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Research Article

Pharmacology and Analytical Chemistry Profile of Dapagliflozin, Empagliflozin and Saxagliptin

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Abstract

Diabetes mellitus is a worldwide disease that requires special and continuous medical care. Many classes of oral hypoglycemic drugs are currently used; however, the treatment strategy depends on the nature of diabetes type, pharmacological properties of the used drugs in addition to the clinical characteristics of the patient. As such, in this literature review, we will shed the light on the pharmacology and analytical chemistry profile of certain oral hypoglycemic drugs specifically Dapagliflozin, Empagliflozin and Saxagliptin that got attention in the last decade. Mode of action and most of up-to-date reported methods that have been developed for determination of these important anti-diabetic drugs in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples are mentioned in detail.

Keywords: diabetes; dapagliflozin; empagliflozin; saxagliptin; pharmacology; analytical chemistry.

Introduction

Diabetes mellitus (DM) is a lifelong disease that requires continuous medical care. Chronic long-term hyperglycemia associated with diabetes that causes serious complications lead to either drug monitoring in the line of treatment single or combined dosage form. Type 2 diabetes mellitus (T2DM) is a worldwide problem that affects about 8% of the adults all over the world, with predictions of more than 400 million cases by 2030 [1]. The prevalence of this type of DM implies an urgent need for finding treatments and preventative strategies. The disease results from progressive β-cell dysfunction in the presence of insulin resistance, and this leads to a progressive decrease in plasma glucose homeostasis, increased glucagon secretion, gluconeogenesis, and renal glucose reabsorption and reduced incretin response. Treatments recommended by the American diabetes association and the European association for the study of diabetes include drugs affecting all of the above processes [2].

Monotherapy using oral medications should be concomitant with management of intensive lifestyle. When glycemic control is no longer maintained with a single drug, the addition of a second or third oral hypoglycemic drugs usually more effective than switching to another single drug.

Hypoglycemic drugs comprise a pharmacologically and chemically heterogeneous drugs groups. There are different classes of oral hypoglycemic drugs and their selection depends on the nature of diabetes, pharmacological properties of the compounds such as efficacy, safety profile and the clinical characteristics of the patient (stage of disease, age and bodyweight) [3]. These drugs, which exhibit different modes of action may be used as a monotherapy or in various combinations.

Gliflozins

Gliflozins are the newest class of approved oral hypoglycemic agents that specifically inhibit sodium glucose co-transporter 2 function in the kidney, thus preventing renal glucose reabsorption and increasing glycosuria in diabetic patients while reducing hyperglycemia with hypoglycemia minimal risk. They reduce glycated hemoglobin and exert favorable effects beyond glucose control with consistent body weight, serum uric acid reductions and blood pressure. The main drugs from this group are dapagliflozin (DGF) and empagliflozin (EGF) [4-8].

Gliptins

Gliptins represent a new class of drugs that improve the health of beta cells and suppress glucagon, resulting in improved post-prandial and fasting hyperglycemia. They function by augmenting the incretin system inhibiting their metabolism by dipeptidyl peptidase 4. They are also safe and do not cause significant hypoglycemia, making it a unique class of drugs. The main drug from this group is Saxagliptin (SXG) [9].

Mechanism of sodium glucose co-transporter 2 Inhibitors

SGLT2 is a human protein that facilitates glucose reabsorption from the kidney. SGLT2 is a low-affinity, high-capacity glucose transporter which is located in the kidney proximal tubules. It is responsible for 90 % of glucose reabsorption. Inhibition of SGLT2 can lead to a decrease in blood glucose due to the increase in excretion of renal glucose. The mechanism of action of this new class of drugs also offers more glucose control by allowing an increase in insulin sensitivity and uptake of glucose in the muscles, decreased gluconeogenesis and improved insulin release from the beta cells. Drugs in the SGLT2 inhibitors class include DGF and EGF, these drugs in this class are FDA approved for the treatment of T2DM.
The usage of studied drugs as tinny amount and very diluted in biological matrix to analyze studied drugs in low levels to be applied in their assay in biological samples and give challenge to find suitable method for analysis of these drugs. Moreover, it is well-known that spectrofluorimetric methods are much more sensitive than spectrophotometric methods [10]. Furthermore, studied drugs analysis in the required low level in plasma samples by measuring the native fluorescence of each DGF and EGF and needed the use of a fluorogenic derivatizing reagent to enhance the sensitivity of the analysis by producing a highly sensitive fluorophore. Therefore, benzofurazan derivative was used in this study for the first time to develop a new validated and sensitive spectrofluorimetric analytical method for studied drug analysis in all sample matrices either pure or biological.

A way to speed up the validation process consists of the use of experimental design, which can be very useful and advantageous for both the evaluation and the optimization of some performance parameters. Experimental design techniques are powerful tools for the exploration of multivariate systems [11-13]. Statistical design is a way of choosing experiments; efficiently and systematically to give reliable and coherent information.

From a statistical standpoint, design means construction of experiments so that the analysis of results yields the maximum amount of information that can be extracted from the experiments. More specifically, experimental design helps the researcher to verify if changes in factor values produce a statistically significant variation of the observed response, and this approach can be used each time it is necessary to have this type of information. Typically, experimental design techniques are used to understand the effect of several variables on a system by a well-defined mathematical model. The strategy is most effective if statistical design is used in most or all stages of development and not only for screening or optimizing the process.

A systematic use of statistical design in developing a method ensures traceability, supports validation, and makes the subsequent confirmatory validation much easier and more certain. In fact, it is difficult to completely separate method optimization from validation since these two areas are linked, and sometimes a compromise has to be found [14].

There is no reported voltammetry study for DGF analysis in the literature. DGF acts as electroactive compound, and it is easily oxidized. The development of electrochemical-based sensors is considered important. Electrochemical sensors have the reputation of being small, quick, cheap, and easy to use for analytical applications, but their designing to be sensitive and selective for analyte of interest is a challenge. The rapid nature of electrochemistry makes it appealing for use in medical applications where quick tests are necessary for medical diagnostics, to ensure drug quality, and to understand dynamics of molecular changes during diseases. Therefore, polymer films modified electrodes received a great attention recently due to their wide applications in the fields of chemical sensors and biosensors [15-19]. Such modified electrodes can significantly improve the electrocatalytic properties of substrates, decrease the over potential, increase the reaction rate and improve the stability and reproducibility of the electrode response in the area of electro analysis [20-29].

The incorporation of metallic nanoparticles (NPs) into conductive polymers is of great interest because of their strong electronic interactions between NPs and the polymer matrices. It has been reported that the electrocatalytic properties and conductivities of NPs could be enhanced by the conductive polymeric matrices [19]. Previously, poly 1,5-diaminonaphthalene (PDAN) was prepared in aqueous and nonaqueous media at glassy carbon (GC) electrode [20, 22]. The electrodeposition of metal NPs in the polymer films improves their tolerance towards electrooxidation of small molecules [30]. Herein, in this perspective, PDAN films were prepared at the surface of GC electrode, followed by monometallic platinum (Pt) or palladium (Pd) NPs electrodeposition. Suitability of these new composite NPs modified polymeric GC electrodes towards the electrocatalytic oxidation of studied drugs have been studied by electrochemical measurements.

On the other hand, The combination therapy of DGF and SXG was shown to be superior in lowering blood glucose when compared with either of the monotherapy regimens [31]. However, this combination therapy leads to a big challenge in pharmaceutical and biomedical analysis area. Therefore, it is important to get a valid analytical separation technique suitable for the analysis of these drugs in presence of each other. Also, the analysis should be valid in presence of their degradation products and also in pharmaceutical dosage form. High performance thin-layer chromatography (HPTLC) has several advantages over HPLC in some analysis. As HPTLC, separations are generally more efficient than HPLC. Also, it takes short time for analysis. Moreover, it requires few nanoliter injection volumes. Furthermore, minimal use of solvent and no prior extraction steps compared to HPLC [32, 33].

Chemistry of the investigated oral hypoglycemic drugs

The chemical structures and pharmacokinetic parameters of the investigated drugs and their chemical names are presented in Tables 1 and 2.

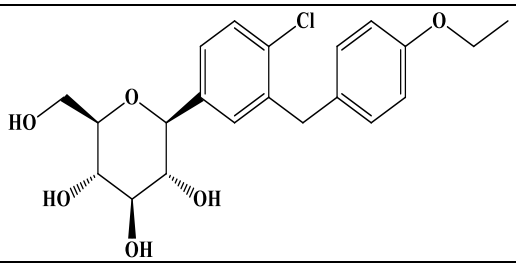
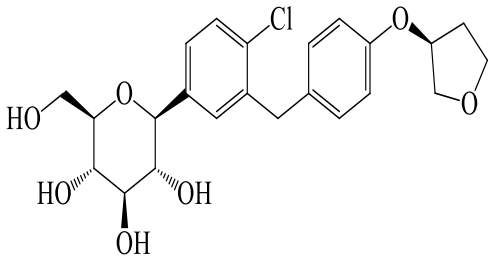
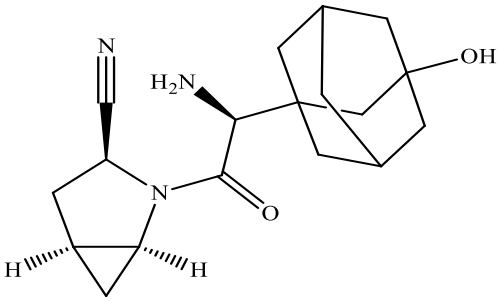
Name	Chemical structure	IUPAC name	Molecular weight
DGF		(2S,3R,4R,5S,6R)-2-(4-chloro-3-(4-ethoxybenzyl)phenyl)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol	408.9 g/mol
EGF		(2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol	450.9 g/mol
SXG		(1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile	315.4 g/mol

Table 1. *The names, chemical structures and nomenclature of the studied oral hypoglycemic drugs.*

Parameters	DGF	EGF	SXG
Bioavailability	78%	78%	67%
Protein binding	91%	86.2%	<10%
Solubility	Soluble in organic solvent (30mg/ml) and sparingly soluble in aqueous (0.111mg/ml)	Soluble in organic solvent (30mg/ml) and sparingly soluble in aqueous (0.111mg/ml)	Soluble in water, 791.8 mg/L
Absorption	Oral DGF reaches a maximum concentration within 1 hour of administration when patients have been fasting	Peak plasma concentrations are reached in approximately 1.5 hours (Tmax). At steady-state, plasma AUC and Cmax were 1870 nmol·h/L and 259 nmol/L, respectively	The corresponding plasma Cmax values were 24 ng/mL and 47 ng/mL for SXG and its active metabolite, respectively.
Route of Elimination	75.2% of DGF is recovered in the urine with 1.6% of the dose unchanged by metabolism. 21% of the dose is excreted in the feces with 15% of the dose unchanged by metabolism	After oral administration of radiolabeled EGF approximately 41.2% of the administered dose was found eliminated in feces and 54.4% eliminated in urine	Both renal and hepatic pathways. Following a single 50 mg dose of 14C-SXG, 24%
Volume of Distribution	118 L	73.8 L	151 L
Metabolism	Primarily glucuronidated to become the inactive 3-O-glucuronide metabolite (60.7%)	The most abundant metabolites are three glucuronide metabolites: 2-O-, 3-O-, and 6-O-glucuronide. EGF does not inhibit, inactivate, or induce CYP450 isoforms.	Cytochrome P450 3A4/5 (CYP3A4/5). 50% of the absorbed dose will undergo hepatic metabolism. The major metabolite 5-hydroxy SXG
Half-life (h)	13.8	12.4	SXG = 2.5 hours;

Table 2. *Pharmacokinetic and physicochemical parameters of the studied oral antidiabetic drugs.*

Analytical methods for the determination of certain antidiabetic drugs:

Pharmaceutical analysis has become one of the most important stages in the therapeutic process. Drug analysis includes analytical investigations

of bulk drug materials, intermediate products, drug formulations, impurities and degradation products. Analytical techniques play a significant role in understanding the chemical stability of the drug, in evaluating the toxicity of some impurities and in assessing the content of drug in formulations.

Also, they are fundamental tools in pharmacokinetic studies where the analysis of a drug and its metabolites in biological fluids must be performed. This review presents analytical procedures such as spectrophotometric (UV/VIS) methods**,** HPLC and HPTLC methods. It is based on a review of the literature from (2009-2020).

The studied drugs (DGF, EGF and SXG) have not an official method in any pharmacopeia. The reported method included;

Spectroscopic methods

Spectrophotometric methods

Ultraviolet and visible spectrophotometric methods:

In literature survey, either spectrophotometric methods have been reported for determination of the studied drugs in pure forms or in their pharmaceutical preparations. These reported methods are summarized in Table 3.

Drug	Solvent	Wavelength (nm)	Linearity range ($\mu g\ mL^{-1}$)	Ref
DGF	Methanol: Water	224 nm	5-40 µg/mL Correlation coefficient :< 1	[34]
DGF	Ethanol: Phosphate buffer (1:1)	233.65 nm	10-35 µg/mL Correlation coefficient :0.9998	[35]
DGF and Metformin HCL	Methanol	DGF-235 nm Metformin HCl-272 nm	DGF-0.5-2.5 µg/mL Metformin-25-125 µg/mL Correlation coefficient: DGF, 0.980 Metformin HCl, 0.982	[36]
DGF	Ethanol	237nm	0.5-0.9 µg/mL Correlation coefficient: 0.994	[37]
SXG and DGF	Methanol	SXG at an absorbance difference between 214.40 nm - 220.0 nm and DGF between 208.0 nm - 209.0 nm.	4-16 µg/mL and 10-22 µg/mL for SXG and DGF respectively.	[38]
Univariate spectrophotometric of DGF and SXG	Methanol	DGF and SXG were highly overlapped at their λmax 224 nm and 209 nm.	DGF and SXG over the range of 2.5–50.0 µg/mL and 2.5–60.0 µg/mL, respectively.	[39]
EGF + Metformin	Methanol	Method A: EGF, 272 and metformin, 234 nm. Method B: EGF, 254 nm metformin, 226 nm	5–25 µg/mL for EGF $r^2 = 0.999$ 2–12 µg/mL for metformin $r^2 = 0.999$	[40]
EGF	Distilled water	Method 1: 247nm Method 1: 438nm Method 1: 782nm	Method 1: 2-12 µg/ml Method 2: 5-30 µg/ml Method 3: 10-60 µg/ml	[41]
EGF and metformin hydrochloride	Methanol	EGF: 224nm metformin: 230nm	EGF, 1-3 µg/ml metformin, 2-10 µg/ml	[42]
Chemometric methods for simultaneous determination of EGF and metformin	Methanol	225 nm and237nm	2-12 $\mu g\ mL^{-1}$ for both drugs	[43]
EGF and Metformin with Linagliptin	Methanol	296 nm for Linagliptin	5- 25µg/ml	[44]

in its ternary mixture				
SXG	Methanol	208	5 – 40 µg/mL r^2 = 0.999 LOD: 0.0607 µg/mL LOQ: 0.1821 µg/mL	[45]
SXG	Methanol	416 nm (DDQ) and 838 nm (TCNQ)	50-300 and 10-110 µg/mL with DDQ and TCNQ, respectively.	[46]
SXG and metformin hydrochloride	Methanol	SXG (274 nm) and Metformin (231 nm) respectively.	50-90 µg/mL for SXG and 2-10 µg/mL for Metformin	[47]
SXG	Methanol	at 208 nm	5–40 µg/mL.	[48]

Table 3. *Spectrophotometric (UV/VIS) methods for the analysis of DGF, EGF and SXG in bulk materials and formulations.*

Spectrofluorimetric methods

The reported spectrofluorimetric methods for the investigated drugs as the following;

Spectrofluorimetric methods of SAX and vildagliptin in bulk and pharmaceutical preparations using NBD-Cl fluorogenic reagent at λex of 468 and 465 nm for SAX and VDG, respectively. Fluorescence intensity at λem of 542 and 540 nm for SAX and VDG, respectively [49]. A simple and highly sensitive and robust spectrofluorimetric method was developed for the determination of sitagliptin phosphate and SAX. The proposed method is based on Hantzsch reaction of both drugs. Fluorescent products in presence of sodium dodecyl sulfate micellar system as additive to enhance the obtained fluorescence at 483 nm after excitation at 419 nm for both drugs[50].

High Performance Thin-Layer Chromatography (HPTLC):

A high-performance thin-layer chromatographic method was developed for simultaneous determination of EGF and Linagliptin. The proposed method was applied successfully to the pharmaceutical analysis using precoated silica plates coated with 0.2 mm layers of silica gel 60 F_{254} and methanol: toluene: ethyl acetate (2:4:4, v/v/v) was selected as mobile phase [51]. Stability indicating HPTLC-MS method for estimation of EGF in pharmaceutical dosage form using silica plates coated with 0.2 mm layers of silica gel 60 F_{254} and toluene : methanol (7:3, v/ v) was selected as mobile phase [52].

HPTLC was developed for the quantitative analysis of SXG in active pharmaceutical ingredients (APIs) and pharmaceutical dosage forms. The method was achieved using silica gel aluminum plate 60 F_{254} (10 × 10 cm) as stationary phase and methanol: chloroform (6:4 v/v) as mobile phase [53]. HPTLC method for the simultaneous determination of metformin, SXG and DGF in pharmaceuticals. Separation was performed using aluminum HPTLC sheets coated with silica gel 60 F_{254} with a mobile phase consisting of a mixture of acetonitrile: 1% w/v ammonium acetate in methanol (9: 1, v/v), scanning was performed at 210 nm[54].

HPTLC analytical method for simultaneous estimation of DGF and SXG in synthetic mixture using silica gel aluminum plate 60F_{254}(10×10cm)as stationary phase and chloroform: ethyl acetate: methanol: ammonia (6:2:2:2 drops) as mobile phase[55]. HPTLC method was developed for the determination of either linagliptin, SXG or vildagliptin in their binary mixtures with metformin in pharmaceutical preparations. Separation was carried out on Merck HPTLC aluminum sheets of silica gel 60F_{254} using methanol: 0.5% w/v aqueous ammonium sulfate (8: 2, v/v) as mobile phase [56].

High Performance Liquid Chromatography (HPLC):

Various HPLC methods had been reported for the determination of the studied drugs either alone or in combination with others active ingredients in dosage forms or in biological fluids. Table 4: summarized the most recent applications of this technique.

Matrix	Column	Mobile phase	Linearity range	Detection	Ref
DGF	RP- C18	Phosphate buffer and acetonitrile in the ratio of 60:40 v/v	10-60 µg/mL	UV 237 nm	[57]
LC–MS/MS of DGF, in normal and ZDF rat plasma	SunFire™ C18 50 × 2.1 mm 5 µm column (Waters, MA, USA)	Mixture of water/acetonitrile (60/40 v/v).	5-2000 ng/mL	LC-TSQ negative ion electrospray ionization mode	[58]
DGF in API	BDS column (250×4.5mm, 5µ)	mixture of ortho phosphoric acid and acetonitrile (45:55 v/v)	25-150µg/mL	UV 245nm	[59]
DGF in raw and tablet formulation.	RP- C18	Methanol: Water (75:25 v/v).	5-25 µg/mL	UV 230 nm	[60]
DGF and Metformin in bulk and synthetic mixture	C18 (4.6mm I.D. × 250mm, 5µm)	Acetonitrile: Water (75:25% v/v)	MET: 20–100 µg/mL Dapa: 10–50 µg/mL	UV 285nm	[61]
DGF and SXG in combined tablet dosage form	RP-HPLC-Xterra C-18 analytical column (150 mm × 4.6 mm i.d., particle size 3.5 µ)	Phosphate buffer and acetonitrile (53 : 47 v/v)	2–14 µg mL^{-1} for all the drugs	UV 230 nm.	[62]

DGF and SXG in bulk and dosage forms	XTerra C 18 column (150mm x 4.6mm x5µm particle size).	Phosphate buffer (pH 4) and Acetonitrile (50:50 v/v)	20-60 ug/mL (SXG), 40-120 ug/mL (DGF).	UV 225 nm	[63]
RP-HPLC for simultaneous estimation of SXG and DGF in human plasma	Eclipse XDB C18 (150 × 4.6 mm × 5 µm)	0.1% ortho phosphoric acid and acetonitrile (50:50) with pH adjusted to 5.0	0.01 to 0.5 µg/mL and 0.05 to 2 µg/mL for SXG and DGF, respectively	UV 254 nm	[64]
DGF and EGF, Canagliflozin, and Metformin	C18 column (250×4.6 mm-5µm p.s) Inertsil® ODS	0.05 M potassium dihydrogen phosphate buffer PH 4 in a ratio [65:35, v/v]	Canagliflozin, DGF, EGF and Metformin was 7.5-225, 5-150, 6.5-187.5 and 10-1000 µg/mL, respectively.	UV 212 nm	[65]
DGF and SAX in API and tablet dosage form	Xterra RP18 (4.6×150 mm, 5 µm particle size)	Acetonitrile: Water (60:40)	100-500 µg/mL for DGF and 50-250 µg/mL for SXG	UV 248 nm	[66]
LC–MS/MS methods of DGF in rat plasma	C18 50 × 2.1 mm 5 µm column (Waters, MA, USA)	Mixture of water/acetonitrile (60/40 v/v)	5–2000 ng/mL	negative ion electrospray ionization mode	[58]
SXG and Metformin in combined-dosage form.	Thermo hypersil BDS C8 (250×4.6×5µ) column	ortho phosphoric acid: methanol in the ratio of (70: 30, v/v)	10-150 µg/mL	UV 241 nm	[67]
SXG and metformin in bulk.	An Agilent, Zorbax CN (250 × 4.6 mm I.D., 5 µm)	Mixture of methanol-50 mM phosphate buffer (pH 2.7)	5.00-125.00 µg mL^{-1} for SXG and 2.50-62.50 µg mL^{-1} for metformin	UV 225 nm	[68]
SXG and its major active 5-monohydroxy metabolite in human plasma	Atlantis® dC18 column (50 mm × 2.1 mm, 5 m)	Mobile phases A and B were 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively.	0.1–50 ng/mL for SXG and 0.2–100 ng/mL for 5-hydroxy SXG	positive ionization mode	[69]
UPLC-MS/MS) assay of SXG in rat plasma	C_{18} column (2.1 × 50 mm i.d., 1.7 µm)	methanol and 0.1% formic acid (40:60, v/v)	0.5–100 ng/mL	positive-ion mode with an electrospray ionization source	[70]
SXG and vildagliptin simultaneously in their binary mixtures with metformin HCl	Inertsil® CN-3 column (250 mm x 4.6 mm, 5 µm)	Potassium dihydrogen phosphate buffer pH (4.6) - acetonitrile (15:85, v: v)	Vildagliptin, SXG and metformin in the ranges of 5-200, 0.5-20 and 50-2000 µg/mL, respectively.	UV 208 nm	[71]
Metformin, SXG and its active metabolite, 5-hydroxy SXG in human plasma	ACE 5CN (150 × 4.6 mm, 5 µm)	acetonitrile and10.0 mm ammonium formate buffer, pH 5.0 (80:20, v/v)	1.50–1500, 0.10–100 and 0.20–200 ng/mL for metformin, SXG and 5-hydroxy SXG, respectively.	Triple quadrupole mass spectrometric using positive ionization mode	[72]
DGF and metformin hydrochloride in bulk and pharmaceutical dosage form.	hypersil BDS C18 (250 mm)	buffer (0.1% orthophosphoric acid) adjusted to pH 6.8 with triethyl amine: acetonitrile in the ratio of 50:50%/v/v	85-510 ng/mL	Photodiode array (PDA) detector at 240 nm.	[73]
SXG drug in its pure and formulated forms.	an Agilent, TC C18 (250 × 4.6 mm) 5µm column	Acetonitrile: Water (pH3), (20:80 v/v)	10-90 µgmL^{-1}	UV 211 nm	[74]
DGF and SXG in bulk and pharmaceutical dosage form	Inertsil-ODS, C_{18} column (250 × 4.6 mm; 5 µm)	a mixture of methanol and potassium dihydrogen phosphate buffer in the ratio of 45:55 v/v	20-70 µg/mL	PDA detector at 210 nm	[75]
SXG and metformin in APIs and tablet dosage forms	Enable C18 G (250 × 4.6 mm; 5 µm particle size) column	0.05 M KH_2PO_4 buffer (pH 4.5): Methanol: Acetonitrile (60:20:20 %v/v)	0.2 - 1.2 µg/mL for SXG and 40 - 240 µg/mL for metformin	UV 220 nm	[76]
HPLC for simultaneous determination of linagliptin–EGF combination	XTERRA® C18 column (250 mm × 4.6 mm, 5 µm)	0.1% aqueous formic acid–methanol– acetonitrile (40:20:40, v/v/v), pH 3.6	2–50 µg mL^{-1} , 4–100 µg mL^{-1} for linagliptin and EGF, respectively	UV 226 nm	[77]

HPLC of EGF	Intersil® C_{18} column (150 mm × 4 mm, 5 µm)	acetonitrile–potassium dihydrogen phosphate buffer pH 4, (50:50, v/v)	5–50 µg/mL	UV 225 nm	[78]
UPLC method of EGF, linagliptin and metformin hydrochloride in the different combinations of their pharmaceutical dosage forms	Symmetry® Acclaim™ RSLC 120 C18 column (100 mm × 2.1 mm, 2.2 mm)	Potassium dihydrogen phosphate buffer pH 4– methanol (50: 50, v/v)	1–32 mg mL^{-1}, 0.5–16 mg mL^{-1} and 1–100 mg mL^{-1} for EGF, linagliptin and metformin hydrochloride, respectively	UV 225 nm	[79]
LC-MS/MS method of EGF in human plasma	ACQUITY UPLC BEH Shield RP C18 column with dimensions (150 mm × 2.1 mm, 1.7 µm)	A mixture of deionized water and acetonitrile in the ratio of (10:90, v/v)	(25–600 ng mL^{-1})	Triple quadrupole detector accompanied with ESI source	[80]
EGF and metformin by RP-HPLC method	Symmetry C18 column (4.6×150mm) 5µ	(70:30 v/v) methanol: phosphate buffer (KH_2PO_4 and K_2HPO_4) phosphate pH 3	5 - 25 µg/mL For EGF 500-5000 ppm For metformin	PDA detector 240 nm	[81]
RP-UPLC-DAD of Metformin and EGF in bulk and tablet dosage form	C18 BEH (Ethylene Bridged Hybrid) UPLC (100mm x 2.1mm ,1.8µm)	0.1% ortho phosphoric acid buffer (pH was adjusted to 3.4 with 0.1 N NaOH) and methanol in the ratio 40:60% v/v	metformin (25-125 µg/ml) and EGF (15-75 µg/mL)	PDA detector 254 nm	[82]
HPLC of process related impurities in EGF drug substances	Inertsil C8 (250mm×4.6 mm, 5µm) column	0.1% orthophosphoric acid and acetonitrile	0.3 - 1.5 µg/mL	UV 230nm	[83]
UPLC of EGF and three related substances in spiked human plasma	Acquity "UPLC® BEH" C18 column (50 mm × 2.1 mm i.d, 1.7 µm particle size)	Aqueous trifluoroacetic acid (0.1%, pH 2.5): acetonitrile (60:40, v/v)	50–700 ng/mL and 40–200 ng/mL for EGF and the three related substances, respectively	DAD detector 210 nm	[84]
HPLC of canagliflozin, DGF or EGF and metformin in presence of metformin major degradation product;1-cyanoguanidine	Prontosil (Lichrosorb 100-5-NH_2)	NaH_2PO_4 buffer (10 mM, pH 2.8): acetonitrile (18.5:81.5, v/v)	12.5–100, 3.75–30, 0.3075–2.46, and 0.3125–2.5 µg/mL for metformin HCl, canagliflozin, DGF and EGF, respectively	UV 225 nm	[85]

Table 4. *HPLC methods for the analysis of DGF, EGF and SXG in bulk materials and formulations.*

Capillary electrophoresis methods

A capillary electrophoretic method coupled to a diode array detector (CE-DAD) was developed and validated for the simultaneous determination of metformin hydrochloride, SAX and DGF. The proposed method was used for the determination of these drugs in combinations namely, SXG/metformin, DGF/metformin and SXG/DGF. CE separation was performed on a fused silica capillary with background electrolyte consisting of 30mM phosphate buffer (pH 6.0). The compounds were detected at 203nm for SXG/DGF and 250 nm for metformin. The method was linear in the concentration ranges of 10-200, 1.25-50 and 7.5-100 µg/mL for SAX, DGF and metformin, respectively [86].

Electrochemical method:

The literature is devoid of any electrochemical methods for the quantitation of the studied drugs. The first, sensitive and accurate potentiometric sensor for the selective determination of SXG in the presence of either its active metabolite 5-hydroxy SXG, other co-administered or structurally related drugs [87].

Conclusion:

This literature review represents the mode of action in addition to an up to date survey about all reported methods that have been developed for determination of Dapagliflozin, Empagliflozin and Saxagliptin in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples such as liquid chromatography, spectrophotometry, spectroflourimetry, electrochemistry, etc.

References:

1. Zheng Y, S.H. Ley, and F.B. Hu , (2018).Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology*, 14(2): 88.
2. Davies M.J., D.A. D'Alessio, J. Fradkin, W.N. Kernan, C. Mathieu, G. Mingrone, P. Rossing, A. Tsapas, D.J. Wexler, and J.B. Buse (2018). Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia, , 61(12): 2461-2498.
3. Inzucchi S.E., R.M. Bergenstal, J.B. Buse, M. Diamant, E. Ferrannini, M. Nauck, A.L. Peters, A. Tsapas, R. Wender, and D.R. Matthews(2015). Management of hyperglycaemia in type 2 diabetes, 2015: a patient-centred approach. Update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia, , 58(3): 429-442.
4. Bonner C., J. Kerr-Conte, V. Gmyr, G. Queniat, E. Moerman, J. Thévenet, C. Beaucamps, N. Delalleau, I. Popescu, and W.J.

Malaisse(2015). Inhibition of the glucose transporter SGLT2 with dapagliflozin in pancreatic alpha cells triggers glucagon secretion. Nature medicine, 21(5): 512.
5. Freeman J.S (2013).Review of insulin-dependent and insulin-independent agents for treating patients with type 2 diabetes mellitus and potential role for sodium-glucose co-transporter 2 inhibitors. Postgraduate medicine, , 125(3): 214-226.
6. Grempler R., L. Thomas, M. Eckhardt, F. Himmelsbach, A. Sauer, D. Sharp, R. Bakker, M. Mark, T. Klein, and P. Eickelmann, Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors. Diabetes, Obesity and Metabolism, , 14(1): 83-90.
7. Neumiller J.J(2014). Empagliflozin: a new sodium-glucose co-transporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes. Drugs in context, 3.
8. Washburn W.N. and S.M. Poucher(2013), Differentiating sodium-glucose co-transporter-2 inhibitors in development for the treatment of type 2 diabetes mellitus. Expert opinion on investigational drugs, , 22(4): 463-486.
9. Scirica B.M, E. Braunwald, I. Raz, M.A. Cavender, D.A. Morrow, P. Jarolim, J.A. Udell, O. Mosenzon, K. Im, and A.A. Umez-Eronini, Heart failure, saxagliptin, and diabetes mellitus: observations from the SAVOR-TIMI 53 randomized trial. Circulation , 130(18): 1579-1588.
10. Derayea S.M., A.A. Hamad, D.M. Nagy, D.A. Nour-Eldeen, H.R.H. Ali, and R. Ali(2018). Improved spectrofluorimetric determination of mebendazole, a benzimidazole anthelmintic drug, through complex formation with lanthanum (III); Application to pharmaceutical preparations and human plasma. Journal of Molecular Liquids, 272: 337-343.
11. Carlson R. and J.E. Carlson(2005). Design and optimization in organic synthesis. Vol. 24.Elsevier.
12. Lewis G., D. Mathieu, and R. Phan-Tan-Luu(1999). Mixtures in a constrained region of interest. Pharmaceutical Experimental Design. New York: Marcel Dekker, 413-54.
13. Montgomery D.C(1997). Design and analysis of experiments John Wiley & Sons. Inc., New York, 2001: 200.1.
14. Furlanetto S., S. Orlandini, P. Mura, M. Sergent, and S. Pinzauti(2003). How experimental design can improve the validation process. Studies in pharmaceutical analysis. Analytical and bioanalytical chemistry, 377(5): 937-944.
15. Ensafi A.A., B. Rezaei, M. Amini, and E. Heydari-Bafrooei(2012). A novel sensitive DNA–biosensor for detection of a carcinogen, Sudan II, using electrochemically treated pencil graphite electrode by voltammetric methods. Talanta, 88: 244-251.
16. Gong W, Z.-Y. Dou, P. Liu, X.-Y. Cai, and X.-q. He(2012). Simultaneous determination of dopamine, ascorbic acid by polyethylene oxide (PEO) covalently modified glassy carbon electrode. Journal of Electroanalytical Chemistry, 666: 62-66.
17. Hadi M. and A. Rouhollahi(2012). Simultaneous electrochemical sensing of ascorbic acid, dopamine and uric acid at anodized nanocrystalline graphite-like pyrolytic carbon film electrode. Analytica chimica acta, , 721: 55-60.
18. Yang L., S. Liu, Q. Zhang, and F. Li(2012). Simultaneous electrochemical determination of dopamine and ascorbic acid using AuNPs@ polyaniline core–shell nanocomposites modified electrode. Talanta, , 89: 136-141.
19. Yuan Y., H. Li, S. Han, L. Hu, S. Parveen, H. Cai, and G. Xu(2012). Immobilization of tris (1, 10-phenanthroline) ruthenium with graphene oxide for electrochemiluminescent analysis. Analytica chimica acta, 720: 38-42.
20. Abdel-Azzem M., U. Yousef, and G. Pierre(1998). A cyclic voltammetric and coulometric study of a modified electrode prepared by electrooxidative polymerization of1, 5-diaminonaphthalene in aqueous acidic medium. European polymer journal, 34(5-6): 819-826.
21. Adzic R., M. Hsiao, and E. Yeager(1989). Electrochemical oxidation of glucose on single crystal gold surfaces. *Journal of electroanalytical chemistry and interfacial electrochemistry*, 260(2): 475-485.
22. Azzem M.A., U. Yousef, D. Limosin, and G. Pierre(1994), Electropolymerization of 1, 5-diaminonaphthalene in acetonitrile and in aqueous solution. Synthetic metals, 63(1): 79-81.
23. Bai Y., W. Yang, Y. Sun, and C. Sun, Sensors Actuators. B, 2008, 134: 471.
24. Barrera C., I. Zhukov, E. Villagra, and F. Bedioui, MA P. aez, J(2006). Costamaga, JH Zagal. Journal of Electroanalytical Chemistry, 589: 212.
25. Jafarian M., F. Forouzandeh, I. Danaee, F. Gobal, and M(2009). Mahjani, Electrocatalytic oxidation of glucose on Ni and NiCu alloy modified glassy carbon electrode. Journal of Solid State Electrochemistry, 2009, 13(8): 1171-1179.
26. Kalimuthu P. and S.A. John(2010), Simultaneous determination of ascorbic acid, dopamine, uric acid and xanthine using a nanostructured polymer film modified electrode. Talanta, 80(5): 1686-1691.
27. Tominaga M., T. Shimazoe, M. Nagashima, H. Kusuda, A. Kubo, Y. Kuwahara, and I. Taniguchi(2006). Electrocatalytic oxidation of glucose at gold–silver alloy, silver and gold nanoparticles in an alkaline solution. Journal of Electroanalytical Chemistry, 590(1): 37-46.
28. Wang C., C. Yang, Y. Song, W. Gao, and X. Xia(2005), Adsorption and direct electron transfer from hemoglobin into a three-dimensionally ordered macroporous gold film. Advanced Functional Materials, 15(8): 1267-1275.
29. Wang H.-S., T.-H. Li, W.-L. Jia, and H.-Y. Xu(2006). Highly selective and sensitive determination of dopamine using a Nafion/carbon nanotubes coated poly (3-methylthiophene) modified electrode. Biosensors and Bioelectronics, 22(5): 664-669.
30. Pournaghi-Azar M. and B. Habibi(2007). Electrocatalytic oxidation of methanol on poly (phenylenediamines) film palladized aluminum electrodes, modified by Pt micro-particles: comparison of permselectivity of the films for methanol. *Journal of Electroanalytical Chemistry*, 601(1-2): 53-62.
31. Singh-Franco D(2015). Potential for dipeptidyl peptidase-4 inhibitor and sodium glucose cotransporter 2 inhibitor single-pill combinations. Expert review of endocrinology & metabolism, 10(3): 305-317.
32. Mohamed A.-M.I., M.A. Omar, S.M. Derayea, M.A. Hammad, and A.A. Mohamed(2018) Innovative thin-layer chromatographic method combined with fluorescence detection for specific determination of Febuxostat: Application in biological fluids. Talanta, 176: 318-328.
33. Saraya R.E., M. Elhenawee, and H. Saleh(2018).Development of a highly sensitive high-performance thin-layer chromatography method for the screening and simultaneous determination of sofosbuvir, daclatasvir, and ledipasvir in their pure forms and their different pharmaceutical formulations. *Journal of separation science*, 41(18): 3553-3560.
34. Mante G.V., K.R. Gupta, and A.T. Hemke(2017). Estimation of Dapagliflozin from its tablet formulation by UV-Spectrophotometry. *Pharm Methods*, 8(2): 102-107.
35. Karuna P., E. China, and M. Basaveswara Rao(2015). Unique UV spectrophotometric method for reckoning of Dapagliflozin

in bulk and pharmaceutical dosage forms. *J Chem Pharm Res*, 7(9): 45-9.

36. Jani B., K. Shah, and P. Kapupara(2015). Development and Validation of UV Spectroscopic First Derivative Method for Simultaneous Estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture. J Bioequiv, 1(1): 102.
37. Manasa S., K. Dhanalakshmi, R. Nagarjuna, and S. Sreenivasa(2014) Method development and validation of dapagliflozin in API by RP-HPLC and UV-spectroscopy. *International Journal of Pharmaceutical Science and Drug Research*, 6: 250-252.
38. Suthar A.M, L.M. Prajapati, A.K. Joshi, J.R. Patel, M.L. Kharodiya, and L. Prajapati(2018). Estimation of Saxagliptin hydrochloride and Dapagliflozin propendiol monohydrate in combined dosage form. JIAPS, 3(2): 01-07.
39. Lotfy H.M, D. Mohamed, and M.S. Elshahe(2019). Novel univariate spectrophotometric determination of the recently released solid dosage form comprising dapagliflozin and saxagliptin via factorized response spectra: Assessment of the average content and dosage unit uniformity of tablets. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.
40. Padmaja N., M.S. Babu, and G(2016). Veerabhadram, 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 , 8(13): 207-213.
41. Jyothirmai N., B. Nagaraju, and M. Anil Kumar(2016). Novel uv and visible spectrophotometric methods for the analysis of empagliflozin a type 2 diabetic drug in bulk and pharmaceutical formulations. Journal de afrikana, 3(1): 177-187.
42. Patil S.D., S.K. Chaure, and S. Kshirsagar(2017). Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs. *Asian Journal of Pharmaceutical Analysis*, 7(2): 117-123.
43. Ayoub B.M(2016,). Development and validation of simple spectrophotometric and chemometric methods for simultaneous determination of empagliflozin and metformin: Applied to recently approved pharmaceutical formulation. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 168: 118-122.
44. Shaker L(2016). Development of Economic UV Spectrophotometric method for Determination of Linagliptin in its Tertiary Mixture with Empagliflozin and Metformin: Comparision to Economic pharmaceutical Analysis Literature. Scholars Research Library, 8(13): 267-269.
45. Kalaichelvi R. and E. Jayachandran(2011). Validated spectroscopic method for estimation of saxagliptin in pure and from tablet formulation. Int J Pharm Pharm Sci, 3(3): 179-180.
46. El-Bagary R.I., E.F. Elkady, and B.M. Ayoub(2012).Spectrophotometric methods based on charge transfer complexation reactions for the determination of saxagliptin in bulk and pharmaceutical preparation. International journal of biomedical science: IJBS, , 8(3): 204.
47. Narendra N. and J. Govinda(2012). Development and validation of uv-vis spectroscopy method for simultaneous estimation of saxagliptin hydrochloride and metformin hydrochloride in active pharmaceutical ingredient. J Pharm Educ Res, 3(2): 19-23.
48. Kalaichelvi R. and E. Jayachandran(2011). Validated Spectroscopic method for the estimation of Saxagliptinin pure and from tablet formulation. Int J Pharm Pharm Sci,3(3): 179-180.
49. Moneeb M.S(2013). Spectrophotometric and spectrofluorimetric methods for the determination of saxagliptin and vildagliptin in bulk and pharmaceutical preparations. Bulletin of Faculty of Pharmacy, Cairo University, 51(2): 139-150.
50. Barseem A., H. Ahmed, Y. El-Shabrawy, and F. Belal(2019). The use of SDS micelles as additive to increase fluorescence analysis of sitagliptin and saxagliptin derivatives in their tablets and human plasma. *Microchemical Journal*, 146: 20-24.
51. Bhole R., S. Wankhede, and M. pandey(2017). Stability indicating HPTLC method for simultaneous estimation of empagliflozin and linagliptin in pharmaceutical formulation. Analytical Chemistry Letters, 7(1): 76-85.
52. Bhole R.P. and F.R. Tamboli(2018). Development and Validation of Stability Indicating HPTLC-MS Method for Estimation of Empagliflozin in Pharmaceutical Dosage Form. Analytical Chemistry Letters, 8(2): 244-256.
53. Srividya S., E. Swetha, and C. Veeresham(2015) Development and validation of a high performance thin layer chromatographic method for quantitative analysis of saxagliptin. *American journal of analytical chemistry*, 6(10): 797.
54. Afnan E. Abdelrahman H.M.M., Nourah Z(2019). Alzoman, HPTLC Method for the Determination of Metformin Hydrochloride, Saxagliptin Hydrochloride, and Dapagliflozin in Pharmaceuticals. Current Analytical Chemistry, 15(1).
55. Shveta H. Parmar* D.S.V.L, Dr. Sachin B. Narkhede(2018). Development and validation of UV-spectroscopic first derivative and high performance thin layer chromatography analytical methods for simultaneous estimation of dapagliflozin propanediol monohydrate and saxagliptin hydrochloride in synthetic mixture,. ejbps,5(5): 668-684.
56. El-Kimary E.I., D.A. Hamdy, S.S. Mourad, and M.A. Barary(2015). HPTLC determination of three gliptins in binary mixtures with metformin. *Journal of chromatographic science*, 54(1): 79-87.
57. Debata J., S. Kumar, S.K. Jha, and A. Khan(2017). A New RP-HPLC method development and validation of dapagliflozin in bulk and tablet dosage form. *Int J Drug Dev & Res*, , 9(2): 48-51.
58. Aubry A.-F., H. Gu, R. Magnier, L. Morgan, X. Xu, M. Tirmenstein, B. Wang, Y. Deng, J. Cai, and P. Couerbe(2010). Validated LC–MS/MS methods for the determination of dapagliflozin, a sodium-glucose co-transporter 2 inhibitor in normal and ZDF rat plasma. Bioanalysis, 2(12): 2001-2009.
59. Sanagapati M., K. Dhanalakshmi, G. Nagarjunareddy, and S. Sreenivasa(2014). Development and validation of a RP-HPLC method for the estimation of dapagliflozin in API. International Journal of Pharmaceutical Sciences and Research, 5(12): 5394.
60. Manoharan G., A.M. Ismaiel, and Z.M. Ahmed (2018).Stabilityindicating RP-HPLC method development for simultaneous determination & estimation of dapagliflozin in raw & tablet formulation. Chemistry Research Journal, 3(2): 159-164.
61. Urooj A., P.S. Sundar, R. Vasanthi, M.A. Raja, K.R. Dutt, K. Rao, And H. Ramana(2017). Development And Validation Of Rp-Hplc Method For Simultaneous Estimation Of Dapagliflozin And Metformin In Bulk And In Synthetic Mixture. *World journal of pharmacy and pharmaceutical sciences*, 6(7): 2139-2150.
62. Singh N., P. Bansal, M. Maithani, and Y. Chauhan(2018), Development and validation of a stability-indicating RP-HPLC method for simultaneous determination of dapagliflozin and saxagliptin in fixed-dose combination. *New Journal of Chemistry*, 2018, 42(4): 2459-2466.

63. Kommineni V., K. Chowdary, and S. Prasad(2017), Development Of A New Stability Indicating Rp-Hplc Method For Simultaneous Estimation Of Saxagliptine And Dapagliflozin And Its Validation As Per Ich Guidelines. *Indo American Journal Of Pharmaceutical Sciences*, 4(9): 2920-2932.
64. Donepudi S. and S. Achanta(2019), Simultaneous Estimation of Saxagliptin and Dapagliflozin in Human Plasma by Validated High Performance Liquid Chromatography-Ultraviolet Method. Turkish Journal of Pharmaceutical Sciences,16(2): 227.
65. Khalil G.A., I. Salama, M.S. Gomaa, and M.A. Helal(2018), Validated Rp-Hplc Method For Simultaneous Determination Of Canagliflozin, Dapagliflozin, Empagliflozin And Metformin. *International Journal of Pharmaceutical, Chemical & Biological Sciences*, , 8(1).
66. Deepan T. and M.D. Dhanaraju(2018), Stability indicating HPLC method for the simultaneous determination of dapagliflozin and saxagliptin in bulk and tablet dosage form. *Current Issues in Pharmacy and Medical Sciences*, 31(1): 39-43.
67. Bhagavanji N(2012). Development and validation of stability indicating LC method for the simultaneous estimation of metformin and saxagliptin in combined dosage form. VSRD International *Journal of Technical & Non-Technical Research*, 3(11): 1-19.
68. Caglar S. and A. Alp(2014), A validated high performance liquid chromatography method for the determination of saxagliptin and metformin in bulk, a stability indicating study. *J Anal Bioanal Tech* S, 12: 2.
69. Demers R., H. Gu, L.J. Christopher, H. Su, L. Cojocaru, D.W. Boulton, M. Kirby, B. Stouffer, W.G. Humphreys, and M.E. Arnold(2012), Liquid chromatography and tandem mass spectrometry method for the quantitative determination of saxagliptin and its major pharmacologically active 5-monohydroxy metabolite in human plasma: method validation and overcoming specific and non-specific binding at low concentrations. *Journal of Chromatography B*, 889: 77-86.
70. Gao J.w., Y.m. Yuan, Y.s. Lu, and M.c. Yao(2012).Development of a rapid UPLC-MS/MS method for quantification of saxagliptin in rat plasma and application to pharmacokinetic study. Biomedical Chromatography, 26(12): 1482-1487.
71. Mohammad M.A.-A., E.F. Elkady, and M.A. Fouad(2012), Development and validation of a reversed-phase column liquid chromatographic method for simultaneous determination of two novel gliptins in their binary mixtures with Metformin. European Journal of Chemistry, 3(2): 152-155.
72. Shah P.A., J.V. Shah, M. Sanyal, and P.S. Shrivastav(2017). LC–MS/MS analysis of metformin, saxagliptin and 5-hydroxy saxagliptin in human plasma and its pharmacokinetic study with a fixed-dose formulation in healthy Indian subjects. *Biomedical Chromatography*, 31(3): e3809.
73. Yunoos M. and D.G. Sankar(2015). A validated stability indicating high-performance liquid chromatographic method for simultaneous determination of metformin Hcl and dapagliflozin in bulk drug and tablet dosage form. *Asian J Pharm Clin Res*, 8(3): 320-326.
74. Daswadkar S.C., M.A. Roy, S.G. Walode, and C. Mahendra Kumar(2016). Quality by design approach for the development and validation of saxagliptin by RP-HPLC with application to formulated forms. *Int J Pharm Sci*, 7(4): 1670-1677.
75. Aswini R.E., MM Babu, P Srinivasa(2018), A Review on Analytical Methods for Estimation of Dapagliflozin and Saxagliptin in Bulk and in Pharmaceutcal Dosage Forms. IJRPC, 8(3): 460-468
76. Prasad P., K. Satyanaryana, and G. Krishnamohan(2015).Development and Validation of a Method for Simultaneous Determination of Metformin and Saxagliptin in a Formulation by RP-HPLC. American Journal of Analytical Chemistry, 6(11): 841.
77. Abdel-Ghany M.F., O. Abdel-Aziz, M.F. Ayad, and M.M. Tadros(2017). New LC–UV methods for pharmaceutical analysis of novel anti-diabetic combinations. Acta Chromatographica,29(4): 448-452.
78. Abdel-Ghany M.F, M.F. Ayad, and M.M. Tadros(2018). Liquid chromatographic and spectrofluorimetric assays of empagliflozin: Applied to degradation kinetic study and content uniformity testing. Luminescence.
79. Ayoub B.M(2015). UPLC simultaneous determination of empagliflozin, linagliptin and metformin. RSC advances, 5(116): 95703-95709.
80. Ayoub B.M., S. Mowaka, E.S. Elzanfaly, N. Ashoush, M.M. Elmazar, and S.A. Mousa(2017), Pharmacokinetic Evaluation of Empagliflozin in Healthy Egyptian Volunteers Using LC-MS/MS and Comparison with Other Ethnic Populations. Scientific reports,7(1): 2583.
81. Godasu S. and S. Sreenivas(2017). 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): 2223-2232.*
82. Padmaja N. and G. Veerabhadram(2017). A Novel Stability Indicating Rp-Uplc-Dad Method for Determination of Metformin and Empagliflozin in Bulk and Tablet Dosage form. *Oriental Journal of Chemistry*, 33(4): 1949-1958.
83. Jaiswal S.H., M. Katariya, V. Katariya, G. Karva, and K. Koshe(2017). Validated Stability Indicating Hplc Method For Determination Of Process Related Impurities In Empagliflozin Drug Substances. World journal of pharmaceutical research, 6: 8741.
84. Mabrouk M.M, S.M. Soliman, H.M. El-Agizy, and F.R. Mansour(2019). A UPLC/DAD method for simultaneous determination of empagliflozin and three related substances in spiked human plasma. BMC Chemistry, 13(1): 83.
85. Hassib S.T, E.A. Taha, E.F. Elkady, and G.H. Barakat(2019). Validated Liquid Chromatographic Method for the Determination of (Canagliflozin, Dapagliflozin or Empagliflozin) and Metformin in the Presence of (1-Cyanoguanidine). *Journal of chromatographic science*.
86. Maher H.M., A.E. Abdelrahman, N.Z. Alzoman, and H.I. Aljohar(2019). Stability-indicating capillary electrophoresis method for the simultaneous determination of metformin hydrochloride, saxagliptin hydrochloride, and dapagliflozin in pharmaceutical tablets. *Journal of Liquid Chromatography & Related Technologies*, 42(5-6): 161-171.
87. Abdallah N.A. and H.F. Ibrahim(2019). Electrochemical determination of Saxagliptin hydrochloride with MWCNTs/CuO/4′ aminobenzo-18-crown-6-ether composite modified carbon paste electrode. Microchemical Journal, , 147: 487-496.