WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 19, 419-430. Review Article ISSN 2277– 7105

A REVIEW ON ESTIMATION OF EMPAGLIFLOZIN AND METFORMIN HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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Article Received on
07 October 2018,

Revised on 28 October 2018,
Accepted on 18 Nov. 2018

DOI: 10.20959/wjpr201819-13730

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ABSTRACT

The aim of this review to focus on comprehensive update of different analytical methods for determination of oral anti-diabetic drugs like Empagliflozin and Metformin hydrochloride for the treatment of type 2 diabetes mellitus (T2DM), such as biguanides and sodium /glucose co –transportaer 2 inhibitors in their bulk materials and in pharmaceutical dosage forms. The review entails about analytical procedures like RP-HPLC, HPLC, UPLC, LC/MS/MS, Spectrophotometric (UV) methods taken from the literature. This review provides detailed information of development and validation for Empagliflozin and Metformin hydrochloride in bulk and in pharmaceutical preparations either alone or in combination with other hypoglycemic agent.

KEYWORDS: Metformin HCL, Empagliflozin, analytical methods, antidiabetic.

INTRODUCTION

Diabetes mellitus (DM) is a chronic condition characterized by high levels of blood glucose due to a defect in insulin production or activity. This disease has been a struggle for many generations.[1] The prevalence of diabetes is expeditiously escalating. Accordingly, the awareness of its treatment has been of a tremendous interest among recent population. Type 1(Insulin dependent Diabetes Mellitus – (IDDM), occur mostly in juvenile and when secretion of insulin is diminished. Management of type 1DMis achieved throught intake of exogenous insulin. Type 2(Non – insulin dependent Diabetes Mellitus –NIDDM) is more common in order adults however its incidence among teenagers have boosted in the current

year mainly due to unhealthy lifestyle. Oral anti-diabetic drugs are initiated in case of type 2 DM that had inadequate response toward lifestyle change including calorie restriction and increase in physical activity.[3]

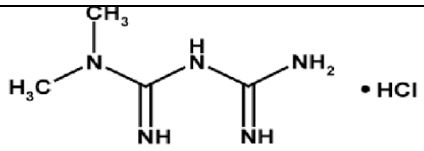
Empagliflozin and metformin hydrochloride are oral diabetes medicine that help control blood sugar levels. Empagliflozin and metformin is a combination medicine used with diet and exercise to improve blood sugar control in adult with type 2 diabetes mellitus.[6] empagliflozin and metformin is not for treating type 1 diabetes. empagliflozin and metformin is also used to lower the risk of death from heart attack, stroke, or heart failure in adults with type 2 diabetes who also heart disease.

Synjardy and Synjardy XR is the combination of empagliflozin and metformin, two medicines with complementary mechanisms of action. Empagliflozin, a sodium glucose co-transporter-2(SGLT2) inhibitor, removes excess glucose throught the urine by blocking glucose re-absorbtion in kidney. Metformin lowers glucose production by the liver and its its absorption in the intestine.[8]

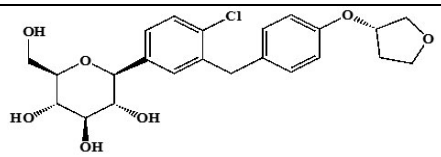
Syjardy and Synjardy XR is specifically indicated an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus who are not adequately controlled on a regimen containing empagliflozin or metformin, or in patients already being treated with both empagliflozin and metformin.[10]

Drug Profile

Metformin hydrochloride

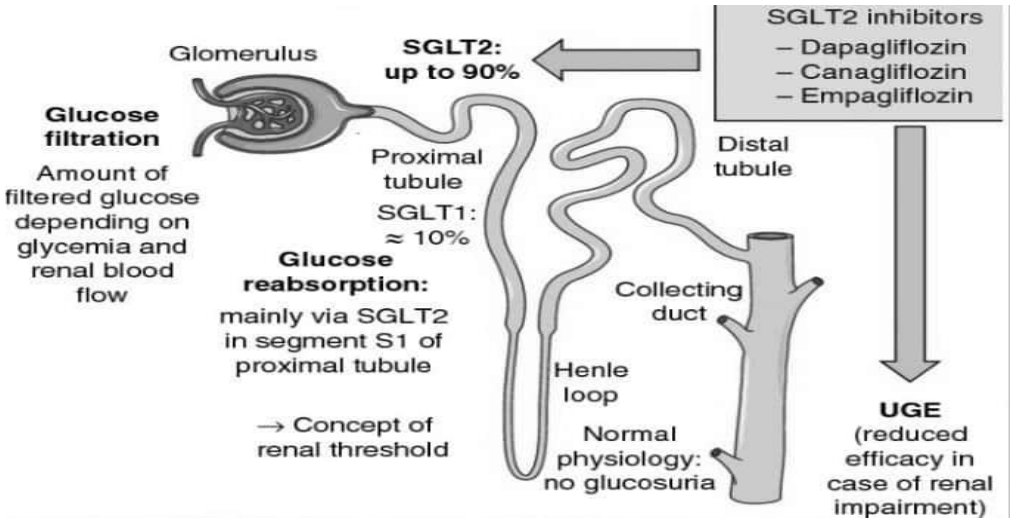
Structure	
Chemical name	1-carbamimidamido-N, N-dimethylmethanimidamide
Molecular formula	$C_4H_{11}N5.HCL$
Molecular weight	165.6g/mol
Appearance	A white, crystalline powder, hygroscopic
Category	Hypoglycaemic
Melting point	222^0C-226^0C
Solublility	Freely soluble in water, slightly soluble in alcohol and acetonitrile; practically insoluble in acetone, ether and chloroform

Empagliflozin

Structure	
Chemical name	D-Glucitol,1,5-anhydro-1-c-[4-chloro-3-[[4-[[(3s)-tetrahydro-3-furanyl]oxy]phenyl]methyl]phenyl]-,(1S)
Molecular formula	$C_{23}H_{27}CLO$
Molecular weight:	450.91g/mol
Appearance	White to yellowish powder
Category	Hypoglycemic
Melting point:	150^0C
Solubility	Very slightly soluble in water, slightly soluble in acetonitrile & ethanol sparingly soluble in methanol;

Mechanism of Action

Empagliflozin: Sodium –glucose co-transporter 2(SGLT2) is the predominant transporter responsible for reabsorption of glucose from the glomerular filtrate back into the circulation. Empagliflozin is an inhibitor of SGLT2. By inhibiting SGLT2, empagliflozin reduces renal reabsorption of filtered glucose and lowers the renal threshold for glucose, and thereby increases urinary glucose excretion.[11] Metformin hydrochloride: Metformin is an antihyperglycemic agent which improves glucose tolerance in patient with type 2 diabetes mellitus, lowering both basal and postprandial plasma glucose.[13] It is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. UnlikeSUs, metformin does not produce hypoglycemia in either patients with type 2 diabetes mellitus or normal subjects (except in special circumstances) and does not cause hyperinsulinemia. with metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decrease.[15]



Reported Analytical Methods

Chromatographic Methods

Title	Method	Description	Detection Mode
[1]Development and validation of RP-HPLC method for empagliflozin and metformin HCL	RP-HPLC	**Mobile Phase -** Methanol: Water (80:20,v/v) **Column:** Grace C_{18} column (250 × 4.6mm. 5µm) **Flow Rate:** 0.8ml/min **Retention Time:** EMPA – 5.133 min MET - 2.630 min	UV at 227nm
[2]Method development and validation of metformin and empagliflozin in Pharmaceutical dosage form in RP-HPLC	RP-HPLC	**Mobile Phase -** Methanol: Phosphate buffer (40:60, v/v) **Column:** C18 (150 mm× 4.6 mm.5µm) **Flow Rate:** 1ml/min **Retention Time:** EMPA – 4.210 min MET – 2.463 min	UV at 255nm
[3]Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of metformin hydrochloride and empagliflozin in bulk and in synthetic mixture	RP-HPLC	**Mobile Phase –** OPA buffer: Acetonitrile (45: 55 ,v/v) **Column:** Kromosil (250 × 4.6mm,5µm) **Flow Rate:** 0.8 ml/min **Retention Time:** EMPA – 3.413 min MET -2.270 min	PDA at 233nm
[4]Development and validation for the simultaneous estimation of metformin and empagliflozin in drug product by RP-HPLC	RP-HPLC	**Mobile Phase –** Water: Acetonitrile: Methanol (200:200:600,v/v) **Column:** Inertsil ODS 3v(250×4.6mm) **Flow Rate:** 0.8ml/min **Retention Time:** EMPA –3.848min MET-2.626min	UV at 265nm

[5]Stability indicating RP-HPLC method development and validation for estimation of empagliflozin and metformin HCL	RP-HPLC	**Mobile Phase –** Phosphate buffer: Acetonitrile (50: 50 ,v/v) **Column:** Inertsil ODS – 2 (250 × 4.6 mm, 5µm) **Flow Rate:** 1.0ml/min **Retention Time:** EMPA – 4.388 min MET- 2.635 min	PDA at 227nm
[6]A New validated RP-HPLC method for determination of metformin HCL and empagliflozin in its bulk and pharmaceutical dosage forms	RP-HPLC	**Mobile Phase –** Methanol: Phosphate buffer (70:30 v/v) **Column:** Inertsil C_{18} (4.6 × 150mm) 5µms **Flow Rate:** 1ml/min **Retention Time:** EMPA – 3.907 min MET -2.403 min	PDA at 240nm
[7]Development and validation of stability indicating RP-HPLC method for simultaneous estimation of metformin and empagliflozin in bulk and tablet dosage form	RP-HPLC	**Mobile Phase -** Phosphate buffer: Acetonitrile: Methanol (15: 80: 5 v/v) **Column:** C_{18} (4.6mm ×250mm ,5µm) **Flow Rate:** 1ml/min **Retention Time:** EMPA – 4.140min MET- 2.528 min	UV at 227nm
[8]Stability indicating RP-HPLC analytical method development and validation for the metformin and empagliflozin in pharmaceutical dosage form	RP-HPLC	**Mobile Phase –** Methanol: Acetonitrile: 0.025M potassium hydrogen phosphate buffer (45:30:25v/v) **Column:** Thermosil C_{18}(4.6mm ×250mm, 5µm) **UV Wavelength:** 225 nm **Flow Rate:** 1.2ml/min **Retention Time:** EMPA – 3.118 min MET- 2.836 min	PDA at 225nm
[9]Development and validation of stability indicating RP-HPLC method for empagliflozin	RP-HPLC	**Mobile Phase –** Methanol: Water (70: 30 ,v/v) **Column:** Phenomenex C_{18}(25 x 4.6mm, 5 µm) **Flow Rate:** 1.0.ml/min **Retention Time:** EMPA –4.808	UV at 224nm
[10]Stability indicating simultaneous estimation of metformin and empagliflozin in pharmaceutical tablet dosage form by RP-HPLC	RP-HPLC	**Mobile Phase -** 0.1%OPA buffer: Acetonitrile (45:55 ,v/v) **Column:** Kromasil C_{18} column (250mm×4.6mm,5µm) **Flow Rate:**1.1ml/min **Retention Time:** EMPA:2.908min MET:2.182min	UV at 226nm
[11]Validation RP-HPLC method for simultaneous determination of	RP-HPLC	**Mobile Phase -**Acetonitrile: 0.05M Potassium dihydrogen phosphate buffer	UV at 212nm

canagliflozin, Dapagliflozin, Emagliflozin and metformin		(PH4) (65:35,v/v) **Column:** C_{18} (250 ×4.6mm µm) **Flow Rate:** 1ml/min **Retention Time:** EMPA:3.004min MET:1.898min	
[12]Stress degradation studies and development of validation stability indicating assay method by RP-HPLC for simultaneous estimation of metformin and empagliflozin in presences of degradation product as per ICH guidelines	RP-HPLC	**Mobile Phase -**Buffer: Methanol (30:70 v/v) **Column:** Inertsil ODS (4.6 × 150mm,5 µm) **Flow Rate:** 1.0ml/min **Retention Time:** EMPA –2.606 min MET-1.788 min	UV at 220nm
[13]New validated stability indicating RP-HPLC method for simultaneous estimation of metformin HCL and empagliflozin in tablet dosage forms	RP-HPLC	**Mobile Phase –**Acetonitrile:0.1% Ortho phosphate acid (50:50,v/v) **Column:** Kromosil C_{18} Column (50×4.6mm; 5µm) **Flow Rate:**1ml/min **Retention Time:** EMPA:3.200min MET:2.192min	UV at 260nm
[14]RP-HPLC method development and validation for the simultaneous estimation of metformin and empagliflozin in tablet dosage form	RP-HPLC	**Mobile Phase –** Buffer: Acetonitrile: Methanol **Column –** ODS (250mm × 4.6 , 5 µm) **Flw Rate:**1ml/min **Retention Time:** EMPA –4.592 min MET-2.211 min	PDA at 233nm
[15]Method development and validation of RP-HPLC method for the estimation of empagliflozin in API	RP HPLC	**Mobile Phase –** 1.01M Acetate buffer: Methanol (50: 70,v/v) **Column:** Inertsil column (150 xz 40mm, 5µm) **Flow Rate:** 2ml/min **Retention Time:** 1.223min	PDA at 260nm
[16]Development and validation novel stability indicating RP – HPLC method for the determination of empagliflozin in bulk and pharmaceutical dosage form	RP-HPLC	**Mobile Phase –** Methanol: Acetonitrile (50:50v/v) **Column:** Inertsil (150 x 4.6mm ,5µm) **UV Wavelength:**265 nm **Flow Rate:** 20 µl/min **Retention Time:** EMPA – 2.184 min	PDA at 265nm
[17]Validate stability indicating RP-HPLC method for determination of empagliflozin	RP-HPLC	**Mobile Phase**-0.1%OPA: Acetonitrile (70:30v/v) **Column:** Hypersil BDS **Flow Rate:**1ml/min	UV at 233nm
[18] A New stability indicating RP-HPLC method for the simultaneous estimation of empagliflozin and metformin in	RP-HPLC	**Mobile Phase -** Water: Acetonitrile: Methanol (200:200:600,v/v) **Column:** Inertsil ODS 3v (250 × 4.6 mm)	UV at 265nm

its pure and pharmaceutical dosage form		**Flow Rate:** 0.8ml/min **Retention Time:** EMPA – 3.848 min MET – 2.626 min	
[19]Validated stability indicating HPLC method for determination of process related impurities in empagliflozin drug substance	HPLC	**Mobile Phase –** 0.1%OPA:Acetonitrile (30:70,v/v) **Column:** Inertsil C_8 (250 mm x4.6mm, 5 µm) **Flow Rate:** 1.2 ml/min	UV at 230nm
[20]UPLC Simultaneous determination of empagliflozin linagliptin and metformin	UPLC	**Mobile Phase**-Potassium dihydrogen phosphate buffer(PH4): Methanol (50:50v/v) **Column:** RSLC 120 C_{18} Column (100mmx2.1mm,2.2µm **Flow Rate:**0.4ml/min	UV at 225nm
[21]A Novel stability indicating RP-UPLC Dad method for determination of metformin and empagliflozin in bulk and tablet dosage form	UPLC	**Mobile phase** -0.1%OPA buffer (PH3.4) with 0.1 N NAOH solution: Methanol (40:60v/v) **Column:** C18 BEH (Ethylene Bridged Hybrid)UPLC (100mmx2.1mm,1.7µm **Flow Rate:** 0.25ml/min **Retention Time:** EMPA-3.471min MET-0.882min	PDA at 254nm
[22]Method development and validation for the determination of metformin HCL and empagliflozin in its bulk and pharmaceutical Dosage Forms by RP-ULTRA performance chromatography method	UPLC	**Mobile phase –**Acetonitrile: Phosphate buffer (PH3)(70:30 v/v) **Column:** BEH C18 Column 2.5x50mm)3µm **Flow Rate:** 0.3ml/min **Retention time:** EMPA – 1.294min MET -0.879min	UV at 220nm
[23]A Validated stability indicating UPLC Method for simultaneous determination of Metformin HCL and Empagliflozin in bulk Drug and tablet dosage form	UPLC	**Mobile Phase –**Phosphate buffer (PH3):Methanol (30:70v/v) **Column:** Dikma C18 (50x2.1mm,1.8µm) **Flow Rate:**1.0mi/min **Retention Time:** EMPA-1.189min MET-1.712min	PDA at240nm
[24]LC/MS /MS Determination of Empagliflozin and Metformin	LC/MS/ MS	**Mobile phase** -0.1%aq formic acid: Acetonitrile (75:25v/v) **Column:** BEH C18 column (50mmx2.1mm,1.7µm) **Flow Rate:**0.2 ml/min	-

Spectrophotometric Methods

Title	Method	Description	Detection Mode
[25]Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and metformin hydrochloride in bulk drugs.	Simultaneous equation method	**Solvent:** Methanol **Linearity:** EMPA-1-3µg/ml MET-2-10µg/ml **%Recovery:** EMPA-99.44% MET-93.27% **LOD:** EMPA-0.036µg/ml MET-0.04µg/ml **LOQ:** EMPA-0.111µg/ml MET-0.1402µg/ml	224nm and 230nm
[26]Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk ,drugs and combined dosage forms	A) Simultaneous Equation method. B) Absorbance ratio method	**Solvent:** Methanol **Linearity:** EMPA-5-25µg/ml MET-2-12µg/ml **%Recovery:** EMPA-98.99% MET-101.12%	A)272 and 234nm B)254nms and 226nm
[27]Novel UV and Visible Spectrophotometric Method for the analysis of Empagliflozin a type 2 diabetic drug in bulk and pharmaceutical formulations	**M1)**Direct UV **M2)**Phenothroline reaction **M3)** K Ferricyanide reaction	**Solvent:** Distil water **Linearity:** **M1)**2-12µg/ml **M2)**5-30µg/ml **M3)**10-60µg/ml **%Recovery:** **M1)** 98.15-100.68% **M2)**98.68-101.25% **M3)**98.25-101.03% **LOD:** **M1)**0.02µg/ml **M2)**0.03µg/ml **M3)**0.30µg/ml **LOQ:** **M1)**0.07µg/ml **M2)**0.10µg/ml **M3)**1.00µg/ml	**M1)**247nm **M2)**438nm **M3)**782nm
[28]Development and Validation of simple spectrophotometric and chemometric methods for simultaneous determination of empagliflozin and metformin:Applied to recently approved pharmaceutical formulation	Simultaneous equation	**Solvent:** Methanol **% Recovery:** EMPA-99.86% MET-100.48% **LOD:** EMPA-0.20µg/ml MET-0.19µg/ml **LOQ:** EMPA-0.59µg/ml	225 nm and237nm

		MET-0.58µg/ml	
[29]Development of Economic UV Spectrophotometric Method for Determination of Linagliptin in its Ternary Mixture with Empagliflozin and Metformin: Comparison to Economic pharmaceutical Analysis Literature.	First Derivative	**Solvent:** Methanol **Linearity:**2-25µg/ml **%Recovery:**97.88-102.11%	296nm
[30]Development and Validation of Analytical Method for the Simultaneous Estimation of Metformin Hydrochloride and Empagliflozin	**A)**Simultaneous equation method **B)**Dissolution in vitro analysis	**Solvent:** Methanol **Linearity:** EMPA-5-25µg/ml MET-2-14µg/ml **% Recovery:** EMPA-98.99% MET-101.12%	**A)**268nm and232nm **B)**238nm and 268nm
[31]Development and validation of Simple UV –Spectrophotometeric Method for the Determination of Empagliflozin	Direct UV	**Solvent:** Water: Methanol (9.0:1.0) **Linearity:** EMPA-1-3µg/ml **%Recovery:** EMPA-99.44% **LOD:**0.036µg/m **LOQ:**0.111µg/ml	224nm

CONCLUSION

The above study presents analytical method for analysis of Empagliflozin and Metformin hydrochloride in bulk materials and pharmaceutical dosage forms by RP-HPLC and UV Spectrophotometry. The various parameters like accuracy, precision, reliability, repeatability, analysis time and sensitivity are performed These methods are adequate to analyse the drugs in single component formulation as well as combination preparation. Literature survey suggested that various RP-HPLC, HPLC, UPLC, LC/MS/MS, UV methods were developed and reported. The published methods were validated for various parameters as per ICH guidelines.

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