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Review article

# Methodical insights into reported sophisticated analytical techniques for the determination of anti-diabetic drug empagliflozin in various pharmaceutical products

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## ABSTRACT

Empagliflozin (EMPA), chemically known as (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol is an oral anti-diabetic drug used for treating type 2 diabetes mellitus that produces hypoglycemia by selective and competitive inhibition of sodium/glucose co-transporter-2 (SGLT2) protein. Currently, EMPA is an emerging drug being prescribed by medical practitioners. The quality-oriented scheduled investigation of diverse commercially obtainable formulations of EMPA is a foremost challenge and recently few sophisticated analytical methods are reported for industrial-scale quantitative analysis. Review of analytical method of antidiabetic drug Empagliflozin. This interesting review article covered the recently published sophisticated instruments-based analytical methods in numerous pharmaceutical databases like PubMed, Google Scholar, Science Direct, etc. of diverse areas like spectrophotometry (Ultraviolet-Visible), Ultra-high Performance Liquid Chromatography (UPLC), High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC), and analytical methods for estimation of EPMA in human plasma. From literature, about 19 HPLC methods, 4 UPLC methods, 15 UV-Vis methods, 3 HPTLC methods, and 4 human plasma-based methods were reported for the estimation of EMPA alone or in combination with other drug substances (Linagliptin, Canagliflozin, Dapagliflozin, and Metformin). The methodical insights outlined the reported characteristics like method, detection wavelength, solvent, % recovery, LOD, LOQ, mobile phase composition, flow-rate, column type, retention time, etc. This review concluded that the developed validated analytical methods have a high degree of accuracy, superior reliability, economical to apply, bears good precision, possess better robustness, and greater reproducible attributes which are compulsory features of an ideal method.

**Keywords:** Empagliflozin, Estimation, Analysis, Quantitative, Formulation, Determination

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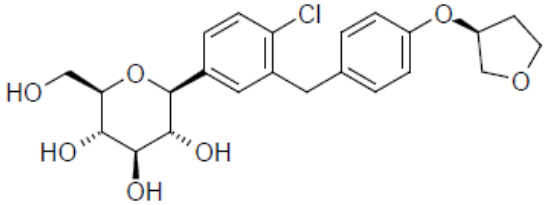
## INTRODUCTION

Empagliflozin (EMPA), chemically known as (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3yl]oxyphenyl] methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol is an oral anti-diabetic drug used for treating type 2 diabetes mellitus that produces hypoglycemia by selective and competitive inhibition of sodium/glucose co-transporter-2 (SGLT2) protein (Figure 1).[1,2,3] The drug is structurally linked with the natural product phlorizin, an O-glucoside that is vulnerable to degradation in the intestines by the enzyme β-glucosidase.[4] EMPA is a C-glucoside and is comprised of a C-C bond between glucose and aglycone moieties which makes it resistant to intestinal degradation and therefore is suitable for oral dosage form.[5]

Figure1. Structure of Empagliflozin.

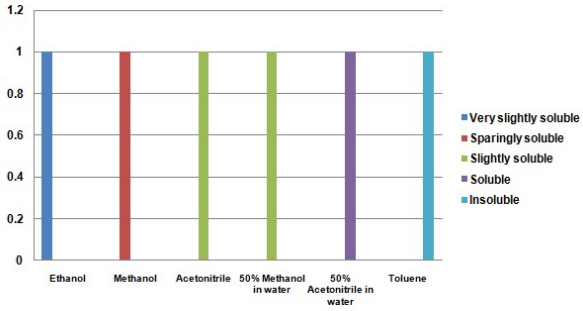



SGLT2 inhibitors are one of the newest categories of anti-diabetic drugs that act by a novel insulin-independent approach for regulating the blood glucose levels.[6] SGLT2 is located in the proximal tubule of the nephron and this drug effectively blocks the reabsorption (Approx. 90%) of glucose in kidneys.[7,8] Additionally, this inhibitor acts as an anti-hypertensive, cardioprotective, and anti-obesity agent.[9,10] EMPA do not produce acute hypoglycemic phases,

this exclusive safety characteristic enhances the patient compliance to several folds.[11]

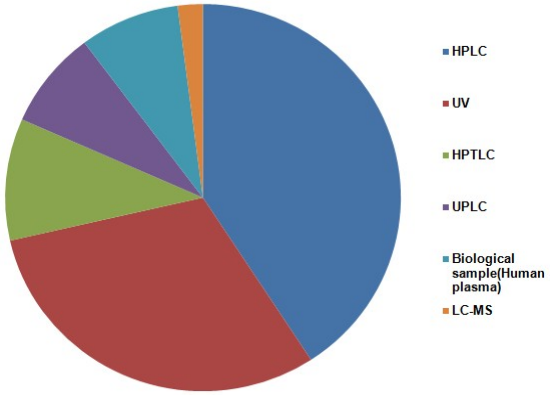
Physically, EMPA is a white to yellowish powder having a molecular formula of C23H27ClO7 and a molecular weight of 450.91 g/mol.[12,13] It has a melting point of 150°C.[14] It is very slightly soluble in ethanol (8 mg/mL), sparingly soluble in methanol (33.5 mg/mL), slightly soluble in acetonitrile (2.6 mg/L), slightly soluble in 50% methanol in water (6.4 mg/mL), soluble in 50% acetonitrile in water (68 mg/mL), and practically insoluble in toluene (<0.001 mg/mL).[15,16] EMPA is a biopharmaceutical classification system (BCS) Class –III drug (Highly Soluble and Low Permeability).[17] The aqueous solubility of this molecule may be further enhanced by preparing agglomerates, solvent evaporation method assisted formulations, solid dispersion, etc.[18,19] The solubility chart of EMPA is described in Figure 2.

Figure 2.Solubility profile of Empagliflozin in various solvents.




In this present review article, we have tried out level best in compiling the so far data depicting the published analytical methods [spectrophotometry (Ultraviolet-Visible), Ultra-high Performance Liquid Chromatography (UPLC), High-Performance Liquid Chromatography (HPLC), and High-Performance Thin Layer Chromatography (HPTLC)] for the determination of EMPA in bulk as well as in various pharmaceutical formulations. From this methodical review, it was observed that HPLC methods have been employed extensively for the analysis of EMPA. An overview of all these reported sophisticated analytical methods is depicted in Figure 3.

Figure 3.Reported sophisticated analytical methods for the determination of Empagliflozin.




## ANALYTICAL METHODS

### High-Performance Liquid Chromatography (HPLC)

In the literature, about 22 methods[20-41] were reported for the estimation of EMPA employing HPLC, of which 4 methods are for determining EMPA alone, while the others are for determining EMPA in combination with other drug substances (Canagliflozin, CANA; Dapagliflozin, DAPA; and Metformin, MET).

Table 1: Illustrated the outline of some reported HPLC methods indicating the mobile phase composition, flow-rate, column type, detection wavelength, retention time.

| Compound | Mobile Phase | Flow Rate | Column | Detection Mode | Retention Time | Reference |
|---|---|---|---|---|---|---|
| Empagliflozin + Linagliptin (Tablet) | 0.1% Perchloric acid : Acetonitrile (60:40 v/v) | 1 ml/min | C18 column [BDS 250mm x 4.6 mm, 5µ.] | UV at 230nm | EMPA- 2.05 min LINA- 4.10 min | 20 |
| Empagliflozin | Methanol: Water (70:30%) | 1.0 ml/min | $C_{18}$ column | UV at 224 nm | EMPA – 4.808 min | 21 |
| Empagliflozin + metformin | Methanol : phosphate buffer (40:60) | 1 ml/min | $C_{18}$ | UV at 255 nm | EMPA – 4.2 min MET – 2.463 min | 22 |
| Empagliflozin + metformin HCL (Bulk dosage form) | OPA buffer : Acetonitrile (95:55 v/v) | 0.8 ml/min | Kromosil | PDA at 233 nm | EMPA – 3.4 min MET – 2.270 min | 23 |
| Empagliflozin + metformin (Drug product) | Water : Acetonitrile : Methanol (200:200 600 v/v) | 0.8 ml/min | Inertsil ODS. | UV at 265 nm | EMPA – 3.848 min MET – 2.62 min | 24 |
| Dapagliflozin + metformin, HCL | Phosphate buffer : Acetonitrile (50: 50 v/v) | 1 ml/min | Inertsil ODS | PDA at 227 nm | Dapagliflozin – 2.633 min MET – 5.620 min | 25 |
| Empagliflozin + metformin HCL (Bulk & Pharmaceutical dosage farm) | Methanol : Phosphate buffer (70:30 v/v) | 1 ml/min | $C_{18}$ | PDA at 240 nm | EMPA – 3.9 min MET – 2.4 min | 26 |
| Empagliflozin + metformin (tablet) | Phosphate buffer : Acetonitrile : Methanol (15:80:5 v/v/v) | 1 ml/min | $C_{18}$ | UV at 227 nm | EMPA – 4.14 min MET – 2.5 min | 27 |
| Empagliflozin + metformin (Dosage form) | Methanol: Acetonitrile: 0.025 M pot. Hydrogen phosph ate (45:30:25, v/v/v) | 1.2 ml/min | Thermosil $C_{18}$ | PDA at 225 nm | EMPA – 3.119 min MET – 2.833 min | 28 |
| Empagliflozin | Methanol : water | 1 ml/min | Phenome nex $C_{18}$ | UV at 224 nm | EMPA – 4.808 min | 29 |
| Empagliflozin + metformin | 0.1 % OPA buffer : Acetonitrile (45:55v/v) | 1.1 ml/min | Kromasil $C_{18}$ | UV at 226 nm | EMPA – 2.908 MET – 2.182 | 30 |
| Canagliflozin + Dapagliflozin + Empagliflozin + metformin | Acetonitrile: 0.05M Potassium dihydrogen phosphate buffer (PH4) (65:35,v/v) | 1ml/min | $C_{18}$ (250 ×4.6mm µm) | UV at 212nm | EMPA – 3.004min MET – 1.89min | 31 |
| Metformin HCL + Empagliflozin (tablet dosage forms) | Acetonitrile:0.1% Ortho phosphate acid (50:50,v/v) | 1ml/min | Kromosil $C_{18}$ Column (50×4.6mm; 5µm) | UV at 260nm | EMPA:3.200min MET:2.192min | 32 |
| Metformin + Empagliflozin (tablet dosage form) | Buffer: Acetonitrile: Methanol | 1ml/min | ODS (250mm × 4.6 , 5 µm) | PDA at 233nm | EMPA –4.592 min MET-2.211 min | 33 |
| Empagliflozin (API) | 1.01M Acetate buffer: Methanol | 2ml/min | Inertsil column (150 xz | PDA at | 1.223min | 34 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | (50: 70,v/v ) | | 40mm, 5µm) | 260nm | | |
| Empagliflozin (bulk and pharmaceutical dosage form) | Methanol: Acetonitrile (50:50v/v) | 20 µl/min | Inertsil (150 x 4.6mm ,5µm) 265 nm | PDA at 265nm | 2.184 min | 35 |
| Empagliflozin (API) | 0.1%OPA: Acetonitrile (70:30v/v) | 1ml/min | Hypersil BDS | UV at 233nm | 3.274 min | 36 |
| Empagliflozin (single dosage form) | 0.1%OPA:Acetonitrile (30:70,v/v ) | 1.2 ml/min | Inertsil C8 (250mm×4.6 mm, 5µm) | UV at 230nm | 11.504 min | 37 |
| Linagliptin , Emagliflozin, Metformin (Solid dosage form) | Buffer: Acetonitrile (45:55v/v) | 1ml/min | Kromosil 250x4.6 mm | 233nm | L-2.370 E-2.787 M-3.419 | 38 |
| Linagliptin and Empagliflozin in Tablets | Water: Acetonitrile (5:95) | 1mL/min | Hypersil ODS 3V, (250 x 4.6 mm.5.0µ) | 225nm | LIN- 5.388 min EMP-8.390 min | 39 |
| Empagliflozin in bulk and pharmaceutical dosage form | Phosphate Buffer: Acetonitrile (45:55v/v) | 1 ml/ min | Develosil ODS HG-5 RP C18, 5µm, 15cm x 4.6mm | 228 nm | EMP 3.873 | 40 |
| Linagliptin and Empagliflozin In Pure and Dosage Forms | phosphate buffer and acetonitrile (65:35, v/v) | 1 ml/ min | C18 reversed-phase column (150 mm×4.6 mm i.d., particle size 5 µm) | photodiode array detector is used at 226 nm | LNG 3.276±0.002 EMP 6.966±0.0006 | 41 |

**Ultra-high Performance Liquid Chromatography (UPLC)**

In the literature, about 4 methods[42-45] were reported for the estimation of EMPA employing UPLC, of which no methods are for determining EMPA alone, while the others are for determining EMPA in combination with other drug substances (Linagliptin, LIN and Metformin, MET).

Table 2: Represented the outline of some reported UPLC methods indicating the mobile phase composition, flow-rate, column type, detection wavelength, retention time

| .Compound | Mobile Phase | Flow Rate | Column | Detection Mode | Retention Time | Reference |
|---|---|---|---|---|---|---|
| Empaglifozin + Linagliptin + Metformin HCl (Pharmaceutical Dosage Form) | potassium dihydrogen phosphate buffer pH(4) : Methanol (50:50 v/v) | 0.4 ml/min | acclaim™ RSLC 120 $C_{18}$ column (100mm × 2.1mm, 2.2µm). | UV at 225 nm | EMPA- 1.5 min LINA- 2.5 min MET- 4.5 min | 42 |
| Metformin + Empaglifozin (Tablet & Bulk Dosage Form) | 0.1% ortho – phosphoric acid buffer : Methanol (40:60 v/v) | 0.25 ml/min | $C_{18}$BEH (ethylene bridged Hybrid) UPLC (100mm × 2.1mm, 1.8µm) | PDA at 254 nm | MET- 0.882 min EMPA- 3.471 min | 43 |
| Empaglifozin (Bulk Dosage Form) | Acetonitrile : phosphate buffer (pH3) (70:30 v/v) | 0.3 ml/min | BEH $C_{18}$ | UV at 220 nm. | MET- 0.879 min EMPA- 1.294 min | 44 |
| Metformin + Empaglifozin (Tablet & Bulk Dosage Form) | phosphate buffer CpH3 : Methanol (30:70 v/v) | 1 ml/min | Dikma $C_{18}$ (50×2.1mm, 1.8 ppm) | PDA at 240 nm | MET- 1.712 min EMP- 1.189 min | 45 |

**Spectrophotometric (Ultraviolet-Visible) Methods**

In the literature, about 7 methods[46-52] were reported for the estimation of EMPA employing spectrophotometry, of which 1 methods are for determining EMPA alone, while the others are for determining EMPA in combination with other drug substances (Linagliptin, LIN and Metformin, MET).

Table 3: Depicts the outline of some reported spectrophotometric methods indicating the method, wavelength, solvent, % recovery, LOD, LOQ

| Compound | Method | Detector | λ max | Solvent | % recovery | LOD (µg/ml) | LOQ (µg/ml) | Reference |
|---|---|---|---|---|---|---|---|---|
| Empagliflozin + Metformin (Bulk drug) | Simultaneous Equation method | UV Detector | 224 & 232 nm | Methanol | E- 99.44% M- 93.27% | At 224nm E= 0.21 M= 0.07 At 232nm E= 0.22 M= 0.12 | At 224 nm, E- 0.41 M- 0.25 At 232 nm E= 0.46 M= 0.27 | 46 |
| Empagliflozin + Metformin HCL (Bulk drugs & combined dosage form) | Simultaneous Equation Method Absorbance Ratio Method | UV Detector | 272 & 234 nm<br>254 nm & 226 nm | Methanol | E- 98.99% M- 101.1% | - | - | 47 |
| Empagliflozin (Bulk & Pharmaceutical formulation ) | Direct UV Phenothroline Reaction Potassium Ferricyanide Reaction | UV Detector | 247 nm 438 nm 782 nm | Distilled Water | 98.15 – 100.86% 98.68 – 101.25% 98.25 – 101% | 0.02 µg/ml 0.03 µg/ml 0.3 µg/ml | 0.07 µg/ml 0.10 µg/ml 1 µg/ml | 48 |
| Lingliptin + MET + EMP (Bulk & Pharmaceutical formulation ) | First Derivative | UV detector | 296 nm | Methanol | 97.88 – 102.11% | - | - | 49 |
| Empagliflozin | Direct UV | UV detector | 224 nm | Water methanol (9:1) | E- 99.44% | 0.036 µg/ml | 0.111 µg/ml | 50 |
| Empagliflozin + lingliptin (Bulk Drugs & combined dosage form) | Vierodt's Method | UV Spectrophoto meter | E- 277 L- 233 | Methanol | E- 99.77 L- 99.86 | - | - | 51 |
| Empagliflozin + Metfpmin HCl(Bulk Drugs) | Simultaneous Equation Method | UV detector | E-224 nm M-230nm | Methanol | 100.377 | 0.036 | 0.111272 | 52 |

**High-Performance Thin-Layer Chromatography (HPTLC) Methods**

In the literature, about 5 methods[53-57] were reported for the estimation of EMPA employing HPTLC, of which 1 method is for determining EMPA alone, while the others are for determining EMPA in combination with other drug substances (Linagliptin, LIN and Metformin, MET).

Table 4: Provided the reports of HPTLC methods indicating the method, retention factor, wavelength, solvent, % recovery, LOD, LOQ.

| Compound | Stationary phase | Mobile phase | Detection (nm) | Rf value | LOD (µg/mL) | LOQ (µg/mL) | % Recovery | Reference |
|---|---|---|---|---|---|---|---|---|
| Empagliflozin + Linagliptin (Pharmaceutical Formulation) | Silica gel 60 F254 | Methanol: toluene: ethyl acetate (2: 4: 4 v/v/v) | 229 | 0.57<br>0.22 | 1.678<br>1.565 | 1.56<br>1.46 | 99.71<br>99.64 | 53 |
| Empagliflozin (Pharmaceutical Dosage Form) | silica gel 60F254 aluminium | Ammonium acetate (2%) : Methanol : Acetonitrile : ethyl acetate (3 : 1 : 4.5 : 1.5v/v/v/v) | 223 | 0.65 | 0.171 | 0.521 | 99.88 | 54 |
| Empagliflozin& Metformin HCL (Tablet) | Aluminum backed pre coated with silica gel 60F254 | Toluene : 3% Ammonium Acetate in methanol : Ethyl acetate : Ammonia in ratio at ( 3 : 5 : 2 : 0.4 v/v/v/v) | 230 | 0.28<br>0.58 | 41.86<br>38.03 | 126.85<br>115.24 | 98.56-101.80<br>99.89-101.91 | 55 |
| Empagliflozin& Metformin (Pharmaceutical Dosage Form) | silica gel 60F254 aluminium | Ammonium acetate (2%) : Methanol : Acetonitrile : ethyl acetate (3 : 1 : 4.5 : 1.5v/v/v/v) | 228 | 1.49<br>0.81 | 109.7<br>3.49 | 330.54<br>& 10.58 | 98<br>102 | 56 |
| Empagliflozin, linagliptin, and metformin HCl in bulk and synthetic mixture. Method: Densitometry | silica gel 60 F254 | n-butanol:Water:Glacial acetic acid (6:3:1, v/v/v) | 223 | 0.73,<br>0.52,<br>and<br>0.33, | 1.696<br>0.009<br>0.019 | 5.139<br>0.027<br>0.059 | 99.56 ± 0.33<br>99.64 ± 0.42<br>100.41 ± 0.46 | 57 |

**Analytical methods for determination of EPMA in Human Plasma**

In the literature, about 4 methods[58-61] were reported for the estimation of EMPA in combination with other drug substances (Linagliptin, LIN; Dapagliflozin, DANA; and Metformin, MET) employing Solid Phase extraction, HPLC-UV, Protein Precipitation, and Solid Phase extraction with LC-MS/MS methods.

Table 5: Summarizes the reports of various methods indicating the mobile phase composition, flow-rate, column type, detection wavelength

| Matric | Method | Mobile phase | Column | Detection mode | Flow Rate | Reference |
|---|---|---|---|---|---|---|
| Empagliflozin + Lingliptin (Human Plasma) | HPLC-UV | Buffer : Acetonitrile (68:32) | $C_{18}$ Column | 218nm | 1 ml/min | 58 |
| Linagliptin + Empagliflozin (Human Plasma) | Solid Phase extraction with LC-MS/MS | Ammonium acetate buffer : Acetonitrile | HSS cyano column | Electrospray ionization | 0.4 mL/min | 59 |
| Empagliflozin + Dapagliflozin (Spiked Human Plasma) | Protcin Precipitation | Trifluoro – acetic acid : acetonitrile (60:40) | UPLC $C_{18}$ Column | DAD at 210 nm | 0.5 ml/min | 60 |
| Empagliflozin + Metformin (Human Plasma) | Solid Phase extraction | Methanol : Ammon trifluoro acetate | Orosi / $C_{18}$ Column | LC-MS | 0.8 ml/min | 61 |

## CONCLUSION

This interesting review provided the existing information of all the sophisticated analytical techniques for the customized analysis of EPMA in pharmaceutical formulations in a single form as well as combined form. The determination of EPMA in plasma, serum, and urine, extensively requires the HPLC method. For the industry-level routine analysis of EPMA in pharmaceutical products as well as in human plasma, liquid chromatography (HPLC/UPLC) method is the most applied part because they provide accurate results and low cost compared to more advanced detection techniques. In contrast to it, the HPTLC technique (an advanced variant of the general TLC technique) and UV-Vis detection method are the most emerging tool for an academic level or laboratory scale-based method development for EPMA. This review concluded that the developed validated analytical methods have a high degree of accuracy, superior reliability, economical to apply bears good precision; possess better robustness, and greater reproducible attributes which are compulsory features of an ideal method.

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## CONFLICT OF INTEREST

The authors stated no conflict of interest for the publication of this review article in the Journal.

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