug	Method	Method Description	Ref. No.
3	Dapagliflozin	Stability indicating HPLC	Mobile phase: Buffer (dipotassium hydrogen phosphate): Acetonitrile (60:40 v/v) Linearity: 50-150 µg/ml r2:0.997 LOD: 5.14 µg/ml 237 nm 6 222 nm 7 LOQ: 15.6 µg/ml	32
4	Dapagliflozin	Stability indicating RP-HPLC	Mobile phase: Acetonitrile: ortho phosphoric acid Linearity: 25-150 µg/ml : 0.999 LOD: 0.6 µg/ml LOQ: 1.8 µg/ml	33

Reported Methods for Dapagliflozin in Combination of Dosage form

Sr. No.	Drug	Method	Method Description	Ref. No.
1	Dapagliflozin and Metformin	UV spectrophotometric method	Mobile phase: Methanol Linearity: Dapa: 0.5-2.5 µg/ml Met – 25-125 µg/ml r^2: Dapa: 0.984 Met: 0.982 LOD: Dapa:0.009 µg/ml nm Met: 0.013µg/ml LOQ: Dapa:0.039 µg/ml Met: 0.041 µg/ml	34
2	Dapagliflozin and Saxagliptin	RP-UPLC method	Column: reverse phase C18 column (2.1 x 100 mm) Mobile phase: 0.1% ortho phosphoric acid and acetonitrile (40:60) Linearity:- 25-150 % r2: Dapa: 0.9997 Saxa: 0.999 LOD: Dapa: 0.53 µg/ml Saxa: 0.13 µg/ml LOQ: Dapa: 1.59 µg/ml Saxa: 0.38 µg/ml	35
3	Dapagliflozin and Metformin	RP-HPLC method	Column: Phenomenex Luna C18 (4.6mm I.D. x 250mm, 5µm) column Mobile phase: Acetonitrile: water (75:25 v/v) Linearity: Dapa: 10-50 µg/ml Met: 20-100 µg/ml 2: Dapa: 0.999 Met: 0.9991 LOD: Dapa: 3.7 µg/ml Met: 5 µg/ml LOQ: Dapa: 11.4 µg/ml Met: 15.2 µg/ml	36

Validation Parameters

Accuracy: The accuracy of the proposed method was checked using recovery studies. Tablet powder equivalent to 2.5 mg of drug was taken, and known amounts of standard drug were added at three levels (50%, 100%, and 150%). These solutions were analyzed again using the developed method. Each level was tested three times to ensure repeatability. The results confirmed that the method is accurate.

Precision: Precision means how close the results are when the same sample is tested multiple times under the same conditions. It is checked in two ways:

- **Intra-day precision**: The sample (20 µg/mL) was tested three times in the same day.

• **Inter-day precision**: The same sample was tested on five different days.
Ruggedness: Ruggedness checks if the method gives consistent results even when tested by different people or on different instruments. For this, Dapagliflozin (20 µg/mL) was analyzed by two analysts using two UV-spectrophotometers (Jasco V-630 and Shimadzu-1700) under the same conditions.
Linearity: Linearity shows if the test results increase in direct proportion to the concentration of the drug. For this, five solutions of Dapagliflozin (80–120% of label claim) were prepared and analyzed, and the results were used to plot calibration curves.
Limit of detection (LOD) and limit of quantification (LOQ): The LOD and LOQ were calculated using following equations as per International Conference on Harmonization guideline for DAPA.
LOD= 3.3 x σ/S
LOQ= 10 x σ/S
Where σ is standard deviation of the response and S is the standard deviation of y-intercept of regression lines.
Specificity: Specificity shows that the method can clearly identify the drug without interference. The chromatogram of Dapagliflozin (DAPA) was checked for peak purity by comparing spectra at the start, middle, and end of the peak, and confirmed by matching its Rf value and spectra with the standard.
Robustness: Robustness checks if small changes in test conditions affect the results. This was tested by slightly changing the mobile phase composition. The sample (800 ng/band) was analyzed three times, and the average peak area and %RSD were calculated and compared with the original conditions. [37-38].

Applications of Developed Methods

• Assay of API and finished dosage forms (content/assay testing):
Dapagliflozin can be routinely quantified in bulk drug and tablets using validated RP-HPLC, UPLC, or spectrophotometric methods. These are applied for content uniformity, release testing, and routine quality control [39].

• Stability-indicating methods & forced-degradation studies:
Methods that separate the intact drug from degradants under acid/base/oxidative/photolytic/thermal stress are essential for shelf-life, formulation development and to support regulatory submissions. RP-HPLC with forced-degradation is commonly reported.
RP-HPLC methods are widely used to separate dapagliflozin from its degradation products under acid, base, oxidative, thermal, and photolytic stress, helping in stability studies, formulation development, and regulatory compliance [39].

• Simultaneous estimation in combination formulations:
RP-HPLC, UPLC, and LC-MS/MS methods enable simultaneous quantification of dapagliflozin with co-formulated antidiabetics like metformin, pioglitazone, linagliptin, and saxagliptin in combination tablets and stability studies [39].

• Bioanalysis (plasma/PK studies) and metabolite quantification:
Sensitive LC-MS/MS or UPLC-MS/MS assays are used for pharmacokinetic, bioavailability, and drug–drug interaction studies — including quantifying dapagliflozin and major metabolites (e.g., D3OG). These methods require thorough validation (LOQ, matrix effects, recovery) [40].

• Multi-analyte quantification across the SGLT2 inhibitor class:
Methods that measure several SGLT2 inhibitors (canagliflozin, empagliflozin, dapagliflozin) in one run support comparative studies, therapeutic drug monitoring and high-throughput screening [41].

• Impurity profiling and degradant identification (LC-MS/MS, HRMS):
When structure elucidation of degradants is needed (e.g., for safety or regulatory reporting), LC-MS/MS or high-resolution MS is used to identify and quantify impurities. Stability-indicating HPLC methods are usually paired with MS for characterization [40].

• Dissolution and content uniformity testing for formulation development:

HPLC/UPLC methods adapted for dissolution samples measure release profiles and help optimize formulation and coating. These methods are often validated for medium compatibility and sampling/time-point robustness [42].

- Green/eco-friendly method adaptations:

Micellar liquid chromatography or methods minimizing organic solvents have been reported as "greener" alternatives for routine QC [40].

- Method transfer, robustness and routine QC adoption:

Many published methods include ruggedness and robustness tests (column, flow, mobile phase composition) so they can be transferred to QC labs and manufacturing sites [43].

Conclusion

Dapagliflozin, an important SGLT2 inhibitor used in diabetes, has been studied through many analytical methods such as HPLC, UPLC, LC-MS/MS, and spectrophotometry. These methods are widely applied for assay of API, dosage forms, stability studies, impurity profiling, and bioanalysis. Most reported methods give accurate and reliable results, but many use buffers and organic solvents that may damage instruments and create environmental concerns.

Therefore, there is a strong need for developing simple, cost-effective, eco-friendly, and robust analytical techniques. Such methods should not only meet regulatory requirements for validation but also be safe for analysts, extend instrument life, reduce waste, and support routine quality control as well as research applications. This comprehensive review highlights existing introduction, Physicochemical properities, Pharmacokinetics, MOA, methods, Validation Parameters, their applications, and the future direction towards greener and more sustainable analytical approaches for dapagliflozin.

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