Systematically Reviewing The High-Performance Liquid Chromatography (HPLC) Analytical Methods For Determining Empagliflozin In Different Pharmaceutical Formulations And Human Samples

[1]Dr. Asawari Pachauri, [2]Shruti Aher, [3]Arya Kulkarni, [4]Sakshi Kamble

[1,2,3,4]JSPM's Rajarshi Shahu College of Pharmacy and Research, Tathawade, Pune, Maharashtra , 411033, India.

Corresponding author: shrutiaher@gmail.com
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Abstract :

Empagliflozin is an oral sodium-glucose cotransporter 2 (SGLT2) inhibitor. Chemically, empagliflozin belongs to C-glucosides, and it works by potently blocking glucose reabsorption in the proximal tubule, increasing urinary glucose excretion. Several methods have been reported in an industrial scale for thorough quantitative analysis. This review article comprehensively examines the published sophisticated instruments-based nearly 30 analytical methods in various pharmaceutical databases like Google Scholar, PubMed, *etc*. of diverse area of High-Performance Liquid Chromatography (HPLC). The determination of Empagliflozin in plasma, serum, and urine, extensively requires the HPLC method. For the determination of Empagliflozin in biological samples, we recommend the LC-MS/MS method since this method combines the HPLC separation ability with MS sensitivity and selectivity, allowing the identification of Empagliflozin and its metabolites. This fascinating review comprehensively highlighted the overview of sophisticated analytical techniques for the method development and validation for Empagliflozin in the most reliable, accurate, precise, economic, reproducible, and robust manner.

KEYWORDS: Empagliflozin, HPLC, UV, LC-MS/MS

INTRODUCTION :

Diabetes mellitus (DM) is a chronic metabolic condition defined by persistent hyperglycemia. It may be due to impaired insulin secretion, resistance to peripheral actions of insulin, or both [5]. More than 382 million individuals are currently affected worldwide, and this number is expected to rise to 592 million by 2035. The latest statistics for the US indicate that over 25 million people, or 8.3% of the population, are affected,3 and another 79 million with prediabetes are at increased risk of developing the disease [2]. T2DM is the most common type of diabetes, accounting for >90% of cases [3]. In T2DM, the insulin response is diminished, and this is defined as insulin resistance. [7]. Insulin is a peptide hormone secreted by the β cells of the pancreatic islets of Langerhans and maintains normal blood glucose levels by promoting cellular glucose uptake, regulating carbohydrate, lipid, and protein metabolism, and promoting cell division and growth through its mitogenic effects.[4].

Major risk factors for T2DM - Older age, Family history of type 2 diabetes mellitus (T2DM), Genetic factors, Components of the metabolic syndrome, Abdominal or central obesity (independent of BMI), Unhealthy dietary factors, Cigarette smoking, History of gestational diabetes mellitus or delivery of neonates >4kg in weight, Some medications, such as statins, thiazides and beta-blockers, Psychosocial stress and depression.

T2DM management: Modification of lifestyle, including weight loss, increasing physical activity, and adopting a healthy diet, remains one of the first-line strategies for the management of T2DM. In addition to lifestyle modification, social support plays a crucial role in T2DM management, as it directly influences the performance of diabetes mellitus self-care behaviours and indirectly affects glycaemic control. [6].

SGLT-2 inhibitors: These drugs, including empagliflozin and dapagliflozin, act by inhibiting glucose reabsorption in the renal tubules, promoting urinary glucose excretion.

GLP-1 receptor agonists, including liraglutide and dulaglutide, enhance glucose-dependent insulin secretion and reduce glucagon release.

DPP-4 Inhibitors: including sitagliptin, enhance glycemic control by preventing the degradation of endogenous incretins.

Dual and triple therapy: Recognizing the heterogeneity of type 2 diabetes, combining different classes of oral antidiabetic medications, such as SGLT-2 inhibitors with DPP-4 inhibitors or GLP-1 receptor agonists, allows for synergistic effects and better glycemic control.[9]

Empagliflozin:

Description: Empagliflozin is an oral sodium-glucose cotransporter 2 (SGLT2) inhibitor [9]. Chemically, empagliflozin belongs to C-glucosides, and it works by potently blocking glucose reabsorption in the proximal tubule, increasing urinary glucose excretion.[11]. Several SGLT2 inhibitors are approved for clinical use in the treatment of T2DM in adults [10]. Among the currently used or tested SGLT2 inhibitors, empagliflozin has the highest selectivity for SGLT2 over sodium–glucose cotransporter 1 [11].

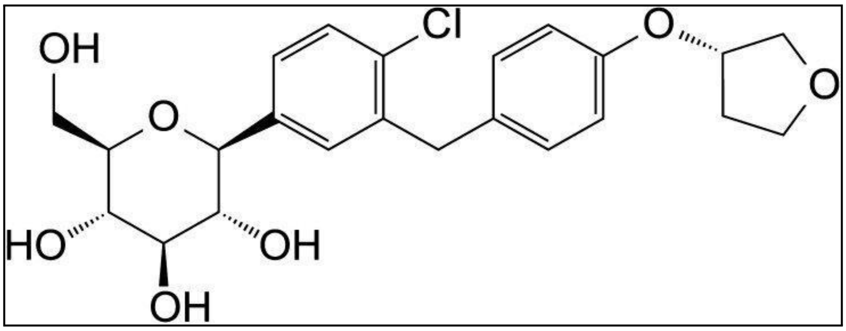

FIG. 1: STRUCTURE OF EMPAGLIFLOZIN

Applications: The principal indication for empagliflozin is T2DM. It is recommended for the treatment of adults with poorly controlled T2DM as an adjunct to diet and exercise. Empagliflozin is the first antidiabetic medicine that has been shown to reduce mortality in the treatment of type 2 diabetes. The agent has been shown to lower mean fasting glucose, postprandial glucose, and when used in monotherapy or with other hypoglycemic drugs, it lowers HbA1c in patients with T2DM. In addition, empagliflozin has been shown to improve pancreatic beta-cell function by reducing pancreatic beta-cell burden and glucotoxicity.[12]

Mechanism of Action: Empagliflozin is considered a potent and selective competitive inhibitor of the SGLT2 protein. Sodium-glucose co-transporter 2 (SGLT2) inhibitors offer an insulin-independent mechanism for improving blood glucose levels, since they promote urinary glucose excretion (UGE) by inhibiting glucose reabsorption in the kidney. In addition to glucose control, SGLT2 inhibitors are associated with weight loss and blood pressure reductions, and do not increase the risk of hypoglycaemia [10]

Pharmacokinetics:

Empagliflozin is an orally active, strong, and selective inhibitor of sodium glucose co-transporter 2 (SGLT2). The single and multiple oral doses (0.5-800 mg), empagliflozin were rapidly absorbed and reached peak plasma concentrations after approximately 1.33-3.0 h, before showing a biphasic decline. The mean terminal half-life ranged from 5.6 to 13.1 h in single rising-dose studies, and from 10.3 to 18.8 h in multiple-dose studies. [8]

Physio-chemical Properties: Empagliflozin is an orally administered sodium glucose co-transporter II inhibitor (SGLT2). chemical name of empagliflozin is (1S)-1,5-anhydro-1-(4-chloro-3- {4 -[(3S)-tetrahydrofuran-3-yloxy] benzyl} phenyl)-D-glucitol, Melting point between 151.0–153.0 °C. It has a molecular formula of $C_{23}H_{27}ClO_7$. It is a white to yellowish non-hygroscopic crystalline solid, very slightly soluble in water, slightly soluble in acetonitrile and ethanol, sparingly soluble in methanol, and practically insoluble in toluene [14]. It has a molecular weight of 450.9 g/mol, water solubility of 0.28 mg/mL, and a partition coefficient of 2. [13].

Need for Quality Control: As per the Literature Survey, it is revealed that very few studies were done on the empagliflozin as a single drug assay (HPLC). Estimations have been made using UV spectroscopy, High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), and Liquid Chromatography-Mass spectrometry (LC-MS), but not an innovative techno-economic MS-compatible HPLC Method development for estimating Empagliflozin in bulk and pharmaceutical formulations. [12]. Therefore, it's important to develop a stability-indicating RP-HPLC method to monitor the purity and stability of empagliflozin in different formulations and under various stress conditions.

Analytical Method: Multiple analytical techniques are reported to have been implemented for stability-indicating assays, with the most common ones being high-performance liquid chromatography (HPLC), gas chromatography, high-performance thin-layer chromatography, capillary electrophoresis, and supercritical fluid chromatography. This method is useful for analyzing pharmaceutical products and active pharmaceutical ingredients (APIs), particularly in the context of forced degradation studies. The ICH Q3B guidelines mandate the provision of evidence for the validation of analytical methods when the method is suitable for the detection and quantification of degradation products and impurities.

TABLE 1: REPORTED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHODS FOR THE QUANTITATIVE ANALYSIS OF EMPAGLIFLOZIN IN FORMULATIONS

Compound	Sample	Mobile phase	Flow rate (ml/min)	Column	Detector	Wavelength (max)	LOD (ǔg/ml)	LOQ (ǔg/ml)	Ref
Empagliflozin	Bulk and Tablet Dosage Form	Methanol: 0.05% pH 3.3 acetic acid (75:25v/v)	1	Agilent C18	DAD	224	0.26	0.78	13
Empagliflozin	Bulk and Tablet Dosage Form	Methanol: acetonitrile: 0.1% OPA (75:20:5v/v/v)	*1*	EC-C18	UV	222	0.05	0.1	14
Empagliflozin And Sitagliptin Phosphate	Bulk and Tablet Dosage Form	Methanol: 0.05% acetic acid in water (45:55 v/v)	*0.9*	Cosmosil C18	UV	254	0.11 and 1.63	0.35 and 4.92	15
Empagliflozin	Bulk	0.1% OPA: Acetonitrile (70:30v/v)	1	Hypersil BDS	PDA	233	0.068	0.207	16
Empagliflozin	Bulk and Tablet Dosage Form	Methanol: Water (70:30 v/v)	1	C-18G	UV	233	---	---	17
Empagliflozin and Metformin	Bulk and Tablet Dosage Form	Methanol: phosphate buffer (KH2PO4 and K2HPO4) (70:30v/v)	1	Symmetry C-18	PDA	240	2.17 and 0.0372	6.60 and 0.1125	18

Empagliflozin	Bulk and Tablet Dosage Form	Acetonitrile: Water (60:40v/v)	1	Shim Pack-C_{18}	UV	223	0.0125	0.0495	19
Empagliflozin and Metformin	Bulk and Tablet Dosage Form	Mixture of phosphate buffer, acetonitrile, and methanol (15:80:5 v/v/v)	1	octadecyl silica C-18	PDA	227	0.31 and 0.10	0.03 and 0.01	20
Empagliflozin	----	Mixture of 0.01 M acetate buffer and methanol (30:70v/v)	2	Intersil column	PDA	260	0.7	1.91	21
Empagliflozin and Impurities	----	mobile phase A - 0.1% orthophosphoric acid, mobile phase B- acetonitrile	1.2	Inertsil C8	UV	230	----	-----	22
Empagliflozin and Linagliptin	Human Plasma	0.1% orthophosphoric acid buffer: acetonitrile (68:32)	1	C-18	PDA	218	----	----	23
Empagliflozin and Metformin	Tablet Dosage Form	0.1% orthophosphoric acid buffer: acetonitrile (50:50v/v)	1	BDS column	PDA	210	0.31 and 0.10	0.03 and 0.01	24
Linagliptin and Empagliflozin	Tablet Dosage Form	Phosphoric acid buffer: Acetonitrile (45:50v/v)	1	ODS column	PDA	245	-----	-----	25

Empagliflozin and Metformin	Bulk and Tablet Dosage Form	Acetonitrile: Water (50:50v/v)	1	C-18	DAD	230	0.30 and 1.12	0.92 and 3.36	26
Empagliflozin and Metformin	Bulk and Tablet Dosage Form	Methanol: KH2PO4 Buffer (60:40v/v)	0.8	Cosmosil C18	UV	227	0.205575 253and 0.030729 6	0.622955 3and 0.09312	27
Empagliflozin	Bulk and Tablet Dosage Form	methanol: acetonitrile (50:50v/v)	1.2	Intersil C18	PDA	265	1.9	0.655	28
Empagliflozin and Metformin HCL	Bulk and Tablet Dosage Form	Methanol: Water pH 3.0 (80:20v/v)	0.8	Grace C-18	UV	227	-----	------	29
Empagliflozin	*Bulk and Tablet Dosage Form*	*Water: Acetonitrile (55:45v/v)*	*1*	Symmetry, Waters C-18	UV	225	0.002669	0.008007	30
Empagliflozin	*Bulk and Tablet Dosage Form*	0.1% solution of trifluoroacetic acid: acetonitrile (70:30 v/v)	0.8	C18 column	UV	224	0.020	0.061	31
Empagliflozin	*Bulk granules and Tablet Dosage Form*	Phosphate buffer: Methanol (70:30%v/v)	1	ODS C18	PDA	224	0.068	0.35	32

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