

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 is a lifelong condition requiring 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 affecting approximately 8% of the adult population, with predictions of more than 400 million cases by 2030 [1]. The prevalence of T2DM implies an urgent need for new treatments and preventative strategies. The disease results from progressive β -cell dysfunction in the presence of chronic insulin resistance, leading to a progressive decline 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 with an oral medication should be started concomitantly with intensive lifestyle management. When glycemic control is no longer maintained with a single drug, the addition of a second or third oral hypoglycemic drugs usually more effective than switching to another single drug.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 chemically and pharmacologically heterogeneous group of drugs. 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 individuals while reducing hyperglycemia with a minimal risk of hypoglycemia. They reduce glycated hemoglobin and exert favorable effects beyond glucose control with consistent body weight, blood pressure, and serum uric acid reductions. The main drugs from this group are dapagliflozin (DGF) and empagliflozin (EGF) [4-8].

Gliptins

Gliptins represent a novel class of agents that improve beta cell health and suppress glucagon, resulting in improved post-prandial and fasting hyperglycemia. They function by augmenting the incretin system (GLP-1 and GIP) preventing their metabolism by dipeptidyl peptidase-4 (DPP-4). Not only are they efficacious but also safe (weight neutral) 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 protein in humans that facilitates glucose reabsorption in the kidney. SGLT2 inhibitors block the reabsorption of glucose in the kidney, increase glucose excretion, and lower blood glucose levels. SGLT2 is a low-affinity, high capacity glucose transporter located in the proximal tubule in the kidneys. It is responsible for 90 % of glucose reabsorption. Inhibition of SGLT2 leads to decrease in blood glucose due to the increase in renal glucose excretion. The mechanism of action of this new class of drugs also offers further glucose control by allowing increased insulin sensitivity and uptake of glucose in the muscle cells, decreased gluconeogenesis and improved first phase insulin release from the beta cells. Drugs in the SGLT2 inhibitors class include DGF and EGF, these drugs in this class approved by the FDA for the treatment of type 2 diabetes

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. Therefore, a new simple and sensitive spectroscopic method was required to achieve the aim of this study. 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.

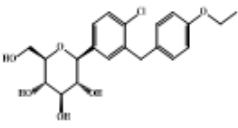
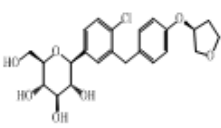
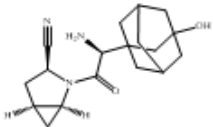
Name	Chemical structure	IUPAC name	Molecular weight
DGF		(2S,3R,4R,5S,6R)-2-(4-chloro-3-(4-ethoxybenzyl)phenyl)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol	408.9 g/mol
EGF		(2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol	450.9 g/mol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

Table 1: The names, chemical structures and nomenclature of the studied oral hypoglycemic drug

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	The most abundant metabolites are three glucuronide metabolites:	Cytochrome P450 3A4/5 (CYP3A4/5). 50% of the absorbed dose will undergo hepatic
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 2

Drug	Solvent	Wavelength (nm)	Linearity range ($\mu\text{g mL}^{-1}$)	Ref
DGF	Methanol: Water	224 nm	5-40 $\mu\text{g/mL}$ Correlation coefficient < 1	[34]
DGF	Ethanol: Phosphate buffer (1:1)	233.65 nm	10-35 $\mu\text{g/mL}$ Correlation coefficient :0.9998	[35]
DGF and Metformin HCL	Methanol	DGF-235 nm Metformin HCl-272 nm	DGF-0.5-2.5 $\mu\text{g/mL}$ Metformin-25-125 $\mu\text{g/mL}$ Correlation coefficient: DGF, 0.980 Metformin HCl, 0.982	[36]
DGF	Ethanol	237nm	0.5-0.9 $\mu\text{g/mL}$ 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 $\mu\text{g/mL}$ and 10-22 $\mu\text{g/mL}$ for SXG and DGF respectively.	[38]
Univariate spectrophotometric of DGF and SXG	Methanol	DGF and SXG were highly overlapped at their λ_{max} 224 nm and 209 nm.	DGF and SXG over the range of 2.5–50.0 $\mu\text{g/mL}$ and 2.5–60.0 $\mu\text{g/mL}$, respectively.	[39]
EGF + Metformin	Methanol	Method A: EGF, 272 and metformin, 234 nm. Method B: EGF, 254 nm metformin, 226 nm	5–25 $\mu\text{g/mL}$ for EGF $r^2 = 0.999$ 2–12 $\mu\text{g/mL}$ for metformin $r^2 = 0.999$	[40]
EGF	Distilled water	Method 1: 247nm Method 1: 438nm Method 1: 782nm	Method 1: 2-12 $\mu\text{g/ml}$ Method 2: 5-30 $\mu\text{g/ml}$ Method 3: 10-60 $\mu\text{g/ml}$	[41]
EGF and metformin hydrochloride	Methanol	EGF: 224nm metformin: 230nm	EGF, 1-3 $\mu\text{g/ml}$ metformin, 2-10 $\mu\text{g/ml}$	[42]
Chemometric methods for simultaneous determination of EGF and metformin	Methanol	225 nm and 237nm	2-12 $\mu\text{g mL}^{-1}$ for both drugs	[43]
EGF and Metformin with Linagliptin in its ternary mixture	Methanol	296 nm for Linagliptin	5- 25 $\mu\text{g/ml}$	[44]
SXG	Methanol	208	5 – 40 $\mu\text{g/mL}$ $r^2 = 0.999$ LOD: 0.0607 $\mu\text{g/mL}$ LOQ: 0.1821 $\mu\text{g/mL}$	[45]
SXG	Methanol	416 nm (DDQ) and 838 nm (TCNQ)	50-300 and 10-110 $\mu\text{g/mL}$ with DDQ and TCNQ, respectively.	[46]
SXG and metformin hydrochloride	Methanol	SXG (274 nm) and Metformin (231 nm) respectively.	50-90 $\mu\text{g/mL}$ for SXG and 2-10 $\mu\text{g/mL}$ for Metformin	[47]
SXG	Methanol	at 208 nm	5–40 $\mu\text{g/mL}$.	[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 λ_{em} of 468 and 465 nm for SAX and VDG, respectively. Fluorescence intensity at λ_{em} of 542 and 540 nm for SAX and VDG, respectively [49]. A simple and highly sensitive spectrofluorimetric method was developed and validated 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 and validated 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 F254 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 F254 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 F254 (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 F254 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 60F254(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 60F254 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.

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 $\mu\text{g/mL}$ 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, spectrofluorimetry, electrochemistry, etc...

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