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Review

A Comprehensive Review On Analytical Methods-For The Quantification Of Empagliflozin In Drug Product And Drug Substance

V. Sravan Kumar[1*], P. Srinivas Babu[2], B. Gnana Sri Siva Naga Lakshmi[1], P. Parimala[1], B. Moushmi[1], U. Lavanya[1], T. Sai Kiran[1]

[1]Department of Pharmaceutical Analysis, Vignan Pharmacy College, Vadlamudi, 522213, Andhra Pradesh, India.
[2]Department of Pharmaceutics, Vignan Pharmacy College, Vadlamudi,522213, Andhra Pradesh, India.

*Author for Correspondence: V. Sravan Kumar
Email: sravankumarvaka@gmail.com

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Abstract

Pharmaceutical analysis plays an important role in the quantification of drugs in pharmaceutical products and it employs a significant role in the analytical research and development and in quality control of finished products and drug substances and also in the bio analytical method development. Empagliflozin, a new oral anti-diabetic drug which is used to treat the type 2 diabetes and it is a selective sodium- glucose co transport–2[SGLT2] inhibitor by inhibiting the reabsorption of glucose from proximal tubules of the kidney and excretes as such through urine by SGLT-2 receptor inhibition. The main advantage of this gliflozin category is that they will improve the functioning of heart and kidneys of the patients who suffering from cardiovascular disease and chronic kidney diseases (CKD). Empagliflozin is available in tablet dosage forms with the strength of 10mg and 25mg. The current review article will provide the analytical data for the quantification of Empagliflozin in various pharmaceutical formulations and in biological samples by using different analytical methods like HPLC, UHPLC, LC MS/MS and UV-Visible Spectrophotometric methods.

Keywords: Empagliflozin, Analytical method development, SGLT-2 receptors, CKD, Cardiovascular disease

INTRODUCTION

Analytical procedures are mandatory for analysis of specific drug substances and drug products. Qualification of analytical methods simply suggests its suitability for the intended use- as do method validations [1]. Type-2 diabetes mellitus [DM] is a disease characterized by the resource of insulin resistance and a progressive decline in pancreatic beta-mobile characteristics associated with growing hyper glycaemia [2]. Empagliflozin, a new oral anti-diabetic drug is a selective sodium- glucose transport protein 2[SGLT2] INHIBITOR. The drug is given as a film-coated pill containing either 10 or 25 mg of empagliflozin as an active pharmaceutical ingredient.

The drug was permitted by the United States food and drug administration [USFDA] in 2014. It is also indicated to reduce the risk of cardiovascular death in adult patients with both type 2 diabetes mellitus and established cardiovascular disease [3]. Empagliflozin was approved for medical use in the United States and in the European union in 2014.It is on the world health organization's list of essential medicines. In 2020, it was the 102nd most commonly prescribed medication in the United States, with more than 6 million prescriptions. It has received approval as a generic medication from the US food and Drug administration [FDA] [4].

The IUPAC Name of empagliflozin is (2s,3r,4r,5s,6r)-2-[4-chloro-3-[[4-[(3s)-oxolan-3-yl] oxyphenyl]methyl]phenyl]-6- (hydroxymethyl)oxane-3,4,5-triol [5].and structure is shown in Fig. 1. 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. Being sodium glucose co transporter (SGLT2) inhibitor in nature, it is probably the latest class of medicine in the treatment of T2DM [6].

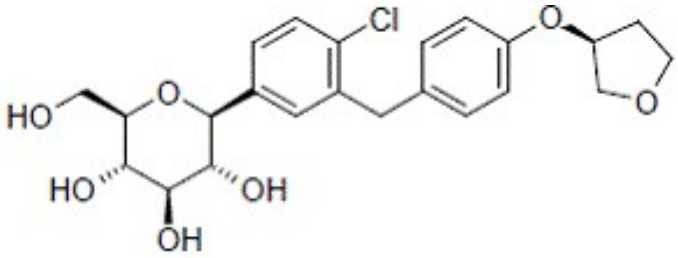

Fig 1: Structure of Empagliflozin

Pharmacokinetic parameters(7)

Empagliflozin is an oral medication used to treat type 2 diabetes by inhibiting the sodium-glucose cotransporter 2 (SGLT2) in the kidneys, leading to increased glucose excretion in urine. Its pharmacokinetics involve absorption, distribution, metabolism, and elimination.

Absorption

The absorption of empagliflozin, an oral medication used to treat type 2 diabetes, plays a crucial role in its pharmacokinetics. Following oral administration, empagliflozin undergoes a rapid absorption process, with the drug entering the systemic circulation from the gastrointestinal tract. This absorption is a pivotal step in the drug's journey through the body, influencing its onset of action and therapeutic effects. Empagliflozin's absorption kinetics are characterized by a relatively quick transition from administration to peak plasma concentration, typically occurring within approximately 1.5 hours. This swift absorption contributes to the medication's ability to inhibit the sodium-glucose cotransporter 2 (SGLT2) in the kidneys promptly, leading to increased glucose excretion in the urine. Understanding the absorption dynamics of empagliflozin is essential for healthcare providers when considering factors such as dosing regimens and therapeutic efficacy. Additionally, the absorption profile contributes to the overall pharmacokinetic profile of empagliflozin, guiding clinicians in optimizing treatment strategies for individuals with type 2 diabetes. (8)

Distribution

The estimated apparent steady-state volume of distribution is 73.8 L based on pharmacokinetic analysis. Empagliflozin is approximately 86.2% protein-bound in plasma. After giving healthy participants an oral empagliflozin solution, the red blood cell partitioning was approximately 36.8%.

Metabolism

Empagliflozin undergoes minimal metabolism. It is primarily metabolized via glucuronidation by 5'-diphospho-glucuronosyltransferases 2B7, 1A3, 1A8, and 1A9 to yield three glucuronide metabolites: 2-O-, 3-O-, and 6-O-glucuronide. Out of the total drug less than 10% of the drug was exposed systemically to each metabolite. Invitro studies was conducted that indicates that glucuronidation by the urine.(9)

Elimination

The primary route of elimination for empagliflozin is renal, with about 90% of the administered dose excreted unchanged in the urine. The elimination half-life is approximately 12.4 hours and oral clearance value of empagliflozin was found to be 10.6L/h according to population pharmacokinetic parameters. After oral administration of radiolabeled empagliflozin approximately 41.2% of the administered dose was found eliminated in feces and 54.4% eliminated in urine. The majority of radioactivity in the feces was due to unchanged parent drug while approximately half of the radioactivity in urine was due to unchanged parent drug.

Analytical methods for determination of empagliflozin

The physical and chemical properties of the drug Empagliflozin was identified in terms of qualitative and quantitative methods.

HIFH performance liquid chromatography

HPLC is a widely used analytical technique for separating and quantifying components in mixtures across scientific disciplines. It operates on chromatographic principles, with a liquid sample passing through a column packed with a stationary phase, interacting with a mobile phase. The high pressure in HPLC allows for efficient separations. It includes a sample injection system, a high-pressure pump, a separation column, a detector, and a data analysis system. HPLC is versatile, analyzing a range of compounds from small organics to large biomolecules. Different modes like reverse-phase, normal phase, ion-exchange, and size-exclusion chromatography provide flexibility. HPLC is vital in pharmaceutical quality control, environmental pollutant monitoring, and ensuring food safety. Its high sensitivity and precision make it indispensable in modern analytical laboratories, driving advancements in research and industry.(10)

Liquid chromatography- mass spectrometry

LC-MS combines liquid chromatography's separation abilities with mass spectrometry's detection and characterization capabilities. Widely used in chemistry, biochemistry, pharmaceuticals, environmental science, and metabolomics, it excels in analyzing complex mixtures with high sensitivity and selectivity. The process involves liquid chromatography separating components in a sample, and mass spectrometry detecting and analyzing these based on mass-to-charge ratio. LC-MS is versatile, handling a broad range of compounds, from small organics to large biomolecules. Applied in drug development, environmental analysis, food safety testing, and clinical research, it provides detailed insights into complex sample compositions, playing a crucial role in modern analytical laboratories and contributing significantly to scientific advancements.(11)

UV- visible spectroscopy

UV-visible spectroscopy is a powerful analytical method used across scientific disciplines to study how a sample absorbs or reflects UV and visible light. It provides insights into the electronic structure of molecules and is valuable for analyzing the concentration of absorbing species. This technique is based on the principle of molecular absorption at specific wavelengths, leading to electronic transitions. The UV range (200-400 nm) and visible range (400-700 nm) are key areas for these transitions, involving the movement of electrons to higher energy orbitals. A UV-visible spectrophotometer comprises a light source, a monochromator or prism for wavelength isolation, a sample holder, and a detector to measure transmitted or reflected light intensity. The resulting absorption spectrum serves as a unique fingerprint for the substance. UV-visible spectroscopy has diverse applications. In chemistry, it quantitatively analyzes substance concentration based on absorbance, while biochemists use it for studying biomolecules like proteins. Environmental scientists apply it to monitor water quality and detect pollutants. In pharmaceuticals, it aids in quality control, and in material science, it helps understand electronic properties.(12)

Mechanism of action

Empagliflozin is an inhibitor of the sodium glucose co-transporter-2 [SLT-2], which is found almost exclusively in the proximal tubules of nephrotic components in the kidneys. SGLT-2 accounts for about 90 percent of glucose reabsorption in the blood. Blocking SGLT-2 reduces blood glucose by blocking glucose reabsorption in the kidney and thereby excreting glucose [i.e.; blood sugar] via the urine [4].

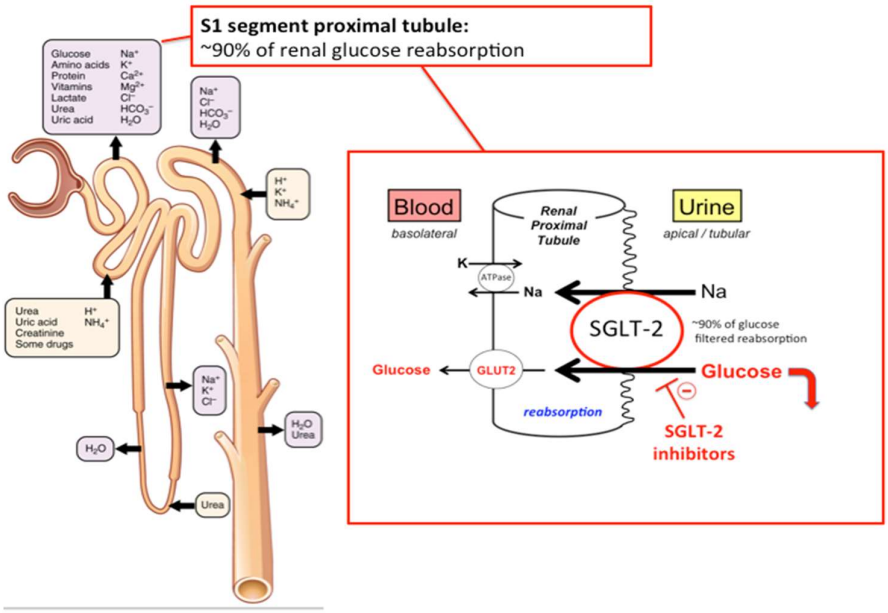

Fig 2: Mechanism of Empagliflozin [7]

Adverse Effects

Adverse effects include Urinary tract infections, Female genital mycotic infections, Upper respiratory tract infections, Increased urination, Dyslipidemia, Male genital mycotic infections, Arthralgia, Nausea, Polydipsia. Post marketing reports like Ketoacidosis, Urosepsis and pyelonephritis, Necrotizing fasciitis of the perineum (Fournier's gangrene), Angioedema, Skin reactions, Acute kidney injury, Constipation, Diabetic ketoacidosis in patients with type 1 diabetes mellitus and other ketoacidosis were observed.

Table 1: Physico-chemical properties [5]

Parameter	Description
Molecular Formula	$C_{23}H_{27}ClO_7$
Molecular Weight	450.9g/mol
Appearance	Crystalline solid
Melting point	151.0-153.0 ^{0}C
Solubility	Methanol, Ethanol, DMSO
Drug type	Approved

Table 2: Marketed formulations of Empagliflozin

S. No	Drug	Trade Name	Company Name	Formulation	Dosage Form
1	Empagliflozin	Jaridance	Boehringer Ingelheim	10mg;25mg	Tablet
2	Empagliflozin	Gibtulio	Lupin	10mg,25mg	Tablet
3	Empagliflozin	oboravo	Cipla Ltd	10mg,25mg	Tablet
4	Empagliflozin	Cospiaq	Torrent Pharmaceutical Ltd	10mg,25mg	Tablet
5	Empagliflozin	Glifaz	Magnus Pvt Ltd	10mg,25mg	Tablet

Table 3: List of HPLC/UHPLC methods for the estimation of Empagliflozin and its impurities

S.No	Column	Mobile phase	Run time (min)	Retention time (min)	Flow Rate (ml/min)	Wavelength (nm)	Linearity range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	Correlation coefficient	Ref.no
1	Shim pack C18 (250×4.6mm,5µm)	Acetonitrile: water (60:40%v\v)	8.0	5.4	1.0	223.0	0.04-25	0.01	0.04	0.999	8
2	C18 (150×4.6mm,5µm)	Phosphate buffer (P^H 3.0): methanol (70:30%v/v)	20.0	5.0	1.0	224.0	25- 125	0.06	0.35	0.996	9
3	Zorbax Eclipse Plus Agilent C18 (250×4.6mm,5µm)	Methanol: acetonitrile: purified water (60:5:35v\v)	20.0	4.8	1.0	225.0	5-150	0.087	0.264	0.999	10
4	Inertsil C8(250×4.6mm, 5µm)	0.1% orthophosphoric acid: acetonitrile (%v/v)	100.0	11.51	1.2	230.0	0.25-2.50	0.01	0.03	0.999	11
5	Poroshell 120EC C18(100×4.6mm,4µm)	Methanol: acetonitrile: 0.1% orthophosphoric acid (75:20:5%v/v/v)	10.0	2.54	1.0	222.0	10-50	0.05	0.1	0.999	6
6	YMC C18 (150×4.6mm,5µm)	Ammonium acetate: [acetonitrile: water (80:20)] (50: 50)	10.0	3.41	1.0	224.0	100-300	3.19	9.67	0.999	12
7	Thermo hypersil gold C18 (250×4.6mm,5µm)	0.1%Trifluoroaceticacid (P^H 4.8): acetonitrile(70:30%v\v)	7.0	5.48	0.8	224.0	0.025-30	0.020	0.061	0.999	13
8	Develosil ODS HG-5 RP C18 (250×4.6mm,5µm)	Phosphate buffer: Acetonitrile (45:55%v/v) (P^H 2.8)	8.0	3.86	1.0	228.0	0-50	0.07	0.21	0.999	14
9	Zorbax Eclipse Plus C18 (50mm×2.1,1.8µm)	Acetonitrile: water (50:50%v/v)	10.0	2.6	0.5	224.0	100-1000 ng/ml	0.15 ng/ml	0.50 ng/ml	0.993	15
10	Symmetry C18 (250×4.6mm,5µm)	Methanol: acetonitrile (70:30%v\v)	7.0	2.76	1.0	245.0	0-14	0.50	1.53	0.999	3
11	Zorbax C18(250×4.6mm,5µm)	Acetate buffer (pH 3.4): Acetonitrile (60:40%v/v)	6.0	2.57	1.0	232.0	10-120	5.0	10.0	0.999	16
12	Inertsil ODS 3V C18 (250 cm×4.6 mm,5µm)	[0.1%v/v Trifluoro acetic acid: water]: Acetonitrile: methanol (200: 200: 600%v/v/v)	5.0	3.84	0.8	265.0	25-75	0.003	0.008	0.999	17
13	Symmetry C18 (150× 4.6 mm, 5µ)	0.1% Ortho Phosphoric acid buffer: Acetonitrile (60:40%v/v)	5.0	2.89	1.0	230.0	25-150	0.01	0.03	0.999	18
14	C18 (150×4.6mm, 5µm)	Phosphate buffer: Acetonitrile (65:35%v/v)	6.0	6.96	1.0	226.0	5-15	0.48	1.50	0.999	19

15	C18 BDS (250×4.6mm,5µm)	0.1% Per chloric acid: Acetonitrile (60:40%v/v)	7.0	2.05	1.0	230.0	25-150	0.03	0.09	0.999	20
16	Eclipse XBD C18 (250×4.6mm,5µm)	0.1% Triethylamine: Acetonitrile (40:60%v/v)	6.0	5.41	1.0	240.0	10-150	1.00	3.30	0.999	21
17	X select-HSS C18 SB (25cm×4.6mm,5µm)	Phosphate buffer: Acetonitrile (60:40%v/v)	8.0	6.40	1.0	255.0	3.13-1.38	0.35	1.05	0.999	22
18	Thermo hypercil C18 (250×4.6mm,5µm)	0.043M Potassium dihydrogen orthophosphate buffer (P^H 3.79): methanol (34.4:65.6%v/v)	6.0	5.17	1.0	255.0	0.05-50	0.017	0.051	0.999	23
19	Zorbax C18 (250×4.6mm,5µm)	Acetonitrile: Orthophosphoric acid buffer (P^H 3.09) (80:20%v/v)	8.0	3.71	0.7	252.0	10-150	5	10	0.999	24
20	Zorbax SB C8 (250×4.6mm,5µm)	Acetonitrile:0.05M Potassium dihydrogen phosphate buffer (P^H 4) (10:90%v/v)	5.0	3.92	1.2	250.0	0.2-8.0	0.05	0.2	0.999	25
21	X-Bridge C18 (250×4.6mm,5µm)	0.1% Trifluoroacetic acid: Methanol: Water (40:40:20%v/v/v)	10.0	5.57	1.0	224.0	2.5-7.5	0.4	2.5	0.999	26
22	Zorbax C18 (25cm×4.6mm,5µm)	Methanol: Phosphate buffer (P^H 3.5) (70:30%v/v)	10.0	6.91	1.0	235.0	2.5-15	0.617	2.5	0.999	27
23	ODS (250×4.6mm,5µm)	Buffer: Acetonitrile (45:50%v/v)	7.0	3.60	1.0	245.0	25-150	10	25	0.999	28
24	Kromosil C18 (50×4.6mm,5µm)	Acetonitrile: 0.1% Phosphoric acid (50:50%v/v)	6.0	3.20	1.0	260.0	3.12-18.75	0.01	0.03	0.999	29
25	Hypercil C18 (25×4.6mm,5µm)	0.1% Formic acid Buffer (P^H 3.7): Acetonitrile (60:40%v/v)	10.0	2.57	1.0	230.0	4-160	0.07	0.25	0.999	30
26	C18 (250×4.6mm,5µm)	0.1% Orthophosphoric acid (P^H 4.5): Acetonitrile (68:32%v/v)	10.0	4.69	1.0	218.0	0.01-10	0.005	0.01	0.998	31
27	Devilosil ODS HG-5 C18 (15cm×4.6mm)	Methanol: Acetonitrile (85:15%v/v)	15.0	2.21	1.0	258.0	0-14	0.08	0.24	0.999	32
28	Prontosil C18 (250×4.6mm,5µm)	Sodium dihydrogen phosphate (P^H 2.8): Acetonitrile (18.5:81.5%v/v)	4.0	2.67	2.0	225.0	0.31-2.5	0.21	0.23	0.999	33
29	ACE C18 (250×4.6mm,5µm)	Orthophosphoric acid buffer: Acetonitrile (P^H 2.7) (30:70%v/v)	7.0	3.20	0.5	230.0	10-100	5	10	0.999	34
30	Kromosil C18 (50×4.6mm,5µm)	Acetonitrile: 0.1% Phosphoric acid (50:50%v/v)	6.0	3.20	1.0	260.0	0.1-20	0.05	0.1	0.999	35

Table 4: List of LC-MS methods for Estimation of Empagliflozin

S.no	M/Z Value	Column	Capillary temperature(^{0}C)	Ionization voltage (V)	Solvent mixture	Mass spectroscopy used	Run time(min)	Retention time(min)	Ref.no
1.	451.72/71.29	UPLC BEH C18 (50×2.1mm,1.7µm)	500.0	16KV	Formic acid (0.01%): Acetonitrile (70:30V/V)	Waters Acquity H-Class TQD-UPLC system (USA) conjugated with an electrospray ionization	3.00	2.23	36
2.	515.2/377.0	ACQUITY UPLC HSS T3 VanGuard Pre-column (50×2.1mm,1.8µm)	350.0	2900	Ammonium acetate20mM, (PH5) with acetic acid: acetonitrile	Thermo Scientific TSQ Quantiva triple-stage quadrupole mass spectrometer	1.00	0.66	37
3.	451.68-1.35	Acquity BEH C18(50×2.5mm,5µm)	200.0	30KV	[5mM ammonium acetate: 0.1% formic acid: water]: [0.1%formic acid: acetonitrile] (10:90%v/v)	Quadrupole mass analyzer Water UPLC H-class, PDA and SQD mass detector	8.00	1.39	38
4	449.01-371.21	WATERS ACQUITY UPLC BEH Shield RP C18 (150×2.1 mm, 1.7 µm)	275.0	40KV	Deionized water: acetonitrile (10:90%v/v)	WATERS ACQUITY UPLC system (S/N F08UPH, USA), TQ detector (S/N QBA530, USA)	1.50	1.16	39
5	451.52-1.32	Phenomenex Synergi (75×4.6mm,5µm)	30.0	30KV	[5Mm Ammonium acetate buffer: 0.1% formic acid]: Acetonitrile (50:50%v/v)	Mass Lynx 4.1 SCN805, auto sampler of Shimadzu (SIL-HTC) coupled with an API 4000 TQM spectrometer	3.00	1.50	40
6	468.00-355.20	Uptisphere C18 (50×4.6 mm,5 µm)	450.0	5000	10 mM ammonium formate in water (mobile phase A) and	AB SCIEX 6500+ TQM spectrometer	2.85	1.13	41

	acetonitrile (mobile phase B) both with 0.1% formic acid

Table 5: List of UV Spectroscopy methods for the estimation of Empagliflozin

S.no	Wavelength (nm)	Linearity range (µg\ml)	LOD (µg\ml)	LOQ (µg\ml)	Correlation coefficient	Ref.No
1	224.0	2-10	3.30	0.93	0.998	42
2	276.0	5-80	0.70	2.15	0.996	43
3	272.0	5-25	3.0	5	0.999	44
4	247.0	2-12	0.02	0.07	0.999	45
5	220.0	2-10	0.04	0.12	0.999	46
6	225.0	5-17.5	1.58	4.79	0.988	47

CONCLUSION

Empagliflozin, a new oral anti-diabetic drug which is used to treat the type 2 diabetes and it is a selective sodium- glucose co transport – 2 [SGLT2] inhibitor by inhibiting the reabsorption of glucose from proximal tubules of the kidney and excretes as such through urine by SGLT-2 receptor inhibition. The main advantage of this gliflozin category is that they will improve the functioning of heart and kidneys who are the patients suffering from cardiovascular disease and chronic kidney diseases (CKD). Empagliflozin is available in tablet dosage forms with the strength of 10mg and 25mg. The current review article will provide the analytical data for the quantification of Empagliflozin in various pharmaceutical formulations and in biological samples by using different analytical methods like HPLC, UHPLC, LC MS/MS and UV- Visible Spectrophotometric methods. This article will be useful for verification of various listed methods by using different analytical techniques and it is also useful for the scientist's/analysts for the development of new analytical methods in research and development and also in Bio analytical development to minimize the time consumption during the study.

ABBREVATIONS

SGLT-2, CKD, HPLC, UHPLC, LC MS/MS, UV-Visible Spectrophotometry.

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CONFLICTS AND INTERST

All authors declare that there do not have any conflicts of interest.

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