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Linagliptin: A Concise Review on Analytical and Bio-analytical Methods

Bassam M. Ayoub^{a,b}

^aCenter for Drug Research and Development (CDRD),

^bPharmaceutical Chemistry Department, Faculty of Pharmacy, British University in Egypt, El-Sherouk city, Cairo 11837, Egypt

ABSTRACT

The presented work is a collective review of the previously published methods concerning the analysis of linagliptin either alone or in binary mixture with metformin. Many spectroscopic and chromatographic techniques were used for linagliptin analysis either in biological fluids or in pharmaceutical formulations including spectrophotometry, spectrofluorimetry, HPLC-UV, LC-MS/MS and HPTLC methods. This review can be used as a guide for analysts to select the most appropriate method for their work.

INTRODUCTION

Linagliptin (figure 1) is one of the Dipeptidyl peptidase-4 (DPP-4) inhibitors that recently used in treatment of type 2 diabetes mellitus by stimulation of glucose-dependent insulin secretion from the beta cells of the pancreas through inhibition of DPP-4 activity in peripheral plasma [1]. Linagliptin was marketed as Tradjenta[®] while its combination with metformin was marketed as Jentaduo[®]. Although many pharmacokinetic studies were reported in the literature for linagliptin analysis in biological fluids [2-17], details about the used analytical procedures were not described by the authors, so it were excluded from the present review article. Many spectroscopic [18-22] and chromatographic techniques [23-48] were used for linagliptin analysis either in biological fluids or in pharmaceutical formulations.

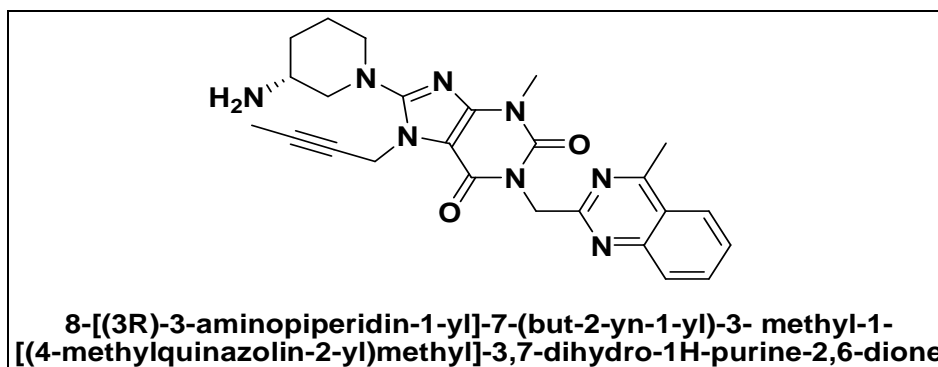


Figure 1: Chemical structure and IUPAC nomenclature of linagliptin

2. Spectroscopic methods for the analysis of linagliptin in pharmaceutical formulations

Different spectrophotometric [18-21] and spectrofluorimetric [22] methods were reported for determination of linagliptin alone in Tradjenta[®] tablets [18, 19 and 22], determination of linagliptin in the presence of metformin in Jentadueto[®] tablets [20] and simultaneous determination of linagliptin and empagliflozin in Glyxambi[®] tablets [21]. All the experimental parameters were described in (figures 2-3) including the applied method, wavelength of detection and the diluents used for preparation of the working solution. Linearity ranges ($\mu\text{g mL}^{-1}$) were found to be (10-35), (2-25), (5-30), (2-12) and (10-110) $\mu\text{g mL}^{-1}$ for [18], [19], [20], [21] and [22] methods, respectively. Limit of detection (LOD) was found to be in the range of (0.19 - 0.84 $\mu\text{g mL}^{-1}$) according to the described methods [18-22] and the dosage form assay resulted in high recovery range (99.83% - 101.2%) with %RSD below 2% confirming the validation of all the methods [18-22]. There is no published work yet regarding derivatization before UV detection as shown with similar gliptins [49] or regarding use of spiking technique as reported with the analysis of some metformin anti-diabetic combinations [50].

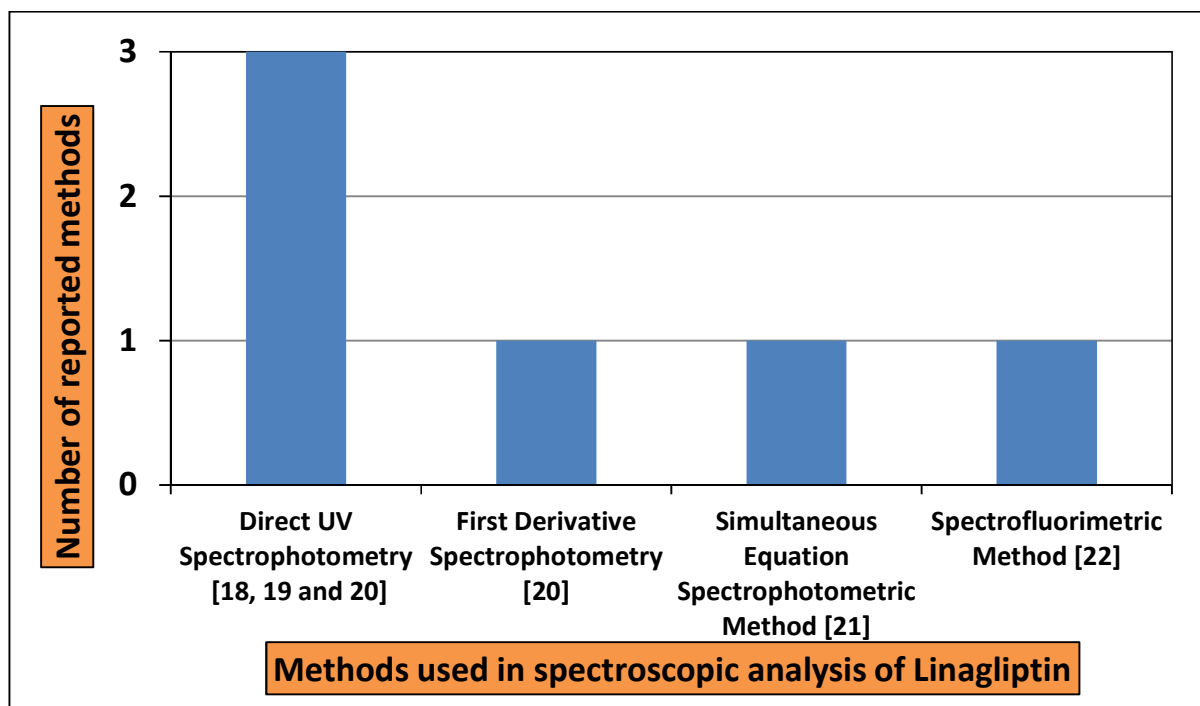


Figure 2: Spectroscopic methods used for linagliptin analysis including direct UV measurement at 241 nm [18], 294 nm [19], 299 nm [20], first derivative at 311 nm [20], simultaneous equation at 277 nm [21] and spectrofluorimetric emission at 435 nm after excitation at 339 nm [22]

3. Bioanalytical methods for determination of linagliptin in biological fluids

3.1. LC-MS method for simultaneous determination of linagliptin with other gliptins in human plasma [23]

LC-MS method was developed and validated to measure the human plasma concentrations of vildagliptin, saxagliptin, sitagliptin, linagliptin and teneligliptin, using pioglitazone as an internal standard. Chromatographic separation of five gliptins was achieved on C-18 column (50×2.1 mm, $1.8 \mu\text{m}$) using a mobile phase consisting of 20 mM ammonium formate and acetonitrile in gradient mode. The detection was performed in positive ESI mode and selective ion monitoring at m/z 304.2 for vildagliptin, 316.2 for saxagliptin, 408.1 for sitagliptin, 473.2 for linagliptin and 427.2 for teneligliptin. Simple protein precipitation was employed for sample extraction from human plasma using acetonitrile. Lower limit of quantification (LLOQ) in plasma was found to be 0.1 ng mL^{-1} for linagliptin [23].

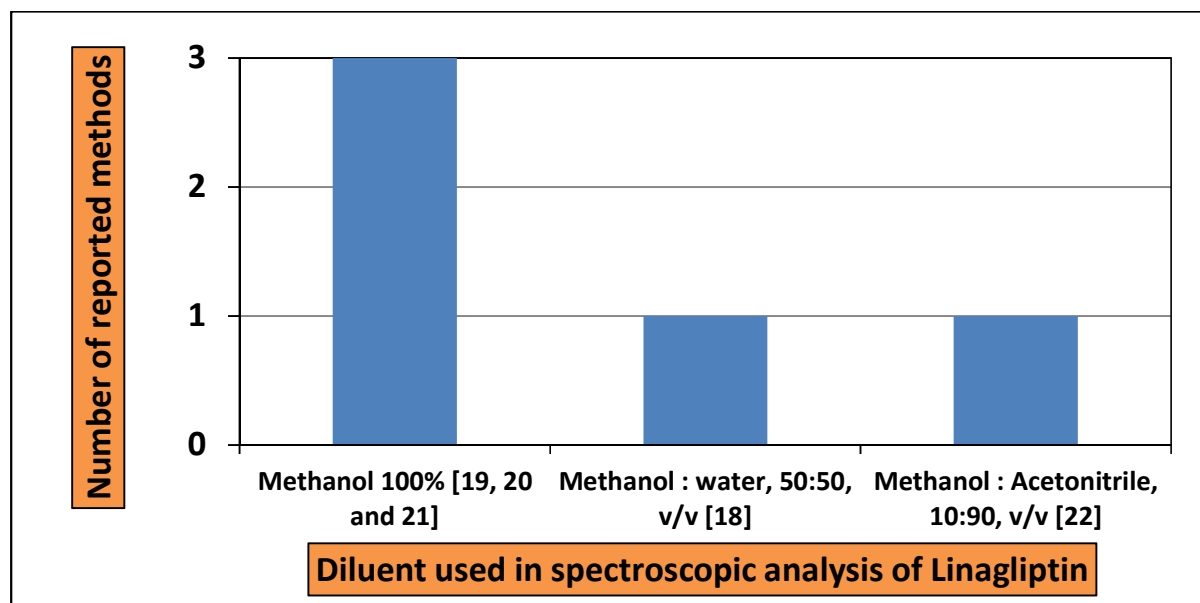


Figure 3: Diluents used in spectroscopic analysis of linagliptin

3.2. LC-MS method for sensitive determination of linagliptin in human plasma applying solid phase extraction [24]

LC-MS method was developed and validated for the quantification of linagliptin in human plasma using linagliptin D4 as an internal standard. Following solid phase extraction in 96 well plate format, linagliptin and linagliptin D4 were run on phenyl hexyl, (100A, 100 X 4.6mm, 2.6 μ) using an isocratic mobile phase consisting of 10mM Ammonium formate buffer (pH 6.5): Methanol, 15:85, v/v. The precursor and productions of the drugs were monitored on a triple quadrupole instrument operated in the positive ionization mode. The [M+H]⁺ peaks were observed at m/z of 473.3 and 477.4 for Linagliptin and Linagliptin D4 respectively. Most abundant product ions were found at m/z of 420.2 and 424.2 for both Linagliptin and Linagliptin D4 providing lower limit of quantification (LLOQ) in plasma equals to 99.5 pg mL⁻¹ for linagliptin [24].

3.3. LC-MS method for determination of linagliptin in human plasma applying liquid-liquid extraction [25]

LC-MS method was developed and validated for the quantification of linagliptin in human plasma using Telmisartan as internal standard. Separation was carried out on Waters, X-Bridge, C18, 5 μ m column having 4.6x50 mm internal diameter and the mobile phase containing acetonitrile and 0.1 % formic acid (90:10 v/v) at a flow rate of 0.6 mL/min. Monitoring of the fragmentation of m/z 473.54 \rightarrow 157.6 performed during MS/MS detection of linagliptin on the mass spectrometer providing lower limit of quantification (LLOQ) in plasma equals to 10 ng mL⁻¹ [25].

3.4. HPLC-UV methods for determination of linagliptin in human plasma [26-27]

HPLC-UV method was developed and validated for quantification of linagliptin in human plasma using a mobile phase consisting of potassium dihydrogen phosphate buffer pH (4.6) - acetonitrile (20:80, v/v) with UV detection at 299 nm. Chromatographic separation was achieved on a Symmetry® cyano column (150 mm x 4.6 mm, 5 μ m). The spiked plasma samples were extracted after direct precipitation of proteins using 100 μ L of perchloric acid (35% w/w) providing lower limit of quantification (LLOQ) in plasma equals to 1.87 μ g mL⁻¹ [26]. In addition, another HPLC-UV method was developed and validated for simultaneous determination of linagliptin and metformin in human plasma. The analytes were extracted by protein precipitation technique using 50 μ L of 2% perchloric acid and 1000 μ L acetonitrile. Mobile phase consisting of acetonitrile and 0.01M di-potassium hydrogen phosphate buffer in ratio of 75:25 was used after adjusting pH to 7.0 with orthophosphoric acid. Grace vyadec genesis CN (150 x 4.6 mm, 4 μ m) column and UV detection at 237 nm was employed providing lower limit of quantification (LLOQ) for linagliptin in plasma equals to 1 ng mL⁻¹ [27].

4. HPTLC methods for determination of linagliptin in pharmaceutical formulations

A high performance thin layer chromatographic (HPTLC) method was developed and validated for the determination of linagliptin, saxagliptin or vildagliptin in their binary mixtures with metformin in pharmaceutical preparations using environmentally preferable green mobile phase system. Separation was carried out on Merck HPTLC aluminum sheets of silica gel 60 F254 using methanol-0.5% w/v aqueous ammonium sulfate (8:2, v/v) as mobile phase. Densitometry measurement of the spots was performed at 225 nm for linagliptin-metformin mixture. The linear regression analysis data were used for the regression line in the range of 0.05-0.5 µg/band for linagliptin [28].

In addition, stability indicating high-performance thin-layer chromatography (HPTLC) method was developed and validated for simultaneous estimation of linagliptin and metformin active pharmaceutical ingredients and fixed dose combination. Linagliptin and metformin densitograms were developed on silica gel 60 F254 HPTLC plates with acetone: methanol: chloroform: formic acid (3:1:5:1v/v) as the mobile phase. Densitometric quantification was performed at 230 nm. Linagliptin and metformin RF values were found as 0.72 and 0.19, respectively. Limit of detection was found to be 5.19 and 8.72 ng per spot for linagliptin and metformin, respectively; lowest possible quantity to be quantified by the proposed method was found to be 15.74 and 26.44 ng per spot for linagliptin and metformin, respectively [29].

Furthermore, economic high-performance thin layer chromatographic method has been established for simultaneous estimation of metformin hydrochloride and linagliptin in formulation. The chromatographic separation was performed on precoated silica gel 60 GF254 plates with acetone-methanol-toluene-formic acid 4:3:2:1 (v/v/v/v) as mobile phase. The plates were developed to a distance of 8 cm at ambient temperature. The developed plates were scanned and quantified at their single wave length of 259 nm. Experimental conditions such as band size, chamber saturation time, migration of solvent front, slit width, etc. was critically studied and the optimum conditions were selected. The drugs were satisfactorily resolved with Rf 0.61 and 0.82 for metformin hydrochloride and linagliptin respectively. The method was validated for linearity, accuracy, precision, and specificity. The calibration plot was linear between 400-2000 (ng/spot) and 20-100 (ng/spot) for metformin hydrochloride and linagliptin respectively. The limits of detection and quantification for metformin hydrochloride and linagliptin are 20 (ng/spot) and 10 (ng/spot) respectively [30].

5. HPLC-UV methods for determination of linagliptin in pharmaceutical formulations

Many HPLC-UV methods were reported for determination of linagliptin either alone or in combination with metformin [31-48, 20 and 26]. All the parameters affecting the chromatographic behavior are described in (table 1) including stationary phase, mobile phase and detection wavelength. Similar column and similar chromatographic conditions were used for determination of similar gliptin, alogliptin, using HPLC-UV method [51].

Table 1: HPLC-UV methods for analysis of linagliptin in different pharmaceutical formulations

Stationary phase	Mobile phase	Detection wavelength	Applications
C18 column (100 mm x 2.1 mm, 2.2 μ m).	Mixture of potassium dihydrogen phosphate buffer (pH 4) and methanol in the ratio of (50:50 %, v/v).	225 nm	Assay of Tradjenta [®] , Jentaduetto [®] and Glyxambi [®] and tablets ⁽³¹⁾
C18 column (150 mm x 4.6 mm, 5 μ m).	Mixture of acetonitrile and 0.02M phosphate buffer (pH 5.0) in the ratio of (35:65 %, v/v).	225 nm	Assay of Jentaduetto [®] tablets ⁽³²⁾
C18 column (250 mm x 4.6 mm, 5 μ m).	Mixture of phosphate buffer (1.625 g of potassium dihydrogen orthophosphate and 0.3 g of dipotassium hydrogen orthophosphate in 550 ml water, pH 4.5) and acetonitrile in the ratio of (60:40 %, v/v).	280 nm	Stability indicating assay of Jentaduetto [®] tablets ⁽³³⁾
C18 column (125 mm x 4 mm, 5 μ m)	Mixture of methanol and 0.05 M potassium dihydrogen orthophosphate (pH 4.6) in the ratio of (70:30 %, v/v).	267 nm	Assay of Jentaduetto [®] tablets ⁽³⁴⁾
C18 column (50 mm x 2.1 mm, 1.8 mm)	Mixture of 0.01 M potassium phosphate (pH 4) and acetonitrile in the ratio of (30:70 %, v/v).	292 nm	Assay of Tradjenta [®] tablets ⁽³⁵⁾
C18 column (150 mm x 4.6 mm, 5 μ m).	Mixture of potassium dihydrogen phosphate buffer (pH 4.6) and methanol in the ratio of (30:70 %, v/v).	260 nm	Assay of Jentaduetto [®] tablets ⁽²⁰⁾
C18 column (250 mm x 4.6 mm, 5 μ m).	Mixture (pH 4.1) of acetonitrile, water and methanol in the ratio of (25:50:25 %, v/v/v).	243 nm	Stability indicating assay of Jentaduetto [®] tablets ⁽³⁶⁾
Cyano column (150 mm x 4.6 mm, 5 μ m).	Mixture of potassium dihydrogen phosphate buffer (pH 4.6) and acetonitrile in the ratio of (20:80 %, v/v).	299 nm.	Assay of Tradjenta [®] tablets ⁽²⁶⁾
C18 column (250 mm x 4.6 mm, 5 μ m).	Mixture (pH 6.4) of acetonitrile, water and methanol in the ratio of (25:50:25 %, v/v/v).	238 nm.	Assay of Tradjenta [®] tablets ⁽³⁷⁾
C18 column (250 mm x 4.6 mm, 5 μ m).	Mixture (pH 3) of water and methanol in the ratio of (60:40 %, v/v).	238 nm	Assay of Tradjenta [®] tablets ⁽³⁸⁾
C18 column (100 mm x 2.5 mm, 3 μ m)	Mixture of methanol and (water containing 0.1% orthophosphoric acid) in the ratio of (70:30 %, v/v) with (pH 6.4).	296 nm	Assay of Tradjenta [®] tablets ⁽³⁹⁾
C18 column (150 mm x 4.6 mm, 5 μ m).	Mixture of phosphate buffer (pH 3), methanol and acetonitrile in the ratio of (45:25:30 %, v/v/v).	237 nm	Assay of Jentaduetto [®] tablets ⁽⁴⁰⁾
C18 column (250 mm x 4.6 mm, 5 μ m).	Mixture of potassium dihydrogen phosphate buffer and acetonitrile in the ratio of (40:60 %, v/v).	250nm	Assay of Jentaduetto [®] tablets ⁽⁴¹⁾
C18 column (150 mm x 4.6 mm, 5 μ m).	Mixture of phosphate buffer (pH 5.6), methanol and acetonitrile in the ratio of (65:10:25 %, v/v/v).	231 nm	Assay of Jentaduetto [®] tablets ⁽⁴²⁾
C18 column (250 mm x 4.6 mm, 5 μ m).	Mixture of methanol and phosphate buffer (pH 4.9) in the ratio of (70:30 %, v/v).	218 nm	Stability indicating assay of Tradjenta [®] tablets ⁽⁴³⁾
C18 column (250 mm x 4.6 mm, 5 μ m).	Mixture of potassium dihydrogen phosphate buffer and acetonitrile in the ratio of (40:60 %, v/v).	236 nm	Assay of Jentaduetto [®] tablets ⁽⁴⁴⁾
C18 column (250 mm x 4.6 mm,	Mixture (pH 4.1) of methanol and water in the ratio of (83:17 %, v/v).	241 nm	Assay of Tradjenta [®] tablets ⁽⁴⁵⁾

5µm).			
C18 column (100 mm × 4.6 mm, 5 µm)	Mixture of phosphate buffer (pH 3.4) and acetonitrile in the ratio of (70:30 %, v/v)	240 nm	Assay of Tradjenta® tablets ⁽⁴⁶⁾
C18 column (250 mm × 4.6 mm, 5µm).	Mixture of phosphate buffer (pH 5.6), methanol and acetonitrile in the ratio of (40:5:55 %, v/v/v).	233 nm	Assay of Jentaduo® tablets ⁽⁴⁷⁾
C18 column (150 mm × 4.6 mm, 5µm).	Mixture of 0.02 M potassium dihydrogen phosphate (pH 5.0) and acetonitrile in the ratio of (70:30 %, v/v).	226 nm	Assay of Tradjenta® tablets ⁽⁴⁸⁾

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