Journal of Pharmaceutical Research International

32(34): 67-83, 2020; Article no.JPRI.63221
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

A Current Review on Analytical Tools for Determination of New Oral Antidiabetic Drugs, Empagliflozin, Linagliptin and Biguanides in Bulk Materials, Pharmaceuticals & Biological Samples

S. Baokar[1,2*], K. Mane[1], A. Bhujbal[1], S. Pharande[1], G. Patil[1], R. Patil[1], P. Jain[3] and A. Pandey[4]

[1]Delonix Society's Baramati College of Pharmacy, Barhanpur, Baramati Mah. 413102, India.
[2]Mansarovar Global University, Bhopal (M. P.) 466111, India.
[3]Scan Rrsearch Laboratories, Bhopal (M. P.) 466111, India.
[4]Mansarovar Global University, Bhopal (M. P.) 46611, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author SB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KM, SP, AB, GP, RP and PJ managed the analyses of the study. Author AP managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i3430966
Editor(s):
(1) Dr. Paola Angelini, University of Perugia, Italy.
Reviewers:
(1) Amina Mohsen Abass, Al-Nahrain University, Iraq.
(2) Manojkumar M. Nitalikar, Rajarambapu College of Pharmacy, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63221

Review Article

Received 15 September 2020
Accepted 21 November 2020
Published 10 December 2020

ABSTRACT

Worldwide the R & D divisions of Pharma industry are actively involved in the development of new therapeutic agents. These agents may be either new entities or partial structural modification of the existing one. The recent FDA statistics represent that the average number of drug filings are increasing every year in the thrust areas like anti-cancer agents, anti-diabetic, antibiotics, cardio-vascular drugs, respiratory drugs etc. Sodium glucose co-transporter-2(SGLT-2) inhibitors, dipeptidyl peptidase-4 (DPP-4) inhibitors and biguanides are effective oral anti-diabetic agents used in treatment of type 2 Diabetes Mellitus. Therefore, the necessity to explore and compare the existing analytical and bioanalytical assays used for determination of such drugs either single or in

**Corresponding author: E-mail: krishnabaokar@gmail.com;*

combination is crucial. Many methods were reported in the literature for the bio-analysis and analysis of four novel gliptins combinations, empagliflozin-linagliptin, empagliflozin-metformin HCl, linagliptin-metformin HCl, empagliflozin-linagliptin-metformin HCl combination with application on Glyxambi®, Synjardy®, Jentadueto®, Trijardy® XR tablets respectively. Furthermore, 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.

Keywords: Anti-diabetic; analytical methods; empagliflozin; linagliptin; metformin HCl.

1. INTRODUCTION

Analysis is vital in any product or service, and it is also important in drug because it involves life [1]. Analytical method development and validation for the analysis therapeutic components and associated substances play an important role in the discovery, development and manufacture of pharmaceuticals and natural medicinal compounds. Analytical instruments play a major role in the process to achieve high quality and reliable analytical data. Thus everyone in the analytical laboratory should be concerned about the quality assurance of equipment. Analytical method could be spectral, chromatographic, electrochemical, hyphenated or miscellaneous. Analytical method development is the process of selecting an accurate assay procedure to determine the composition of a formulation. It is the process of proving that an analytical method is acceptable for use in laboratory to measure the concentration of subsequent samples. Analytical methods should be used within GMP and GLP environments and must be developed using the protocols and acceptance criteria set out in the ICH guidelines Q2(R1) [2-6]. Diabetes mellitus is one of the chronic metabolic disorders that have reached to epidemic proportions worldwide [6]. It may be defined as a group of physiological impairments or dysfunctions distinguished by chronic hyperglycemia as a consequence of the inability of cells to use glucose for essential biological processes. The underlying causes of diabetes mellitus include insulin insensitivity, impaired insulin secretion or excessive glucagon secretion [7]. In addition to diabetes mellitus being responsible for an increased rate of mortality and morbidity incidences in patients [6], it is usually also associated with a number of different damaging effects on various tissue, cellular and even organ functions. Some of the complications of diabetes mellitus include hypertension [7], cardiovascular diseases [8], diabetic retinopathy [9], renal nephropathy [10] etc. Type 1 (Insulin dependent Diabetes Mellitus-IDDM), occur mostly in juvenile and when secretion of insulin is diminished. Management of type 1 DM is achieved through intake of exogenous insulin. Type 2 (Non-insulin dependent Diabetes Mellitus - NIDDM), is more common in older adults however its incidence among teenagers have boosted in the current years mainly due to unhealthy lifestyle. The clinical management as well as prevention of diabetes requires superlative control of factors like blood glucose, blood pressure, lipid concentrations, body weight etc. that have the potential to cause complications. This can be accomplished by maintaining a strict diet regime and regular exercise or by the use of anti-diabetic medications or a combination therapy including both [7]. 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. SGLT-2 inhibitors, DPP-4 inhibitors and biguanides are effective oral anti-diabetic agents used in treatment of type 2 Diabetes Mellitus. Therefore, the necessity to explore and compare the existing analytical and bioanalytical assays used for determination of such drugs either single or in combination is crucial. Diverse techniques like spectrophotometry, capillary electrophoresis (CE), high-performance liquid chromatography (HPLC), liquid chromatography--mass spectrometry (LC-MS) and high performance thin layer chromatography (HPTLC) have been used for the analysis of different formulations. These techniques offer a sensitive and rapid determination of these 3 drugs in bulk drug, pharmaceutical formulations, in biological matrices and could be used for quality control assay and pharmacokinetic assays. In the present review we have compiled the published

analytical methods reported for the determination of empagliflozin, linagliptin, metformin HCl in bulk drug, various pharmaceutical formulations and in biological samples.

1.1 Oral Hypoglycemic

Oral hypoglycemic are chemical agents, which are used to control the blood glucose level of diabetes patients. These drugs are also called anti-hyperglycemic agents that act by increasing insulin secretion, promoting organs sensitivity towards insulin and sometimes reducing the absorption of glucose from the gastrointestinal tract into the blood [11].

1.2 Biguanides

The discovery of biguanide and its derivatives in the management of diabetes started in the middle ages. *Galega officinalis*, an herbaceous plant was found to contain guanidine, galegine and biguanide, which was found to decrease blood glucose levels [12]. Biguanides are widely prescribed antihyperglycemic agents that suppress hepatic glucose production, increase peripheral glucose uptake and moderately reduce LDL cholesterol and triglyceride levels. Glucose control with the aid of biguanides appears to decrease the risk of diabetes-related complications, and is not associated with weight gain. The most common adverse effect of biguanides is gastrointestinal distress, including diarrhoea, cramps, nausea, vomiting, and increased flatulence. Long-term use of biguanides has been associated with decreased absorption of vitamin B12 [13].

1.3 Metformin

Metformin (MET Fig. 1A) chemically 3-(diaminomethylidene)-1, 1-dimethylguanidine, lowers plasma glucose level via four distinct mechanisms: inhibits hepatic gluconeogenesis and glycogenolysis, stimulates peripheral glucose uptake, utilization and increases insulin sensitivity in and delays/inhibits intestinal glucose absorption stimulates intracellular glycogen synthesis via glycogen synthase and increases membrane glucose transporters capacity [14]. MET is used basically in case of obese patients to increase fatty acid oxidation, reduce glucose absorption rate from GIT as well as, increase glucose uptake by phosphorylating GLUT-enhancer factor [15,16]. Moreover, a research revealed that MET actives AMP-activated protein kinase that promotes gluconeogenic genes in liver [17]. MET is used as monotherapy, but it can be used as a combination with sulfonylureas agents. However, if the patients has renal failure syndrome, it may lead to lactic acidosis [18].

1.4 Dipeptidyl Peptidase-4 (DPP-4) Inhibitors

Dipeptidyl peptidase-IV (DPP-4) inhibitors are a class of oral antidiabetic drugs that considered as oral alternatives to the GLP-1 agonist. They increase circulating concentrations of the incretin gastrointestinal hormones; GLP-1 and GIP polypeptides, by preventing their degradation by DPP-4 enzyme. This enhances insulin secretion from pancreatic β-cells, lowers glucose levels in an insulin-dependent manner and reduces plasma glucagon secretion from pancreatic α-cells. Thus, reducing endogenous glucose production [19,20]. The most common used DPP-4 inhibitors are: saxagliptin, vildagliptin, sitagliptin, linagliptin and alogliptin. Their effectiveness is comparable with the other hypoglycemic agents present in current times; even sometimes, these drugs are more effective with very little chances of hypoglycemia [21].

1.5 Linagliptin

Linagliptin (LIN Fig. 1B) chemically 1H-Purine-2, 6-dione, 8-[(3R)-3-amino-1-piper-idinyl]-7-(2-butyn-1- yl)-3, 7-dihydro-3-methyl-1-[(4-methyl-2-quinazolinyl) methyl] is a DPP-4 inhibitors in the treatment of type-II diabetes. DPP-4 is an enzyme that degrades the hormones, glucagon like peptides -1 (GLP-1) and glucose dependent insulin tropic polypeptide (GIP).Both GLP-1 & GIP increases the insulin biosynthesis and secretion from pancreatic α-Cells resulting in the reduction of hepatic glucose output [22]. Amongst all the members of DPP peptidase family, linagliptin is approximately 40,000 fold more selective towards the enzyme DPP-4. After binding, its dissociation rate from the active site of DPP-4 enzyme is also slow owing to its sustained release. Linagliptin mediates competitive DPP4 inhibition which is reversible at the same time [23,24].

1.6 Sodium Glucose Co-Transporter 2 Inhibitors

Sodium-glucose co-transporter 2 (SGLT-2) inhibitors, also known as gliflozins, are a novel class of pharmacologic agents developed for the management of T2DM. They play an important

role in glucose homeostasis by filtering and reabsorbing glucose in the proximal tubules. The mechanism of action of SGLT-2 inhibitors is independent of β-cell function and has shown favorable effects on blood pressure, body weight and albuminuria [25,26]. These benefits have resulted in considerable reduction in cardiovascular and renal events in patients with T2DM as evident from several cardiovascular outcome trials [27-30]. Currently there are four SGLT-2 inhibitors namely dapagliflozin (Farxiga), empagliflozin (Jardiance), canagliflozin (Invokana) and ertugliflozin (Steglatro) that are approved by the FDA and are widely studied in large cardiovascular outcome trials [27-30]. Besides, there are others such as ipragliflozin, luseogliflozin, janagliflozin, sotagliflozin, remogliflozin and tofogliflozin which are at different stages of clinical investigation leading to possible approval. Due to their unique mechanism of action, SGLT-2 inhibitors have the potential for use in combination therapy with other glucose lowering agents. They have shown clinically meaningful reductions in glycated hemoglobin, body weight and systolic blood pressure when given as add-on or fixed-dose combination therapies such as Invokamet (canagliflozin-metformin), Xigduo XR (dapagliflozin-metformin), Qtern (dapagliflozin-saxagliptin), Synjardy XR (empagliflozin-metformin) and Glyxambi (empagliflozin-linagliptin) for patients with T2DM [25,26].

1.7 Empagliflozin

Empagliflozin (EMP Fig. 1C) chemically (2S,3R,4R,5S,6R)-2-[4-chloro-3- [[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol.

SGLT2 is the predominant transporter responsible for reabsorption of glucose from the glomerular filtrate back into the circulation. EMP is an inhibitor of SGLT2 reduces renal reabsorption of filtered glucose and lowers its renal threshold and thereby increases urinary glucose excretion. EMP increases urinary excretion of glucose by markedly reducing the renal tubular threshold for glycosuria. This leads to excretion of 60-100 g/day of glucose, improving glucose control with low risk of hypoglycemia and results in loss of 240-400 kCal/ day into the urine with associated weight reduction. In addition, a decrease in blood pressure is seen due to osmotic diuresis of glucose and natriuresis of co-transported sodium [31,32].

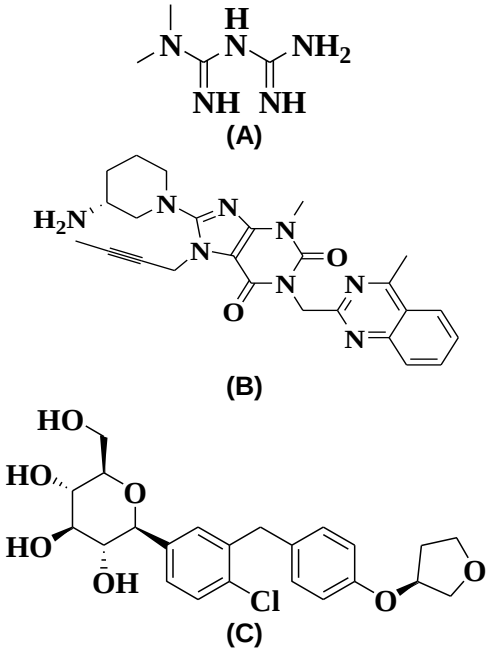

Fig. 1. Chemical structure of (A) Metformin, (B) Linagliptin, (C) Empagliflozin,

2. ANALYTICAL METHODS

2.1 HPLC/UPLC Method

Determination of MET, EMP and LIN alone or in combination with other drugs in biological samples as well as pharmaceutical formulations carried out by HPLC/UPLC are listed in Table 1 [33-61].

2.2 Spectrophotometric Method

Various spectrophotometric methods used in MET, EMP and LIN in pharmaceutical formulations and biological samples are presented in Table 2 [62-74].

2.3 HPTLC Method

MET, EMP and LIN estimation in various pharmaceutical dosage forms was successfully carried out using HPTLC methods and is summarized in Table 3 [75-80].

2.4 Stability-Indicating and LC-MS/MS Method

Stability-indicating HPLC and UPLC methods for determination of MET, EMP and LIN in pharmaceutical formulation were given in Table 4 [81-99] and LC-MS/MS method for determination of MET, EMP and LIN in biological matrix and pharmaceutical dosage form was given in Table 5 [100-110].

Table 1. RP-HPLC/UPLC methods for determination of MET, EMP and LIN

Sr. no	Name of Drug/ Formulation/ Biological Fluid	Stationary Phase	Mobile Phase	Detection wavelength (Nm)	Ref.
1	EMP-Tablet	Hypersil BDS 150mm X 4.6 Mm, 5µ	0.1% OPA: Acetonitrile (70:30% V/V)	233 nm	[33]
2	EMP-Tablet	Enable C_{18}G (250 X 4.6 Mm,5µ)	Methanol: Water (70:30 % V/V)	233 Nm	[34]
3	EMP-Tablet	Shim Pack C_{18} (250 Mm×4.6 Mm,5 µm)	Acetonitrile: Water (60:40 V/V)	223 Nm	[35]
4	EMP-Plasma	Acquity UPLC BEH C_{18} (50 Mm×2.1 Mm,1.7 µm)	Trifuoroacetic Acid (0.1%, Ph 2.5): Acetonitrile (60:40 V/V)	210 Nm	[36]
5	EMP-LIN-Synthetic Mixture	Agilent C_{18} (4.6×150mm) 5µ	Methanol: Phosphate Buffer (KH_2PO_4 And K_2HPO_4) Ph 3 (70:30 V/V)	254 Nm	[37]
6	EMP-LIN-Tablet	Agilent C_{18} (4.6×150mm) 5µ	Methanol: Phosphate Buffer (KH_2PO_4 And K_2HPO_4) Ph 3 (70:30 V/V)	254 Nm	[38]
7	EMP-LIN-Plasma	Discovery C_{18} (250×4.6×5)	Buffer: Acetonitrile (68:32 V/V)	218 Nm	[39]
8	EMP-LIN-Tablet	Thermo C_{18} (250mmx4.6mm, 5µm)	Phosphate Buffer Ph 3.4: Methanol (70:30 V/V)	240 Nm	[40]
9	EMP-LIN-Tablet	Equisil BDS C_{18} (4.6 × 250 Mm) 5µ	Methanol: Water (40:60 V/V)	224 Nm	[41]
10	EMP-LIN-Tablet	Youglin C_{18} (250 Mm X 4.6 Mm) 5 µm	Methanol: Water (80:20 V/V)	233 Nm	[42]
11	EMP-LIN-Tablet	Kromosil (250 X 4.6 Mm, 55 µm)	Buffer Ph 4.8: Acetonitrile (70:30 V/V)	286 Nm	[43]
12	EMP-LIN-Tablet	Kromasil 250 X 4.6 Mm, 5mm	0.1% O-Phosphoric Acid Buffer: Acetonitrile (60:40%V/V)	230 Nm	[44]
13	EMP-MET-Tablet	C_{18} (150 Mm X 4.6 Mm;5µ)	Methanol: Phosphate Buffer (40:60 V/V)	255 Nm	[45]
14	EMP-MET- Canagliflozin-Dapagliflozin- Tablet	Inertsil ODS C_{18} (250×4.6 Mm-5µm)	Acetonitrile: 0.05 M Phosphate Buffer Ph 4 (65:35 V/V)	212 Nm	[46]
15	EMP-MET- Canagliflozin-Dapagliflozin- Tablet	Prontosil (Lichrosorb 100-5- NH_2)	Nah_2po_4 Buffer (10 Mm, Ph 2.8):Acetonitrile (18.5:81.5, V/V),	225 Nm	[47]
16	EMP-MET-Tablet	Symmetry C_{18} (4.6×150mm) 5µ	Methanol: Phosphate Buffer (KH_2PO_4 And K_2HPO_4) Ph 3(70:30 V/V)	240 Nm	[48]
17	EMP-MET-Tablet	X-Select-HSS C_{18} SB (4.6-Mm X 25-Cm; 5-µm)	Phosphate Buffer : Acetonitrile (60:40 V/V)	255 Nm	[49]

Sr. no	Name of Drug/ Formulation/ Biological Fluid	Stationary Phase	Mobile Phase	Detection wavelength (Nm)	Ref.
18	EMP-MET-LIN-Tablet	Acclaim TM RSLC 120 C_{18} (100 Mm × 2.1 Mm, 2.2 µm)	Phosphate Buffer Ph 4: Methanol (50:50, V/V)	225 Nm	[50]
19	LIN-MET-Plasma	Grace Vyadyec Genesis CN (150 × 4.6 Mm, 4 µm)	Acetonitrile: 0.01M Phosphate Buffer Ph 7.0 (75:25 V/V)	237 Nm	[51]
20	LIN	Chiralpak AD-H (250*4.6 Mm*5 µm)	Ethanol: Methanol : Diethylamine (90:10:0.1 V/V/V)	225 Nm	[52]
21	EMP-MET-LIN-Saxagliptin-Teneligliptin-Pioglitazone-Dapagliflozin- Gliclazide- Tablet	Waters Reliant™ (250 Mm × 4.6 Mm, 5 µm).	Acetonitrile: 0/01% V/V Formic Acid Buffer	-	[53]
22	LIN-MET-Plasma	Lichrosphere 100 RP 18e (125 Mm × 4.0 Mm, 5 Mm)	Potassium Dihydrogen Orthophosphate (0.05 M, Ph 4.6): Methanol (70:30 V/V)	267 Nm	[54]
23	MET- Sitagliptin-Tablet	Symmetry C_{18} (100 Mm × 2.1 Mm, 2.2 Mm)	(Methanol 20%), Ph 3.5	220 Nm	[55]
24	MET-Teneligliptin- Tablet	Cosmosil C_{18}, 250X4.6mm, 5µm	Methanol: Water Ph 3.5 (50:50 V/V)	242 Nm	[56]
25	MET-Sitagliptin- Vildagliptin	Symmetry® Cyanide (150 Mm × 4.6 Mm, 5 Mm)	Phosphate Buffer Ph4.6: Acetonitrile (30:70 V/V)	210 Nm	[57]
26	MET-Gliclazide-Tablet	Alltima CN (250 Mm 4.6 Mm X5µm)	20 Mm Ammonium Formate Buffer Ph 3.5: Acetonitrile (45:55 V/V)	227 Nm	[58]
27	MET- LIN-Plasma	Onyx C_{18} Monolithic (100mm× 5µm)	CAN: Meoh: 0.01% HCOOH Ph 3 (30:13.59:56.41 V/V)	220 Nm	[59]
28	MET- Canagliflozin	HPLC: C_{18} (100 Mm × 2.1 Mm, 3 μm) UPLC: Hypersil Gold (50 Mm × 2.1 Mm, 1.9 μm).	HPLC: Methanol: 0.03 M Phosphate Buffer (75:25 V/V) At Ph 3.2 UPLC: Methanol: 0.03 M Phosphate Buffer (80:20 V/V)	240 Nm	[60]
29	EMP-LIN-Tablet	ODS (250 X 4.6mm, 5µ)	Buffer: Acetonitrile (45:50 V/V)	245 Nm	[61]

Table 2. Spectrophotometric methods used for determination of MET, EMP and LIN alone and in combined dosage form

Sr. no	Name of drug	Sample matrix	Method	Detection (nm)	Ref.
1	LIN-pioglitazone	Synthetic mixture	I-First derivative, II-Area under curve III-isosbestic point	I-LIN247.80,PIO 258.40 nm; II-LIN292-305, PIO265-279nm; III-273.05 nm	[62]
2	EMP-MET	Tablet	I-simultaneous equation II-Absorption ratio	I-EMP224,MET233nm II-MET233,EMP266 nm	[63]
3	EMP-MET	Tablet	simultaneous equation	EMP-225, MET- 237 nm	[64]
4	EMP-MET	Bulk drug	absorption correction	EMP224,MET230nm Both- 227 nm	[65]
5	EMP-LIN	Tablet	simultaneous equation	EMP233, LIN277nm	[66]
6	EMP-MET	Tablet	I- absorbance corrected II-area under curve III- dual wavelength	I-EMP224,MET232, intercept 203nm II-EMP219-229,MET 227-238nm III-EMP224-238,MET 232-244nm	[67]
7	EMP-LIN	Tablet	simultaneous equation	EMP233, LIN277nm	[68]
8	EMP-MET	Tablet	I-simultaneous equation II- Absorbance ratio	I-EMP-272, MET- 234 II-254nm and 226nm	[69]
9	MET	Tablet	Zero order	234	[70]
10	EMP-LIN	Tablet	I-zero-order II-ratio spectra	I-EMP225.4,LIN295 II- EMP236.8,LIN353.8	[71]
11	MET-LIN	Tablet	I-Third derivative II-Derivative ratio III-Ratio difference IV-Factorized dual wavelength	I-268.7nm II-264 nm III-262 and 268 nm IV-264 and 268nm	[72]
12	EMP-LIN	Tablet	I-Ratio subtraction coupled with extended ratio subtraction	I-EMP225,LIN226 nm	[73]
13	EMP	Tablet	Zero order	247	[74]

Table 3. HPTLC methods for determination of MET, EMP and LIN

Sr. no	Name of drug/formulation	Stationary phase plates	Mobile phase composition	Detection (nm)	Rf	Ref.
1	MET-LIN-Tablet	Silica gel 60 F254	Acetone: methanol: chloroform: formic acid (3:1:5:1v/v)	230	MET-0.19, LIN-0.72	[75]
2	EMP-LIN-Tablet	Silica gel 60 F254	Methanol: toluene: ethyl acetate (2: 4: 4v/v/v)	229	EMP- 0.57, LIN- 0.22	[76]
3	MET-EMP-Tablet	Silica gel 60F254	Toluene: 3% ammonium acetate in methanol: ethyl acetate: ammonia (3: 5: 2: 0.4 % v/v/v/v)	230	MET- 0.28, EMP-0.58	[77]
4	EMP-MET-LIN-Synthetic mixture	Silica gel 60 F254	n-Butanol: water: glacial acetic acid (6:3:1 v/v)	223	EMP-0.73, LIN-0.52, MET-0.33	[78]
5	LIN-MET saxagliptin- vildagliptin- Synthetic mixture	silica gel 60 F254	methanol-0.5% w/v aqueous ammonium sulfate (8 : 2, v/v)	225, 208 nm	MET-0.22 LIN-0.44 - SGP-0.51 VGP-0.46	[79]
6	MET-LIN-Tablet	silica gel 60 GF254	Acetone-methanol toluene-formic acid 4:3:2:1 (v/v/v/v)	259	MET- 0.61 LIN-0.82	[80]

Table 4. Stability-indicating HPLC and UPLC methods for determination of MET, EMP and LIN

Sr. no	Name Of Drug/ Formulation	Stationary Phase Plates	Mobile Phase	Detection Wavelength (Nm)	Ref.
1	LIN	Zorbax SB-Aq 250 × 4.6 Mm, 5 µm	A: (0.02M) KH_2PO_4 Buffer Ph 3.0; B: ACN: Meoh (90:10 V/V)	225 Nm	[81]
2	MET-EMP-Tablet	C_{18} BEH(Ethylene Bridged Hybrid) UPLC (100mm X 2.1mm ,1.8µm)	0.1% Ortho Phosphoric Acid Buffer (Ph 3.4): Methanol (40:60% V/V)	254 Nm	[82]
3	EMP-LIN-Tablet	Agilent C_{18} (4.6×150mm)5µ,	Methanol: Phosphate Buffer (KH2PO4 And K2HPO4) Phosphate Ph 3(70:30 V/V)	254 Nm	[83]
4	MET-EMP-Tablet	Kromosil C_{18} (250x4.6 Mm; 5.6 µ)	Acetonitrile: 0.1% Orthophosphoric Acid Buffer Ph 2.8 (50:50 V/V)	260 Nm	[84]
5	MET-EMP-Tablet	Kromasil C_{18} (250mm×4.6mm, 5 µm)	Buffer: Acetonitrile (45: 55 V/V)	226nm	[85]

Sr. no	Name Of Drug/ Formulation	Stationary Phase Plates	Mobile Phase	Detection Wavelength (Nm)	Ref.
6	EMP-Tablet	Develosil ODS HG-5 RP C_{18}, 5µm, 15cm X 4.6mm	Phosphate Buffer Ph-2.8: Acetonitrile (45:55 V/V)	228 Nm	[86]
7	LIN-Tablet	C18 Column	Acetonitrile: Methanol (50:50 (V/V)	238 Nm	[87]
8	MET-EMP-Synthetic Mixture	Kromosil 250 X 4.6 Mm, 5µm	0.1% Ortho Phosphoric Acid Buffer: Acetonitrile (45:55v/V)	233 Nm	[88]
9	MET-LIN-Tablet	BDS Hypersil C_8 (250 × 4.6 Mm, 5 µm)	Acetonitrile: Water: Methanol (25:50:25 V/V/V)	243 Nm	[89]
10	EMP-Tablet	Intersil® C_{18} (150 Mm × 4 Mm, 5 Mm)	Acetonitrile: Phosphate Buffer Ph 4, (50:50 V/V)	225 Nm	[90]
11	EMP-LIN-Tablet	BDS C_{18} (250mm X 4.6 Mm 5µ)	0.1% Perchloric Acid: Acetonitrile (60:40 V/V)	230nm	[91]
12	EMP-MET-Tablet	Inertsil ODS (4.6 X 150mm, 5 µM)	Buffer (Ph 3): Methanol (30:70 V/V)	220 Nm	[92]
13	EMP-LIN-Tablet	Luna Omega Polar C_{18}, 100x2.1mm, 1.6µ	A: 10mm Potassium Dihydrogen Orthophosphate Ph-3.0; B: Acetonitrile And Methanol (55:45%V/V)	225nm	[93]
14	MET-LIN-Tablet	Inertsil ODS2, 150 Mm X 4.6mm, 5µ	Ammonium Phosphate Buffer (Ph 3.00): Methanol (40:60 V/V)	233nm	[94]
15	LIN-Tablet	Thermo Scientific® RP-8 (100 Mm × 4.6 Mm; 5µm)	A: 0.1% Formic Acid Ph 3.5; B:Acetonitrile	294 Nm	[95]
16	MET-EMP-Tablet	BDS (250 Mm × 4.6 Mm, 5 M)	0.1% Orthophosphoric Acid Buffer: Acetonitrile (50:50 V/V)	210 Nm	[96]
17	MET-EMP-Tablet	Thermosil C_{18} (4.6mmx250mm, 5µ).	Methanol: Acetonitrile: 0.025M Potassium Hydrogen Phosphate Buffer (Ph: 3) (45:30:25, V/V/V)	225nm	[97]
18	MET-EMP-Tablet	Kromasil C_{18} (250mm×4.6mm, 5µm),	Buffer: Acetonitrile (45:55 V/V)	226nm	[98]
19	EMP- LIN-Tablet	Hypersil ODS 3V, 250 X 4.6 Mm.5.0µ	A: Potassium Di-Hydrogen Phosphate Ph2.20 B: Water: Acetonitrile (5:95)	225nm	[99]

Table 5. LC-MS/MS method for determination of MET, EMP and LIN

Sr. no	Name of drug/formulation	Stationary phase plates	Mobile phase	MS/MS transitions (m/z)	Ref.
1	MET-Alogliptin-Tablet	Hypersil gold 50 mm × 2.1 mm (1.9 µm)	Acetonitrile:0.2 % formic acid (10: 90, v/v)	MET-130.12-71.32, ALN-340.33-116.32	[100]
2	EMP-Human plasma	UPLC BEH Shield RP C_{18}(150 ×2.1 mm, 1.7 µm)	Water: acetonitrile (10:90, v/v)	449.01- 371.21	[101]
3	EMP-MET-Tablet	C_{18} (50 × 2.1 mm, 1.7µm	0.1% Aqueous formic acid: acetonitrile (75:25, v/v)	EMP-451.04–71.07, MET-130.11–71.14	[102]
4	EMP	Eclipse plus C_{18} RRHD (2.1 × 50 mm, 1.8 µm)	Water with 0.1% formic acid: methanol in 50:50 (v/v)	Base peak-451.1516	[103]
5	LIN- tadalafil- plasma	Zorbax Eclipse $XDBC_{18}$ (4.6 × 150 mm, 5 µm)	Methanol:0.05% aqueous formic acid (50:50)	Base peak-LIN-473, TDL-390	[104]
6	LIN-MET- human plasma	Symmetry® C_{18} (3.5 µm x 4.6 mm x 50)	Methanol: 10 mM ammonium formate buffer (containing 0.2 % formic acid) (95: 5, v/v)	LIN-473.24-419.94 MET-130.14-60.18	[105]
7	EMP-MET- Canagliflozin-Dapagliflozin-Plasma	Xbridge C_{18} column (50 × 2.1 mm, 5 µm)	A: water; B: acetonitrile containing 1 mM ammonium formate and 0.1% formic acid.	MET- 130.1-71.1 EMP-468.0-354.9 CAN-462.0-191.2 DAP-426.1-167.1	[106]
8	LIN-Plasma	Waters, X-Bridge, C_{18}, (5µm 4.6×50 mm)	Acetonitrile: 0.1 % formic acid (90:10 v/v)	473.54-157.6	[107]
9	EMP-MET-Plasma	Orosil C_{18} column (150 4.6 mm, 3 m).	Methanol:10 mM ammonium trifluoroacetate (90:10, v/v)	EMP-468.0-355.1 MET-130.0-71.2	[108]
10	LIN-Plasma	Gemini C_{18} (100 mm × 4.6 mm, 3µ)	10 mM Ammonium formate: methanol (20:80 v/v)	473.3-420.1	[109]
11	EMP-LIN-Plasma	XSelect HSS Cyano (50 ×2.1 mm,3.5µm)	A: 2Mm ammonium acetate, pH 5.0 B: acetonitrile	EMP- 451.3-71.1 LIN- 473.2-420.2	[110]

3. CONCLUSION

There are a extensive choice of analytical techniques were accessible for the analysis of MET, EMP and LIN alone or in combination with other drugs in pharmaceutical and biological samples. The available data it was revealed that HPLC technique was comprehensively used for the estimation of MET, EMP and LIN in various matrices like plasma and serum. HPLC MS/MS method is also recommended in the determination of MET, EMP and LIN in biological samples because HPLC separation ability with MS sensitivity and selectivity allows the unambiguous identification of MET, EMP and LIN and its metabolites. HPLC with UV detection is applicable in the case of analysis of MET, EMP and LIN in pharmaceuticals which provide us cost effective accurate method when compare with more advance techniques. Various bioanalytical method development and validation has been performed in order to study the bioavailability and bioequivalence of the drug which would help the chemists/analyses to formulate a stabilized drug.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hema, Swati Reddy G. A review on new analytical method development and validation by RP-HPLC. Int Res J Pharm Biosci. 2017;4:41-50.
2. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Topic Q7: Good Manufacturing Practices for Pharmaceutical Ingredients, 2000.
3. Current Good Manufacturing Practices for finished Pharmaceuticals, 21 CFR, Parts 210 and 211, US Food and Drug Administration.
4. European Commission. Final Version of Annex 15 to the EU Guide to Good Manufacturing Practice: Qualification and validation. 2001;4:1-10.
5. McDowall RD, Effective and Practical risk management options for computerized system validation, Quality Assurance Journal. 2005;9 (3):196-227.
6. Xiao-Wu Che, Zhi-Xu He, Zhi-Wei Zhou, Tianxin Yang, Xueji Zhang, Yin-Xue Yang, Wei Duan, Shu-Feng Zhou. Clinical pharmacology of dipeptidyl peptidase 4 inhibitors indicated for the treatment of type 2 diabetes mellitus. Clinical and Experimental Pharmacology and Physiology. 2015;42(10):999-1024.
7. Mehanna A. Antidiabetic agents: Past, present and future. Future Medicinal Chemistry. 2013;5(4):411–430.
8. Potenza MA, Nacci C, Gagliardi S, Montagnani M. Cardiovascular Complications in Diabetes: Lessons from Animal Models. Current Medicinal Chemistry. 2011;18(12):1806– 1819.
9. World Health Organization. Global data on visual impairment 2010. Geneva: World Health Organization; 2010.
10. World Health Organization. Global status report on noncommunicable diseases. World Health. 2010;176.
11. Pagkalos EM. Combinations of insulin and oral hypoglycemic agents in diabetes mellitus type 2. Diabetes Res Clin Pract, 93(Suppl 1):S100-101. DOI:10.1016/S0168-2011; 8227(11)70022-5
12. Michael GF, Abd El M. Analytical Study of Some Drugs Used in Treatment of Diabetes. Phd thesis, Faculty of Pharmacy Cairo University; 2019.
13. Sofer S et al. A diet with carbohydrates eaten primarily at dinner: an innovative, nutritional approach to end the vicious cycle of abdominal obesity. Nutr Pre Treat Obe. 2014;401-414.

14. UKPAR, in: Metformin 500 mg and 800 mg Tablets BP, MHRA, United Kingdom. 2007; 38.
15. Igel LI, Sinha A, Saunders KH, Apovian CM, Vojta D, Aronne LJ. Metformin: an Old Therapy that Deserves a New Indication for the Treatment of Obesity. Curr Atheroscler Rep. 2016;18(4):16.
16. Jackson RA, Hawa MI, Jaspan JB, Sim BM, Disilvio L, Featherbe D, Kurtz AB. Mechanism of metformin action in non-insulin-dependent diabetes. Diabetes. 1987;36(5):632-640.
17. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001; 108(8):1167-1174.
18. Fimognari FL, Corsonello A, Pastorell R, Antonelli-Incalzi R. Metformininduced pancreatitis: A possible adverse drug effect during acute renal failure. Diabetes Care. 2006;29(5):1183.
19. Scheen AJ. Dipeptidylpeptidase-4 (DPP-4) inhibitors are favourable to glucagonlike peptide-1 (GLP-1) receptor agonists: Yes. Eur J Intern Med. 2012;23(2):126-131.
20. Ahren B, Schmitz O. GLP-1 receptor agonists and DPP-4 inhibitors in the treatment of type 2 diabetes. Horm Metab Res. 2004;36(11-12):867-876.
21. Crepaldi G, Carruba M, Comaschi M, Del Prato S, Frajese G, Paolisso G. Dipeptidyl peptidase 4 (DPP-4) inhibitors and their role in Type 2 diabetes management. J Endocrinol Invest. 2007;30(7):610-614.
22. Mark Kirby, Denise M T Yu, Steven O'Connor, Mark D Gorrell. Inhibitor Selectivity in the Clinical Application of Dipeptidyl peptidase-4 Inhibition. Clinical science. 2009;118(1):31-41.
23. Eckhardt M, Hauel N, Himmelsbach F, Langkopf E, Nar H, Mark M, Lotz R. 3,5-Dihydro-imidazo[4,5-d]pyridazin-4-ones: A class of potent DPP-4 inhibitors. Bioorganic and Medicinal Chemistry Letters. 2008;18(11):3158–3162.
24. Gallwitz, B. Small molecule dipeptidylpeptidase IV inhibitors under investigation for diabetes mellitus therapy. Expert Opinion on Investigational Drugs. 2011;20(6):723–732.
25. Lo KB, Gul F, Ram P, et al. The effects of SGLT2 inhibitors on cardiovascular and renal outcomes in diabetic patients: A systematic review and meta-analysis. Cardiorenal Med. 2020;10(1):1–10.
26. Giugliano D, Esposito K. Class effect for SGLT-2 inhibitors: A tale of 9 drugs. Cardiovasc. Diabetol. 2019;18:94.
27. Zinman B, Wanner C, Lachin JM et al. Empagliflozin, cardiovascular outcomesand mortality in Type 2 diabetes. N. Engl. J. Med. 2015;373(22):2117–2128.
28. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in Type 2 diabetes. N. Engl. J. Med. 2017;377(7):644–657.
29. Wiviott SD, Raz I, Bonaca MP et al. Dapagliflozin and cardiovascular outcomes in Type 2 diabetes. N. Engl. J. Med. 2019; 380(4):347–357.
30. Perkovic V, Jardine MJ, Neal B et al. Canagliflozin and renal outcomes in Type 2 diabetes and nephropathy. N. Engl. J. Med. 2019;380(24):2295–2306.
31. Gita Chawla, Krishna Kr Chaudhary. A complete review of empagliflozin: Most specific and potent SGLT2 inhibitor used for the treatment of type 2 diabetes mellitus. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2019:13(3): 2001-2008.
32. Inzucchi SE, Zinman B, Wanner C, Ferrari R, Fitchett D, Hantel S, et al. SGLT-2 inhibitors and cardiovascular risk: proposed pathways and review of ongoing outcome trials. Diabetes Vasc Dis Res. 2015;1;12(2):90-100.
33. Shyamala, M. Soumika, E. Sangeetha, L. Mahender. Method development and validation of empagliflozin by rp-hplc in bulk and pharmaceutical dosage form. An International Journal of Advances in Pharmaceutical Sciences. 2016;7(1):3040-3042.
34. Mounika P Siridevi, Hemant T Kumar, Srinivasa Y Rao, Vara Prasad K Rao. RP-HPLC Method for Quantification of Empagliflozin in Pharmaceutical Formulation. Asian J. Pharm. Tech. 2019; 9(3):208-211.
35. Madhurima Basak, Santhosh Reddy Gouru, Animesh Bera, Krishna Veni Nagappan. A rapid and sensitive rp-hplc method for the quantitative analysis of empagliflozin in bulk and pharmaceutical dosage form. Int J App Pharm. 2019;11: 5 60-65.
36. Mokhtar M, Mabrouk SM, Soliman HM, El-Agizy, FR, Mansour A. UPLC/DAD method for simultaneous determination of empaglifozin and three related substances

in spiked human plasma. BMC Chemistry. 2019;13:83.

37. Jayalaxmi M, Rajesh T, Gampa VK. A Validated RP- HPLC Method for the Simultaneous Estimation of Empagliflozin and Linagliptin in its Bulk and Pharmaceutical Dosage Forms.
38. Suresh B M. Simultaneous estimation and validation of empagliflozin and linagliptin bulk drug and it's dosage form by rp-hplc. International Journal of Pharmaceutical Research and Novel Sciences. 2017;3(2): 377-384.
39. Sharmila D, Suneetha A. Validated hplc-uv method for simultaneous estimation of linagliptin and empagliflozin in human plasma. Int J App Pharm. 2018;10(3):56-61.
40. Anjali B, Mounika A, Shweta B, Bhagvan R. Simultaneous estimation of empagliflozin and linagliptin by rp-hplc method. World Journal of Pharmacy and Pharmaceutical Sciences. 2018;7(8):1062-1071.
41. Abhijeet NR, Shailesh G, Jawarkar, Vaibhav SK, Vivek AK. Method development, validation by simultaneous estimation of empagliflozin and linagliptin by rp-hplc method. J. Pharm. Sci. Innov. 2020;9(1):1-4.
42. Sharma AS, Agrawal KS, Gandhi LR. Development and validation of rp-hplc method for simultaneous determination of empagliflozin and linagliptin in bulk & its formulation. Indian Research Journal of Pharmacy and Science. 2018;19:1747.
43. Sruthi V, Poornima B. Analytical Method Development and Validation for Simultaneous Estimation of Linagliptin and Empagliflozin in Bulk and Pharmaceutical Formulation by RP-HPLC. Int. Pharm. Pharm. Res. 2019;16(2): 555-568.
44. Lakshmana RA, Prasanthi T, Anusha EL. RP-HPLC method development and validation for simultaneous estimation of linagliptin and empagliflozin. Indian Drug. 2019;55(5):68-71.
45. Jaffar Hussain B, Karuppasamy Y, Suresh G, Somasekhar M, Jyothsna A, Venkatesh. Method development and validation of metformin and empagliflozin in pharmaceutical dosage forms in rp-hplc. Asian Journal of Research in Chemistry and Pharmaceutical Sciences. 2016;4(3): 91-100.
46. Khalil GA, Salama I, Gomaa MS, Helal MA. Validated rp-hplc method for simultaneous determination of canagliflozin, dapagliflozin, empagliflozin and metformin. International Journal of Pharmaceutical, Chemical & Biological Sciences. 2018;8(1):1-13.
47. Sonia T, Hassib L, Elham A, Taha Y, Ehab F, Elkady O, Ghada H. Validated Liquid Chromatographic Method for the Determination of (Canagliflozin, Dapagliflozin or Empagliflozin) and Metformin in the Presence of (1-Cyanoguanidine). Journal of Chromatographic Science. 2018;1–11.
48. Godasu SK, Sreenivas SA. 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. 2017;8(5):2223-2232.
49. Alaa S, Amin SF, Mohamed MM. Abo-Taleb. Simultaneous For the Estimation of Metformin and Empagliflozin in Pharmaceutical Dosage Form by HPLC Method. IOSR Journal of Pharmacy and Biological Sciences. 2019;14:1 75-80.
50. Bassam M B. UPLC Simultaneous Determination of Empagliflozin, Linagliptin and Metformin New Combinations. RSC Adv; 2015.
    DOI: 10.1039/C5RA17231D
51. Rutvik HP, Rajeshwari R, Dilip GM. Bioanalytical method development and validation for simultaneous determination of linagliptin and metformin drugs in human plasma by rp-hplc method. Pharmacophore. 2014;5(2):202-218.
52. Cholleti VK, Pasula A, Pavan KVY, Ravindra K, Nitin HD. A New and Enantioselective Chromatographic Method for Linagliptin on Amylose Coated Chiral Stationary Phase. American Journal of Analytical Chemistry. 2016;7:556-567.
53. Ravi K, Ramesh BB, Garima K, Rubina B. Optimization of a single HPLC-PDA method for quantifying Metformin, Gliclazide, Pioglitazone, Dapagliflozin, Empagliflozin, Saxagliptin, Linagliptin and Teneligliptin using central composite design. Bioorganic Chemistry. 2019;91: 103111.
54. Ali G, Fahimeh B, Mohammad F. Sensitive Simultaneous Measurement of Metformin and Linagliptin in Plasma Samples by Couple of Nano Graphene Oxide-based

Dispersive Solid Phase Extraction Method and Liquid Chromatography. IJPR (Accepted Manuscript).
55. Moataz SH. Improved Chromatographic Separation of Sitagliptin Phosphate and Metformin Hydrochloride. Int J Biomed Sci. 2015;11(4):190-194.
56. Sawant P, Gandhi LR, Bhajipale NS. RP-HPLC method development and validation of teneligliptin and metformin in pharmaceutical dosage forms. Indian Research Journal of Pharmacy and Science. 2018;19:1733.
57. Ramzia IEB, Ehab FE, Bassam MA. Development and validation of a reversed phase liquid chromatographic method for the determination of three Gliptins and Metformin in the presence of Metformin impurity (1-cyanoguanidine). European Journal of Chemistry. 2013;4(4):444-449.
58. Ahmed G, Hani AS, Crispin RD. Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide. Journal of Food and Drug Analysis. 2019;27:315 -322.
59. Balamurugan K, Kirtimaya M, Suresh R. simultaneous estimation of linagliptin and metformin hcl in human plasma by rp-hplc method. Int. Res. J. Pharm. 2019;10(1): 167-170.
60. Wafaa AZ, Shereen M, Moataz SH. Comparative Liquid Chromatographic Study for Concurrent Determination of Canagliflozin and Metformin in Combined Tablets. Journal of Analytical Methods in Chemistry; 2017.
Article ID 9197230
61. Madhusudhan P, Radhakrishna MR, Devanna N. RPHPLC Method Development and Validation for Simultaneous Determination of Linagliptin and Empagliflozine in Tablet Dosage Form. International Advanced Research Journal in Science, Engineering and Technology. 2015;2(2):95-99.
62. Heba AA, Sherin FH, Mohammed SE, Khaled MD. Determination of Novel Promising Combination of Linagliptin and Pioglitazone HCl in Bulk and Laboratory Synthetic Mixture by Earth-Friendly Three Spectrophotometric Methods. Rec. Pharm. Biomed. Sci. A 2020;4(2):1- 12.
63. Potdar A, Jorige A, Mogili S. Development and validation of uv spectrophotometric method for simultaneous estimation of empagliflozin and metformin hydrochloride in combined dosage form. International Journal of Pharmaceutical Sciences and Research. 2020; 11(5): 2173-2180.
64. Bassam M. Development and validation of simple spectrophotometric and chemometric methods for simultaneous determination of empagliflozin and metformin: Applied to recently approve pharmaceutical formulation. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2016;168: 118–122.
65. Patil SD, Chaure SK, Kshirsagar S. Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs. Asian J. Pharm. Ana. 2017;7(2):117-123.
66. Padmaja N, Veerabhadram G. Development and validation of analytical method for Simultaneous estimation of Empagliflozin and Linagliptin in bulk drugs and combined dosage forms using UV-visible spectroscopy. Der Pharmacia Lettre, 2015;7(12):306-312.
67. Sushil DP, Swapnapurti VB, Tejashri RD, Sanjay K. Spectrophotometric simultaneous determination of Empagliflozin and Metformin in combined tablet dosage form by absorbance corrected method, area under curve method, and dual wavelength spectrophotometry. Asian J. Research Chem. 2019;12(2):94-98.
68. Pravin C, Yogita T, Chandrakala P, Priya M, Monika J, Rutuja M, Komal H, Vidya G, Kajal S. Comparative study of two different marketed preparation (tablets) containing empagliflozin & linagliptin and developing novel method for simultaneous estimation of empagliflozin and linagliptin using uv visible spectrophotometer. World Journal of Pharmacy And Pharmaceutical Sciences. 2019;8(4):1227-1235.
69. Padmaja N, Mulagiri SB, Veerabhadram G. Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs and combined dosage forms. Der Pharmacia Lettre, 2016;8(13):207-213.
70. Yuvraj DD, Sandip MH, Somnath DB, Vijay RS, Dhanraj RJ. Development and Validation of UV-Spectrophotometric Method for Estimation of Metformin in Bulk and Tablet Dosage Form. Indian Journal of

Pharmaceutical Education and Research, 2017; 51(4S):S 754-S760.
71. Maha FAG, Omar AA, Miriam FA, Mariam MT. Comparative Study Between Multivariate and Univariate Analysis of Two Antidiabetic Combinations. Journal of AOAC International. 2017;100(5):1379-1390.
72. Ola MA, Ahmed MAM, Ahmed SS, Shymaa SS. Simultaneous spectrophotometric determination of compounds having relatively disparate absorbance and concentration ranges; application to antidiabetic formulation of linagliptin and metformin; 2017. DOI:10.1016/j.saa.2018.05.064.
73. Bahia AM, Marianne AM, Michael G. Different resolution techniques for management of overlapped spectra: Application for the determination of novel co-formulated hypoglycemic drugs in their combined pharmaceutical dosage form; 2018. DOI:10.1016/j.saa.2018; 06.072
74. Jyothirmai N, Nagaraju B, Anil KM, Novel UV. Visible Spectrophotometric methods for the analysis of Empagliflozin a type 2 diabetic drug in bulk and pharmaceutical formulations. Journal de Afrikana. 2016; 3(1):177-187.
75. Jillala S, Balekari U, Ciddi V. Development and validation of stability indicating hptlc method for simultaneous determination of linagliptin and metformin. Int J Pharm Pharm Sci. 2016;8(1):112-115.
76. Bhole RP, Wankhede SB, Madhu P. Stability Indicating HPTLC Method for Simultaneous Estimation of Empagliflozin and Linagliptin in Pharmaceutical Formulation, Analytical Chemistry Letters. 2017;7:1,76-85.
77. Dedhiya PP, Singh RS, Shah PJ, Shah SA. Development and validation of high performance thin layer chromatographic method for estimation of metformin hydrochloride and empagliflozin in combined tablet dosage form using quality by design approach. Pharma Science Monitor 10(2), Apr-Jun. 2019;83-100.
78. Ishita MP, Usmangani KC, Harsha DJ, Devansh K, Dimal A. Densitometric simultaneous estimation of combination of empagliflozin, linagliptin and metformin hydrochloride used in the treatment of type 2 diabetes mellitus. Journal of Planar Chromatography – Modern TLC, 2020;33:109–118.
79. Eman IEK, Dalia AH, Sara SM, Magda AB. HPTLC determination of three gliptins in binary mixtures with metformin. J Chromatogr Sci. 2016;54(1):79-87.
80. Rajasekaran A, Kavitha R, Arivukkarasu R. Development and validation of HPTLC method for simultaneous estimation and stability indicating study of metformin HCl and linagliptin in pharmaceutical formulation. World J Pharm Sci. 2014;2(4): 317-327.
81. Sushant BJ, Sunil RP, Kalyanaraman LN, Popatrao NB. Development of RP-HPLC, Stability Indicating Method for Degradation Products of Linagliptin in Presence of Metformin HCl by Applying 2 Level Factorial Design; and Identification of Impurity-VII, VIII and IX and Synthesis of Impurity-VII. Sci. Pharm. 2017;85:25.
82. Nenavath P, Guttena Veerabhadram. A Novel Stability Indicating Rp-Uplc-Dad Method for Determination of Metformin and Empagliflozin in Bulk and Tablet Dosage form. Orient. J. Chem. 2017;33(4):1949-1958.
83. Srinivasa RM, Anusha K, Syed M. Stability indicating rp-hplc method for simultaneous estimation of empagliflozin and linagliptin in bulk and pharmaceutical dosage forms. J Syn Natu Chem. 2017;2(1):1-6.
84. Vinay KD, Seshagiri Rao JVLN. A New Validated Stability Indicating RP-HPLC Method for Simutaneous Estimation of Metformin Hydrochloride and Empagliflozin in Tablet Dosage Forms. International Research Journal of Pharmacy and Medical Sciences. 2018;1(5):16-22.
85. Ali SI, Kumar P, Bharath R. Stability Indicating Simultaneous Estimation of Metformin and Empagliflozin in Pharmaceutical Tablet Dosage form by RP-HPLC. Asian Journal of Research in Chemistry. 2017;10(6):783-788.
86. Shirisha V, Bolle K, Santosh I, Rao KNV, Rajeswar DK. A New Simple Method Development, Validation and Forced Degradation Studies of Empagliflozin by Using RP-HPLC. International Journal of Pharmacy and Biological Sciences. 2019;9 (1):25-35.
87. Maruthi R, Chandan RS, Anand KT. Development and Validation of Stability Indicating Rp-Uflc Method for the Estimation of Linagliptin in Active Pharmaceutical Ingredients. Research & Reviews: Journal of Pharmaceutical Analysis. 2018;7(1):12-23.

88. Rupasi P C, Bhagavan RM. Development and validation of stability indicating rp-hplc method for the simultaneous estimation of metformin hydrochloride and empagliflozin in bulk and in a synthetic mixture. Int J Pharm. 2016;6(4):138-147.
89. Kavitha KY, Geetha G, Hariprasad R, Kaviarasu M, Venkatnarayanan R. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of linagliptin and metformin in pure and pharmaceutical dosage form. Journal of Chemical and Pharmaceutical Research, 2013;5(1):230-235.
90. Maha FAG, Miriam FA. Mariam MT. Liquid chromatographic and spectrofluorimetric assays of empagliflozin: Applied to degradation kinetic study and content uniformity testing. Luminescence. 2018;1–14.
91. Naazneen S, Sridevi A. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of empagliflozine and linagliptin in tablet formulation. Der Pharmacia Lettre, 2016;8 (17):57-65.
92. Madan MRM, Gowri SD, Seshagiri RJVLN. Stress degradation studies and development of validated stability indicating assay method by rp-hplc for simultaneous estimation of metformin and empagliflozin in presence of degradation products as per ich guidelines. J Sci Res Pharm, 2017;6(2):20-33.
93. Varaprasad J, Nagendra KPV, Srinivasu P, Ramaprasad LA, Nagaraju D. A novel stability-indicating rp-uplc method for the quantification of impurities and a new qda mass detector coupled with lc-pda for identification of mass of degradation products in a fixed dose combination of empagliflozin and lanigliptin tablets used as second-line therapy for type-2 diabetes, Int. Res. J. Pharm. 2018:9(7):192-201.
94. Shaikh JSA, Mohamad A, Pathan MAAK. Validation of stability indicating high performance liquid chromatographic method for simultaneous determination of assay of Linagliptin and Metformin drugs in the pharmaceuticals tablet formulations using bupropion as a common internal standard. Journal of Innovations in Pharmaceutical and Biological Sciences. 2018;I:5(1):21-28.
95. Raquel BHF, Jonathaline AD, Fla DF, Luis FSO, Michel MM, Marcelo DM. Biological Safety Studies and Simultaneous Determination of Linagliptin and Synthetic Impurities by LC-PDA. Journal of Analytical Methods in Chemistry; 2019. Article ID 7534609.
96. Geetha SA, Rajitha G, Ramya YY, Uma P. Analytical method development and validation of new stability-indicating reverse-phase high-performance liquid chromatography method for simultaneous estimation of metformin hydrochloride and empagliflozin in tablet dosage form. Asian J Pharm Clin Res. 2019;12;1:241-244.
97. Padmaja N, Mulagiri SB, Veerabhadram G. Stability-indicating RP-HPLC analytical method development and validation for the Metformin and Empagliflozin in pharmaceutical dosage form. Journal de Afrikana. 2016;3(5):314-328.
98. Syed IA, Bharath RK P. Stability indicating simultaneous estimation of metformin and empagliflozin in pharmaceutical tablet dosage form by rp-hplc. International Journal of Life Sciences and Review. 2017;3(9):92-99.
99. Nagunath S, Siva SN, Naveen KR G. Stability Indicating Method Development and Validation for Simultaneous Estimation of Linagliptin and Empagliflozin in Tablets by HPLC. Saudi J. Med. Pharm. Sci. 2018;4(8):884-896.
100. Mowaka S, Ayoub BM. Comparative study between UHPLC-UV and UPLC-MS/MS methods for determination of alogliptin and metformin in their pharmaceutical combination. Pharmazie. 2017;72:67-72.
101. Bassam MA, Shereen M, Eman SE, Nermeen A, Mohamed ME, Shaker AM. Pharmacokinetic Evaluation of Empagliflozin in Healthy Egyptian Volunteers Using LC-MS/MS and Comparison with Other Ethnic Populations. Scientific Reports. 2017;7:2583.
102. Bassam MA, Shereen M. LC–MS/MS Determination of Empagliflozin and Metformin. Journal of Chromatographic Science. 2017;1–6.
103. Prakash N, Abhijeet SK. Structural characterization of forced degradation products of empagliflozin by high resolution mass spectrometry, Journal of Liquid Chromatography & Related Technologies. 2019;42:13-14, 417-428.
104. Sara SM, Eman IEK, Magda AB, Dalia AH. Pharmacokinetic interaction between linagliptin and tadalafil in healthy Egyptian

males using a novel LC–MS method. Bioanalysis; 2019.
(Epub ahead of print)

105. Abbas MB, Mahrouse MA, Fawzy MG, A validated HPLC-MS/MS method for simultaneous determination of linagliptin and metformin in spiked human plasma coupled with solid phase extraction: Application to a pharmacokinetic study in healthy volunteers, Journal of Pharmaceutical and Biomedical Analysis; 2018.
Available:https://doi.org/10.1016/j.jpba.2018.09.052
106. Dias BCL, Fachi MM, de Campos ML, Degaut FLD, Peccinini RG, Pontarolo R. A new HPLC–MS/MS method for the simultaneous quantification of SGLT2 inhibitors and metformin in plasma and its application to a pharmacokinetic study in healthy volunteers. Biomedical Chromatography. 2019;33:e4663.
107. Mahamad SSS, Arifa B, Saradhi NDVR. Bioanalytical method development and validation of linagliptin in plasma through LCMS/MS. Int. J. Bioassays. 2014;3(07): 3146-3151.
108. Tejas W, Ashish M, Supriya H, Nancy P. Development of simultaneous determination of empagliflozin and metformin in human plasma using liquid chromatography–mass spectrometry and application to pharmacokinetics. European Journal of Mass Spectrometry. 2019;1–14.
109. Nagaraj KN, Sunil SJ, Rajendraprasad M, Sunil KS. An ultra high performance liquid chromatography - tandem mass spectrometry method for quantification of linagliptin in human plasma. RSC Advances. 2016;6(71):66756-66766.
110. Priyanka AS, Pranav SS, Archana G. Mixed-mode solid phase extraction combined with LC-MS/MS for determination of empagliflozin and linagliptin in human plasma. Microc; 2018.
Available:https://doi.org/10.1016/j.microc.2018;11.0

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63221