IJBPAS, May, 2022, 11(5): 2279-2286

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF EMPAGLIFLOZIN IN HUMAN PLASMA BY CHROMATOGRAPHIC METHOD: A REVIEW

JAMBHULKAR Y* AND GOKHALE N

Oriental University, Department of Pharmacy, Indore 453555- India

***Corresponding Author: Dr. Yogeshwari T. Jambhulkar: E Mail: yognandini20@gmail.com**

Received 18th May 2021; Revised 17th July 2021; Accepted 3rd Aug. 2021; Available online 1st May 2022

https://doi.org/10.31032/IJBPAS/2022/11.5.6071

ABSTRACT

Bioanalysis is the method used to determine the concentration of drugs, their metabolites in the biological matrices such as blood plasma, serum, cerebrospinal fluid, urine and saliva. Bioanalytical method employed for the quantitative determination of drugs and their metabolites in biological fluids plays a significant role in the evaluation and interpretation of bioequivalence, pharmacokinetics and toxic kinetic studies. For various types of drug approval processes at different timings to regulate and harmonize bioanalytical method development and validation is required. Gas chromatography, high-pressure liquid chromatography, LC and GC, combined with mass spectrometric (MS) procedures such as LC-MS, LC-MS-MS, GC-MS, and GC-MS-MS are used for quantitative analysis. Techniques such as high pressure liquid chromatography (HPLC) and liquid chromatography coupled with double mass spectrometry (LCMS-MS) can be used for the bioanalysis of drugs in body. Empagliflozin(EMPA) is an orally active, potent and selective inhibitor of sodium glucose co-transporter 2 (SGLT2), currently in clinical development to improve glycaemic control in adults with type 2 diabetes mellitus (T2DM). SGLT2 inhibitors, including empagliflozin, are the first pharmacological class of antidiabetes agents to target the kidney in order to remove excess glucose from the body and, thus, offer new options for T2DM management. SGLT2 inhibitors exert their effects independently of insulin. This review covers most recent bioanalytical methods such as various chromatographic methods and other methods for determination of Empagliflozin in various pharmaceutical dosage forms were reported.

Keywords: Antidiabetic drug, Empagliflozin (EMPA), Bioanalytical methods

INTRODUCTION

Hyperglycemia plays an important role for the pathogenesis of type 2 diabetes mellitus, i.e., glucotoxicity, and it also is the major risk factor for microvascular complications inhibitors of the renal sodium-glucose cotransporter have been developed to produce glucosuria and reduce the plasma glucose concentration. These oral antidiabetic agents have the potential to improve glycemic control while avoiding hypoglycemia, to correct the glucotoxicity, and to promote weight loss **[1, 2]**. Diabetes mellitus", is one of the most common non-communicable diseases worldwide. India faces several challenges in diabetes management, including a rising prevalence in urban and rural areas, lack of disease awareness among the public, limited health care facilities, high cost of treatment, suboptimal glycaemic control and rising prevalence of diabetic complications. Insulin therapy for diabetes is most commonly delivered via subcutaneous injections, up to four times a day. Long-term insulin therapy, compounded by the invasive nature of its administration, has caused problems with patient compliance, ultimately influencing patient outcomes. There is an increase in the prevalence of type 1diabetes also, but main cause of diabetic epidemic is type 2 diabetes mellitus, which accounts for more than 90 percent of all diabetes cases. Type 2 diabetes is a serious and common chronic disease resulting from a complex inheritance- environment interaction along with other risk factors such as obesity and sedentary lifestyle **[3]**.

MECHANISM OF ACTION OF EMPAGLIFLOZIN (EMPA)

The chemical name of empagliflozin is D-Glucitol,1,5-anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetrahydro-3furanyl]oxy]phenyl]methyl]phenyl]-, (1S) is an inhibitor of sodium-glucose co-transporter-2 (SGLT2) transporters primarily responsible for the reabsorption of glucose in the kidney used clinically as an adjunct to diet and exercise, often in combination with other drug therapies for the management of type 2 diabetes mellitus. Empagliflozin is the first pharmacological class of antidiabetes agents to target the kidney in order to remove excess glucose from the body and, thus, offer new options for T2DM management. SGLT2 inhibitors exert their effects independently of insulin **[4]**.

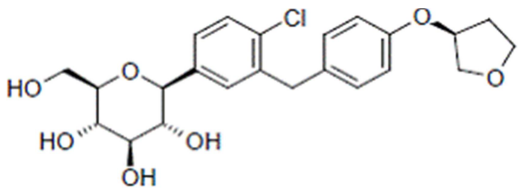

Figure 1: Chemical structure of EMPA

DRUG PROFILE [5] (Table 1, 2)

Table 1: Chemical Profile of EMPA

Sr. No.	Parameters	Empagliflozin
1.	Molecular weight	450.91
2.	Molecular Formula	$C_{23}H_{27}ClO_7$
3.	Melting point	151-153°C
4.	Solubility	It is very slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol and acetonitrile; soluble in 50% acetonitrile/water and practically insoluble in toluene. DMSO (Slightly), Methanol (Slightly)
5.	Storage	Hygroscopic, Refrigerator, under inert atmosphere
6.	Color	White powder
8.	Odour	Characteristic

Table 2: Pharmacokinetic Profile of EMPA

Sr. No.	Parameters	Empagliflozin
1.	Absorption	Orally absorbed
2.	Metabolism	Liver
3.	Bioavailability	78%
4.	Half life	12.4 hrs
5.	Plasma protein binding	86.2%

Table 3: Marketed formulation of EMPA

Sr. No.	Brand name	Company name	Formulation	Dose
1.	Jardiance	Boehringer Ingelheim Pharma	Tablet	25mg, 10mg, 12.5mg

REPORTED BIOANALYTICAL METHOD DEVELOPMENT BY CHROMATOGRAPHY

Literature survey reveals various methods are reported for EMPA in single or in combination. DonepudiaS., *et al*. Chromatography was performed on waters 2695 HPLC equipped with a quaternary pump. The separation was carried using discovery C18 (250×4.6×5) column, buffer: acetonitrile (68:32) as mobile phase with 1 ml/min flow rate. The analyte detection was monitored at 218 nm. Retention time of linagliptin, empagliflozin and internal standard was found at 6.421, 4.696, and 4.074 min respectively. The method is validated over a dynamic linear range of 0.01-10.0 µg/ml for both drugs with a correlation coefficient of 0.998 **[6]**.

Bassam M., *et al*. The main pharmacokinetic parameters estimated were Cmax, Tmax, t1/2, elimination rate constant, AUC0-t and AUC0-inf. administration of 25mg empagliflozin to Egyptian population. A new LC-MS/MS method was developed and validated, allowing sensitive estimation of empagliflozin (25–600ng mL−1) in human plasma using dapagliflozin as an internal standard (IS). The method was applied successfully on the underlying pharmacokinetic study with enhanced sample preparation that involved liquid-liquid extraction. Multiple Reaction Monitoring (MRM) of the transition pairs of m/z 449.01 to 371.21 for empagliflozin and m/z 407.00 to 328.81 for dapagliflozin

(IS) was employed utilizing negative mode Electro Spray Ionization (ESI) **[7]**.

Godasu S., *et al*., the chromatographic conditions were successfully developed for the separation of Metformin and Empagliflozin by using Symmetry C18 column (4.6×150mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer (KH_2PO_4 and K_2HPO_4) phosphate pH 3. The retention times were found to be 2.403 mins and 3.907 mins. The % purity of Metformin and Empagliflozin was found to be 99.87% and 100.27% respectively. The linearity study of Metformin and Empagliflozin was found in concentration range of 50µg-250µg and 5µg-25µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.3and 0.3, % RSD for intermediate precision was 1.3 and 0.4respectively. LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively **[8]**.

Khalil G., *et al*. Chromatographic separation was achieved on C18 column (250×4.6 mm-5µm p.s) Inertsil ODS through isocratic elution using acetonitrile and 0.05 M potassium dihydrogen phosphate buffer PH 4 in a ratio [65:35, v/v] as a mobile phase at flow rate of 1ml/min. UV detection was operated at 212 nm and injection volum was 10 µl. Linearity range for Canagliflozin, Dapagliflozin, Empagliflozin and Metformin was 7.5-225, 5-150, 6.5-187.5 and 10-1000 µg/ml, respectively. The proposed method showed good linearity, accuracy, precision and was successfully applied for determination of the four drugs in laboratory prepared mixtures and in the seven pharmaceutical dosage forms and so it is suitable for quality control of them **[9]**.

Rahul S., *et al*., Empagliflozin is a potentially highly selective Sodium-glucose co-transportace-2 (SGLT-2) inhibitor used for the treatment of type 2 diabetes mellitus alone or in combination with the metformin or dipeptidyl peptidase-4 (DPP-4) inhibitors. The literature entitles the various analytical techniques like UV spectroscopy, High Performance Liquid Chromatography, High Performance Thin Layer Chromatography, Liquid Chromatography-Mass spectrometry the various analytical, stability studies, impurity profiling and bio-analytical methods used for the estimation of Empagliflozin, Limit of detection (LOD), Limit of Quantification (LOQ), Standard Curve, Accuracy & Precision for the analysis of Empagliflozin alone or in combination with the Linagliptin or Metformin. Result shown as per ICH guidelines **[10]**.

Susmita A., *et al*. The chromatographic conditions were optimized and it was run through Std. BDS (250 mm Ã-4.6 mm, 5

m) column with mobile phase consisting of 0.1% orthophosphoric acid buffer: acetonitrile in the ratio of 50:50. The flow rate was 1 ml/min and optimized wavelength was 210 nm. Temperature was maintained at 30Â°C.The retention times of metformin and empagliflozin were found to be 2.588 min and 3.679 min and percentage relative standard deviation (RSD) of the metformin and empagliflozin was found to be 0.59 and 1.2, respectively. Percentage recovery was in the range of 100.01â€“100.65% for metformin and empagliflozin, respectively **[11]**.

Borkar S., *et al*. This review offered an overview of different methods used for determination of every drug alone as empagliflozin from SGLT-2 inhibitors, linagliptin from DPP-4 inhibitors and metformin from biguanides in a tabulated comparative way. Moreover, the current review emphasizes the most common stability indicating assays to be of interest to the analysts in the area of drug control. This review helps in understanding the further need for the development of analytical methods for the estimation of such drugs **[12]**.

Mabrouk M., *et al*., The chromatographic separation was achieved on an Acquity "UPLC® BEH" C18 column (50 mm × 2.1 mm i.d, 1.7 µm particle size), and a mobile phase consisting of aqueous trifluoroacetic acid (0.1%, pH 2.5): acetonitrile (60:40, v/v) at a flow rate of 0.5 mL/min. Upon using the UPLC system, the run time could be reduced to less than 1.2 min, and the solvents consumption decreased to 0.36 mL of acetonitrile per run. The response was linear over a concentration range of 50–700 ng/mL and 40–200 ng/mL ($r^2 = 0.9994–0.9999$) with lower limits of detection and quantification (LOD/LOQ) of 15/50, 11.5/40, 12/40 and 12.5/40 ng/mL for EMPA and the three related substances, respectively. Good accuracy was obtained with mean percentage recoveries ≥ 96.97% for the studied compounds **[13]**.

Other methods

Munde M., *et al*., Four new UV spectrophotometric methods was developed. In simultaneous equation method, absorbance was measured at 224 and 232 nm for both the drugs. Empagliflozin and Metformin hydrochloride was estimated using 224 and 232 nm in absorbance ratio method. In Area under curve method both drugs were estimated at 224 and 232 nm respectively. First derivative (zero crossing) method was based on the transformation of UV spectra in to first derivative spectra followed by measurement of first derivative signal at 224 and 232 nm for Empagliflozin and Metformin hydrochloride, respectively using 2 nm as wavelength interval **[14]**.

Ayoub B., *et al*. A simple, economic, green, and sensitive bioanalytical method for empagliflozin bioassay in rats' plasma was employed successfully owing to the empagliflozin native fluorescence behaviour. Enhanced liquid-liquid extraction, using diethyl ether (DEE), was successfully employed for the improved extraction of empagliflozin from rats' plasma based on its high value of logP as 1.8 that boosted the drug migration from plasma to the organic layer. The relative fluorescence intensity for empagliflozin was recorded at emission (299.4 nm) after excitation at 226.5 nm, HQC samples, respectively. Bias values for the trueness ranged between −10.62 and +14.95, while %RSD values for the precision ranged between 5.39% and 9.33% **[15]**.

Padmaja N., *et al*. Two simple, precise and economical UV spectrophotometric methods have been developed. Method A is simultaneous equation method (Vierodt's Method), which is based on measurement of absorption at 272nm and 234nm. Method B is Absorbance ratio (Qanalysis method) which is based on measurement of absorption at wavelength of 254nm and 226nm. Linearity was observed in the concentration range of 5-25µg/ml for Empagliflozin and 2-12µg/ml for Metformin hydrochloride. The accuracy of methods was assessed by recovery studies and was found to be within range of 98.99-101.12% for both Empagliflozin and Metformin hydrochloride **[16]**.

Sushil D., *et al*., A new, simple, accurate and sensitive UV-spectrophotometric absorption correction method has been developed. The method is based upon determination of Empagliflozin at 224 nm & Metformin HCl at 230 nm methanol as a solvent. Overlay spectra of both drugs shows absorbance at 227 nm. Linearity was observed in range of 10-50 µg/ml and 1-3 µg/ml for Metformin HCl and Empagliflozin respectively **[17]**.

CONCLUSION

From all above information it should be concluded that various chromatographic methods and other methods were used for determination of Empagliflozin with plasma or without plasma which has been successfully used on a routine basis and allows the quantification of the drug in various pharmaceutical dosage form and in short analytical time.

REFERENCES

[1] Abdul-Ghani MA, Norton L, Defronzo RA, Role of sodium–glucose cotransporter 2 (SGLT inhibitors in the treatment of type 2 diabetes, Endocr Rev, 2011, 32, 515–531.

[2] Toubro S, Cefalu WT, Xie J, Canagliflozin, A sodium glucose co-transporter 2 inhibitor reduces body weight mainly through loss of

fat mass in subjects with type 2 diabetes, Diabetologia, 2012, 55, S313.

[3] Singh N, Kesherwani R, Tiwari A, Patel D, A review on diabetes mellitus, The Pharma Innovation, 5(7), 2016, 36-40.

[4] Scheen. A, Pharmacokinetic and pharmacodynamic profile of empagliflozin, a sodium glucose co-transporter 2 inhibitor, National library of medicine, 53(3), 2014, 213-225.

[5] 5. Ndefo U, Anidiobi N, Basheer E, Eaton A, Empagliflozin (Jardiance): A Novel SGLT2 Inhibitor for the Treatment of Type-2 Diabetes, A prereviewed journal for Managed care and Hospital Formulary Management, 40(6), 2015, 364–368.

[6] Donepudia S, Achantab S, Validated hplc-uv method for simultaneous estimation of linagliptin and empagliflozin in human plasma, 10(3), 2018,1-5.

[7] Bassam M, AyoubShereenMowaka, Eman S, Nermeen A, Mohamed E, Shaker M, Pharmacokinetic Evaluation of Empagliflozin in Healthy Egyptian Volunteers Using LC-MS/MS and Comparison with Other Ethnic Populations, National library of Medicine, 7(1), 2017, 2583.

[8] Godasu S, Sreenivas S, A new validated RP-HPLC method for the determination of Metformin HCL and Empagliflozin in its bulk and pharmaceutical dosage forms, International Journal of Pharmaceutical Sciences and Research, 8(5), 2017, 2223-32.

[9] Khalil G, Salama I, Gomaa M, H Mohammed, Validated RP-HPLC method for simultaneous determination of canagliflozin, Dapagliflozin, Empagliflozin and Metformin, International Journal of Pharmaceutical Chemical and Biological Sciences, 8(1), 2018, 1-13.

[10] Rahul S, Kumar B, Avinash H, Empagliflozin: a review on analytical and bio-analytical methods, International Journal of Pharmaceutical Sciences and Research, 11(9), 2021, 4144-51.

[11] Susmita G, R Yadav, Analytical method development and validation of new stability-indicating reverse-phase high-performance liquid chromato-graphy method for simultaneous estimation of metformin hydro-chloride and empagliflozin in tablet dosage form, Asian Journal

of Pharmaceutical and Clinical Research, 12(1), 2019, 241-4.

[12] Borkar S, Mane K, Bhujbal A, Pharande S, Patil G, Patil R, Jain P,Pandey A,A Current Review on Analytical Tools for Determination of New Oral Antidiabetic Drugs, Empagliflozin, Linagliptin and Biguanides in Bulk Materials, Pharmaceuticals & Biological Samples, Journal of pharmaceutical Research International, 32(34), 2020, 67-83.

[13] Mabrouk M, Soliman S, El-Agizy H, Mansour F, A UPLC/DAD method for simultaneous determination of empagliflozin and three related substances in spiked human plasma, BMC Chemistry,13(1), 2019,1-9.

[14] Manojkumar K. Munde S, Khiste R, Sen D, Development and Validation of Novel Analytical Method for Empagliflozin and Metformin Hydrochloride in Bulk and Pharmaceutical Dosage Form by Four Different Simultaneous Estimation Approaches using UV Spectroscopy, 13(3), 2020,1-5.

[15] Ayoub B, Zahar N, Michel H, Tadros M, Economic Spectrofluorometric Bioanalysis of Empagliflozin in Rats' Plasma, Journal of analytical methodsin chemistry 2021, 1-7.

[16] Padmaja N, Mulagiri S, Veerabhadram G, Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs and combined dosage forms, 8(13), 2016, 207-213.

[17] Sushil D , K Sayali, Chaure, Kshirsagar S, Development and validation of UV Spectrophotometric method for simultaneous estimation of empagliflozin and Metformin HCL in bulk drugs, Asian journal of Pharmaceutical Analysis, 7(2),2017, 117-123.