ERURJ **2023, 2, 1, 110-120**
Mini Review

Mini review on different analytical methods for quantitative determination of linagliptin and empagliflozin either in a biological sample or in the pharmaceutical dosage forms

Israa M. Nour [1,*], Ahmed R. Mohamed [1], Mohamed Badrawy [1]

[1]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Egyptian Russian University, Badr City, 11829, Cairo, Egypt.

*Corresponding author(s): Israa M. Nour, E-mail: Israa–mamdouh@eru.edu.eg, Tel: 0201121936325

Received 19th January 2023, Revised 21st January 2023, Accepted 29th January 2023

DOI:10.21608/ERURJ.2023.188531.1006

ABSTRACT

This article discussed several analytical techniques concerned with the determination of linagliptin and empagliflozin to help every analyst to know the latest analytical methods used for the determination of the studied drugs especially those not recorded in pharmacopoeia and also mentioned the physiochemical characters and the pharmacological action of the studied drugs. Linagliptin is a dipeptidyl peptidase-4 inhibitor that is used for the treatment of type-II diabetes and empagliflozin is a sodium-glucose co-transporter-2 (SGLT-2) inhibitor and also is used for type-II diabetes treatment. The review discussed techniques used for the determination of each drug quantitively involving spectrophotometric (UV and visible), spectrofluorimetric, electrochemical, and chromatographic (TLC and HPLC) methods either in a biological sample or in the pharmaceutical dosage forms. All methods included in the review are validated and constructed according to ICH guidelines. Distribution of analytical methods for empagliflozin and linagliptin was performed and it was obvious that the HPLC technique was the most developed method followed by spectroscopy and electrochemical methods. Spectrofluorimetric methods were the most suitable methods for the determination of either linagliptin or empagliflozin in biological samples as the spectrofluorimetric technique has high sensitivity.

Keywords: Linagliptin; Empagliflozin; Analytical methods; Type II diabetes.

1. Introduction

Linagliptin (**Figure. 1A**) is a dipeptidyl peptidase-4 (DPP-4) inhibitor used to treat type II diabetes. It works by inhibiting the action of the DPP-4 enzyme, which otherwise destroys the hormone GLP-1, which aids in increasing the secretion of insulin. It also prevents the release of glucagon, which lowers blood glucose levels **[1-2]**. Chemical name of linagliptin is 8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4- methylquinazolin-2-yl) methyl] purine-2, 6-dione) with molecular weight 472.5. It is somewhat soluble in isopropanol, barely soluble in ethanol, and soluble in methanol **[3].**

Empagliflozin (**Figure. 1B)** is an inhibitor of the SGLT-2 sodium-glucose co-transporter. As a result, it is used to treat type II diabetic patients by preventing the kidneys from reabsorbing glucose and encouraging the elimination of excess glucose in urine. **[4-6**]. It is used in combination with proper diet and exercise to help people with T2DM lower their blood sugar levels **[7].** Its chemical name is (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl] oxyphenyl] methyl] phenyl]-6-(hydroxymethyl) oxane-3, 4, 5-triol **[3],** its molecular weight is 450.9. Physically it was found as a white to off-white solid powder and it is spontaneously soluble in water, slightly soluble in methanol and ethanol; nearly insoluble in carbon tetrachloride, ether, and chloroform **[3].**

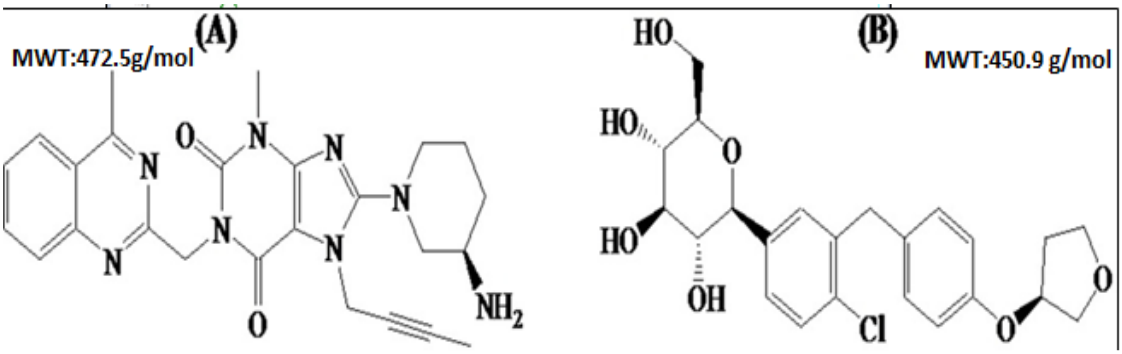

Figure 1. Chemical structure of (A) Linagliptin and (B) Empagliflozin.

This article discussed several analytical techniques concerned with the determination of linagliptin and empagliflozin either alone or in a combination with each other. These techniques included spectrophotometric, spectrofluorimetric, electrochemical,

and chromatographic methods and the distribution of analytical methods for empagliflozin and linagliptin was presented in **Figure.2**.

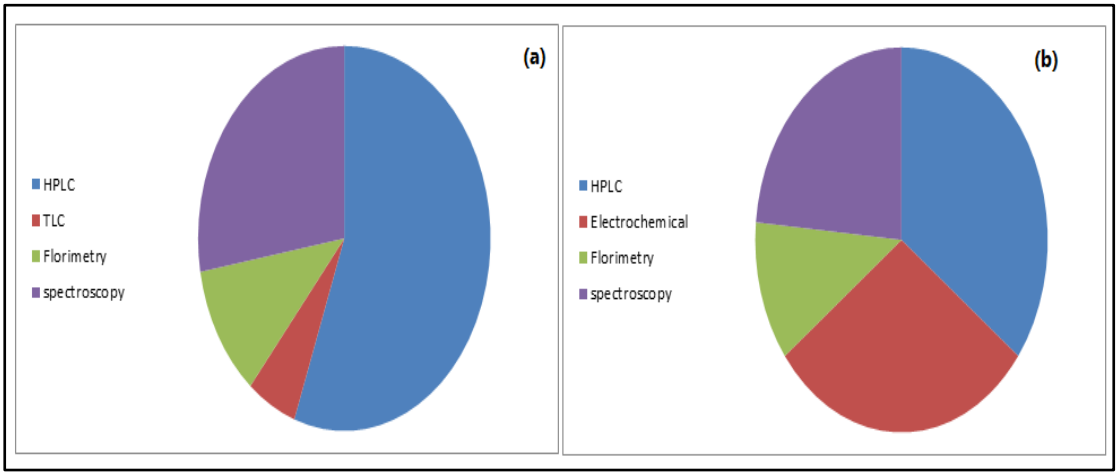

Figure 2. proportion of analytical methods for determination of (a) empagliflozin (b) linagliptin.

2. Analytical methods

2.1. Determination of empagliflozin

2.1.1. Spectrophotometric methods

Empagliflozin is directly determined at 224 nm **[8]**, 247 nm **[9],** and 223 nm **[10]**.
Colorimetric determination by coupling with potassium ferrocyanide and 1,10 phenanthroline was measured at 782 nm and 438 nm, respectively **[9]**.

2.1.2. Spectrofluorimetric methods

Spectrofluorimetric method depends on the formation of a yellow fluorescent agent between benzo furazan reagent and empagliflozin in a slightly alkaline medium and measured at $\lambda_{emission}$ = 521 nm after excitation at $\lambda_{excitation}$ = 455 nm **[11]**.
The fluorescence intensity for empagliflozin was measured at $\lambda_{emission}$ = 299.4 nm after $\lambda_{excitation}$ at 226.5 nm **[12]**.

2.1.3. Chromatographic techniques

Thin layer chromatographic method

Development of HPTLC- mass densitometric method for determination of empagliflozin and its degradation product using mobile phase (toluene-methanol 7:3 v/v) **[13]**.

High-performance liquid chromatographic (HPLC) methods

Table 1. HPLC methods for the determination of empagliflozin.

Sample matrix	Column	Mobile phase	Detection	Reported methods
Human plasma	C18 column	Aqueous trifluoroacetic acid (0.1% at pH 2.5): acetonitrile 60:40, v/v	DAD at 210 nm.	**[14]**
Pharmaceutical Formulations	Shim-pack phenyl column	Acetonitrile: water (72 : 28 v/v)	UV at 230 nm.	**[15]**
Pharmaceutical Formulations	Zorbax Eclipse Plus C18 column	The mobile phase is composed of different proportions of acetonitrile and water, gradient elution	QTOF-MS mass analyzer	**[16]**
Pharmaceutical Formulations	a particle size Poroshell 120 EC-C18	Methanol: acetonitrile: 0.1%OPA 75: 20: 5 v/v.	DAD at 220 nm.	**[17]**
Pharmaceutical Formulations	Enable C18G	Methanol: water (70: 30 v/v)	UV at 233 nm	**[18]**
Pharmaceutical Formulations	Develosil ODS HG-5 RP C18	Phosphate Buffer: Acetonitrile (45:55 v/v) (pH2.8)	UV at 228 nm.	**[19]**
Pharmaceutical Formulations	Intersil® C18 column	Acetonitrile: potassium dihydrogen phosphate buffer (pH 4) (50:50, v/v)	UV at 225 nm.	**[20]**
Pharmaceutical Formulations	Cosmosil C18	Methanol: water 80: 20 v/v	UV at 224 nm	**[21]**
Pharmaceutical Formulations	C18 column	0.1% trifluoroacetic acid solution: acetonitrile (70:30 v/v) (pH 4.8)	UV at 224 nm	**[22]**
Pharmaceutical Formulations	C18 column	Methanol: acetonitrile: water (60:5:35 v/v)	QTOF-MS mass	**[23]**

2.2. Determination of Linagliptin

2.2.1. Spectrophotometric methods

Oxidative coupling reaction of LIN with MBTH (3-methyl-2-benzothiazoline hydrazine) in the presence of ferric chloride forming green-colored chromogen and charge transfer reaction of the primary amino group of LIN with picric acid forming orange-colored ion-pair chromogen. The formed complexes were measured at 660 and 490 nm for MBTH and picric acid, respectively **[24]**.

Condensation reaction of the primary amine group of linagliptin with vanillin (Schiff base formation) forming purple-colored chromogen complex at 463 nm and with NQS (1, 2-naphtho quinine 4- sulphonic acid sodium salt) reagent forming orange-colored chromogen at 454 nm. The formed complexes were measured at 463 and 454 nm for vanillin and NQS, respectively **[25]**.

2.2.2. Spectrofluorimetric methods

The fluorescence intensity of linagliptin was determined at $\lambda_{emission}$ = 435 nm and $\lambda_{excitation}$ = 339 nm **[26]**.

Interaction of the primary amino group of linagliptin with fluorescamine reagent in aqueous borate buffer (pH 8.5) to create a highly fluorescent product. After excitation at 390 nm, the emission of the produced product was detected at 479 nm. **[27]**.

2.2.3. Electrochemical techniques

Square Wave Adsorptive Anodic Stripping Voltammetric for the determination of linagliptin Using Pencil Graphite Electrode **[28]**.

Voltametric determination of linagliptin using an electrochemical sensor based on L-cysteine modified 1T-MoS_2 nanosheets **[29]**.

Adsorptive Stripping Voltammetry for the Determination of linagliptin using A Glassy Carbon Electrode **[30]**.

Square Wave Anodic Voltammetric Determination linagliptin by Microparticles Copper Pencil Graphite Electrode **[31]**.

Measurement of linagliptin using an improved carbon paste electrode (CPE) with graphitic carbon nitride/β-cyclodextrin nanocomposite (gCN-βCD/CPE) as an electrochemical sensor **[32]**.

Square wave voltammetry using graphene oxide modified glassy carbon electrode for determination of linagliptin in dosage form, biological fluids, and rats' feces **[33]**. Voltametric determination of linagliptin using imprinted sensor based on CuBi2O4/rGO@MoS2 nanocomposite **[34]**.

2.2.4. Chromatographic techniques

High-performance liquid chromatographic methods

Table 2. HPLC methods for the determination of linagliptin.

Sample matrix	Column	Mobile phase	Detection	Reported methods
Pharmaceutical formulations	InertSustain C8 column	(A) aqueous phase containing 10 mM ammonium acetate at pH 5.5 and (B) an organic phase containing a mixture of ACN and methanol (80:20 v/v)	QTOF-MS mass	**[35]**
Pharmaceutical formulations	Tracer Extrasil Silica	Water: acetonitrile 10:90 v/v containing 10.0 mM ammonium acetate pH 6	UV at 298 nm.	**[36]**
Pharmaceutical formulations	PrimesilC18	0.3% TEA: methanol. 60:40 v/v at pH 4.5	UV at 292 nm	**[37]**
Pharmaceutical formulations	Thermo Scientific® RP-8 column	a mixture of 0.1% formic acid (pH 3.5) and acetonitrile, gradient elution	UV at 294 nm	**[38]**
Pharmaceutical Formulation	SB-C18 RRHD	0.01 M potassium phosphate and acetonitrile (30:70 v/v)	PDA at 292 nm	**[39]**
pharmaceutical and human plasma	Symmetry® cyanide column	potassium dihydrogen phosphate buffer at pH 4.6: acetonitrile (20:80, *v/v*)	UV at 299 nm	**[40]**

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3. Conclusion

Simple and clear highlights for the collected information about the most analytical methods used for the determination of linagliptin and empagliflozin either alone or with each other. It was obvious that the HPLC technique was the most developed method followed by spectroscopy and electrochemical methods. linagliptin and empagliflozin were only present in a combined form with each other and a review for simultaneous determination of them was previously presented. New coming analytical techniques will be developed and need future evaluation.

- **Conflict of Interest**

The Authors declare no conflict of interest

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