IJBPAS, July, 2021, 10(7): 2321-2334

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANALYTICAL METHODS OF SAXAGLIPTIN A DPP-4 INHIBITOR: A REVIEW

PATHAK S AND BANSAL K*

Institute of Pharmaceutical Research (IPR), GLA University, NH-2, Mathura - Delhi Road, Mathura, Uttar Pradesh (India), Pin – 281406

***Corresponding Author: Keshav Bansal: E Mail: keshav.bansal@gla.ac.in; Mob: +91 9997592453**

Received 18th Aug. 2020; Revised 17th Sept. 2020; Accepted 16th Oct. 2020; Available online 1st July 2021

https://doi.org/10.31032/IJBPAS/2021/10.7.5551

ABSTRACT

Saxagliptin is dipeptidyl peptidase protein-4 and active renal glucose reabsorption inhibitors. It is used in type 2 diabetes patients. They are administered as tablets. It has numerous analytical papers for Reverse Phase-High Performance Liquid Chromatography and Ultraviolet Spectroscopy estimation of the Active Pharmaceutical Ingredient or drug formulation. The use of chemical, drug and solvent separation methods used for green chemistry in pharmaceutical products is very difficult. Phosphate buffer and other toxic reagents were mainly used for estimation in this analysis and these agents damage tools as well as the environment and a lot of waste, so that novel analytical techniques for quantifying and identifying Saxagliptin should be developed for the person and the community as easily and safely as possible. This review focuses on the essential physicochemical state, properties, intervention, and aims to concentrate on various analytical methods for Saxagliptin estimation in pharmaceutical formulation.

Keywords: Saxagliptin, RP-HPLC methods, UV methods, Pharmacokinetic

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a progressive, chronic condition. Absolute or relative insulin-characterized metabolic disorder inadequacy. It is known that diabetes has a prevalence of around 220 million people around the world and it is estimated that it will affect by 2030, about 440 million. Of these, roughly 90 to 95 percent of there is cases of T2DM **[1]**. The predicted increase in diabetes prevalence is

Due to improved treatment, primarily due to an extended life span Facilities and increase in diabetic risk factors, especially physical inactivity and obesity due to sedentary lifestyle **[2]**. In T2DM patients, pancreatic β cell function is progressively degraded and reflected into long-term ineffective glycemic control **[3]**.Glycemic regulation that has been poorly achieved contributes to micro-vascular and macro-vascular (retinopathy, nephropathy) Complications (cardiovascular). These are liable not just for a person, the enormous burden of disease but also at the economic level, and at the social level. Inhibitors of DPP-4 are administered orally and tolerated well. Besides the Improving the work of β-cells, stimulating the secretion of insulin and these agents minimize appetite by inhibiting glucagon secretion, weight stabilization and/or weight loss promotion T2DM in patients. Glucose-dependent because they are more effective in reducing the mechanism of action, postprandial hyperglycemia, especially early stage hyperglycemia, Disease while T2DM patients continue to work pancreatic β cells. Guidelines AACE / ACE, released in DPP-4 inhibitors were recommended in late 2009 as an alternative for Used as first-line mono therapy (6.5 to 7.5 percent HbA1C) and in combination therapy (7.6 to 9 percent HbA1C). Saxagliptin is the recently approved agent in this class by US FDA in July 2009 as an adjunct to diet and exercise to improve glycemic control in adults with T2DM **[4-6]**. The characteristics of Saxagliptin are shown in **Table 1**. The structure shown in **Figure 1**.

Table 1: Characteristic of Drug Saxagliptin

S.no	Parameter	Specification
1.	IUPAC Name	(1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile
2.	Molecular formula	C_{18} H_{25} N_3 O_2
3.	CAS number	361442-04-8
4.	Molecular weight	315.4 g/mol
5.	Solubility	Sparingly soluble (in water with 791.8 mg/L at 25'C)
6.	Vapor Pressure	4.19X10-11 mm Hg at 25 °C
7.	Dissociation Constant	pKa= 7.90
8.	Volume of distribution	151L
9.	Clearance	Renal Clearance (single 50mg dose with 14L/h)
10.	Biological half life	2.5 hours
11.	Pharmacotherapeutic class	Antidiabetic
12.	Appearance	White to yellow or light brown being non-hygroscopic and crystalline powder

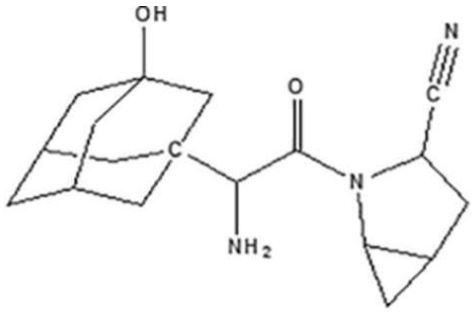

Figure 1: Structure of Saxagliptin

Mechanism of Action

Saxagliptin and its active metabolite M2 (two times less, respectively) Inhibitors of DPP-4 that are potent as parent drugs are by avoiding inactivation, the improvement of glycemic regulation

GLP-1 and glucose-dependent incretin hormones Polypeptide insulinotropic. This raises GLP-1 levels, Stimulates the secretion of insulin and decreases post-prandial the levels of glucagon and glucose. GLP-1's detailed behavior are Saxagliptin and M2 are more selective, as depicted in **Table 2**. Inhibition of DPP-4 as opposed to DPP-8 (400- and 950-fold) Or DPP-9 enzymes (75- and 160-fold) or a wide panel of other enzymes (> 4000-fold) proteases **[7]**. It has been observed that inhibition of Alopecia produced by DPP-8/9, thrombocytopenia, splenomegaly, splenomegaly, multiorgan pathology, leading to death, and thrombocytopenia in mice, and in dogs for gastrointestinal toxicity **[8]**.

Table 2: Action of (GLP-1) [9]

Organ	Effect
Heart	Increases cardiac output
GIT	Delays gastric emptying
Brain	Decreases appetite
Liver	Decreases neoglucogenesis
Muscle	Improves insulin sensitivity

Pharmacokinetics

Saxagliptin shows a bioavailability of 67% and is orally absorbed. Kidney and intestinal tissues shows its highest concentration and are distributed through the extra vascular tissues **[10]**. It shows both renal as well as hepatic excretion having a percentage excretion of 22% in feces and 75% through urine majorly **[11]**. Since the parent route of administration of the drug is found to be renal, its dosage should be proper in patients suffering from renal disorders. Being first order in kinetics, the mean plasma level termination of Saxagliptin shows a half-life of 3.1h **[12]**.

Dosage and Administration

In patients suffering from T2DM, the prescribed dosage is 2.5-5 mg which is done orally regardless of meals **[13]**.

Analytical Methods of Saxagliptin

- **High Performance Liquid Chromatography Methods**

Daswadkar *et al* developed a method using acetonitrile and water at pH-3 in a 20:80 ratio respectively with 1mL/min at 211nm wavelength[14]. Another method given by Scheeren *et al* using a 0.1% phosphoric acid at pH-3 and methanol (70:30) with 1mL/min at 225nm wavelength **[15]**. Zengad *et al* gave a new method using a methanol and water in 80:20 ratio at 0.8mL/min with detection at 212nm **[16]**. Islam *et al* exhaust a novel method with using phosphate buffer and acetonitrile in 80:20 ratio and pH-2.7 of buffer adjusted with ortho phosphoric acid at 210nm wavelength **[17]**. Recently Lokhande developed a latest method using methanol and phosphate buffer (pH-4.8) in a 70:30 ratio at o.8 mL/min with detection at 212 nm **[18]**. Tekkeli *et al* gave a method in human plasma with fluorescence detection using acetonitrile and 10mM ortho phosphoric acid at 1.3 mL/min with detection of wavelength at 378 nm **[19]**. Above methods are for only Saxagliptin drugs after this method has been developed in combination also so there is two combination is very much important in analysis. One is Saxagliptin and metformin and another one is Saxagliptin and Dapagliflozin.

For Saxagliptin and metformin combination there are some method available out of these methods one is given by Hanan *et al* with using acetonitrile and phosphate buffer (pH-4.5) adjusted with ortho phosphoric acid 1.5 mL/min with detection at 2220nm **[20]**. Another study done by Nyola for the same combination with using 0.02M potassium dihydrogen phosphate, acetonitrile and methanol in a ration of (50:25:25) pH-4.3 at 1 mL/min at 240 nm wavelength **[21**. Barge *et al* gave a new method for this combination with using methanol: phosphate buffer (pH-5) in a ratio of 1 mL/min at 228nm wavelength **[22]**. Caglor *et al* produce a sensitive method using a methanol and 50mM phosphate buffer (pH-2.7) at 1mL/min at wavelength of 225nm **[23]**. Bangaruthalli *et al* develed a robust method with using Acetate buffer and acetonitrile and methanol (70:20:10) at 225nm with flow rate 1 mL/min **[24]**. Recently Gurav *et al* gave a specific method using ammonium dihydrogen phosphate at pH-2.5 and methanol (70:30) at 210nm with 1.8mL/min flow rate for the same combination**[25]**.

Another combination that is Saxagliptin and Dapagliflozin have a number of papers. One study done by Kommineni *et al*. gave a novel stability method in combination of Saxagliptin and Dapagliflozin with phosphate buffer (50) and Acetonitrile (50) maintaining pH 4 at 225nm **[26]**. Patel *et al*. produce an easy, fast, exact, rugged and robust stability method in combination of Saxagliptin hydrochloride and Dapagliflozin using Potassium dihydrogren phosphate Buffer (45): Acetonitrile (55) at 220 nm with maintain pH 6 **[27]**. Singh *et al*. presented an easy and novel stability method for the combination of Dapagliflozin and Saxagliptin with buffer (53) and acetonitrile (47) at 230 nm **[28]**. Aswini *et al* gave a method using methanol and potassium dihydrogen phosphate buffer (45:55) at 210nm with 1 mL/min flow rate **[29]**. Another study done by padmaja *et al* using potassium dihydrogen phosphate and acetonitrile (55:45) pH-3.8 adjusted with dilute orthophosphoric acid at 210nm with 1 mL/min flow **[30]**. Suthar *et al* developed a new method with 0.05M Potassium dihydrogen phosphate pH-6.0 and Acetonitrile (70:30) at 275nm with 1 mL/min flow rate **[31]**. Swamy *et al* gave a specific method using same mobile phase but the ratio is different that is (45:55) at 247nm with 1 mL/min flow rate **[32]**. Patel *et al* using potassium dihydrogen phosphate and methanol (85:15) at 222nm with 1 mL/min flow **[33]**. Patel *et al* using potassium dihydrogen phosphate (pH-6.0) and methanol (70:30) at 275nm with 1 mL/min flow **[34]**. Kadam *et al* gave simple method of methanol and water (75:25) at 270nm with flow rate 0.9 mL/min **[35]**. Usman *et al* gave simple method of methanol and ortho phosphoric acid 0.1% (60:40) at 270nm with flow rate 0.9 mL/min **[36]**. Deepan *et al* gave a method using acetonitrile and water (60:40) at 248nm with flow 1 mL/min **[37]**. Gundala gave a robust method with using acetonitrile and orthophosphoric acid (0.1%) in a ratio of 50:50 **[38]**. Phani *et al* use a buffer of ammonium dehydrogen phosphate and methanol (65:35) at 280nm with flow rate 1.5mL/min **[39]**. Rao gave a new method using 10mM phosphate buffer pH-6.8 and acetonitrile (40:60) at 260nm with 1 mL/min flow rate **[40]**. Donepudi *et al* gave a method in human plasma using 0.1% ortho phosphoric acidand acetonitrile in a ratio of 50:50 at 1mL/min **[41]**. All of above methods are shown in **Table 3**.

- **Spectrophotometric methods of Saxagliptin**

Kalaichelvi *et al* gave a simple method at 208 nm in methanol **[42]**. Koli *et al* gave a

method at 212 nm with using acetonitrile as solvent **[43]**. Recently Deshpandey produce a two different method of UV. Method A used at 211nm and method B used at 204nm in methanol **[44]**. For combination Saxagliptin and metformin Cholke and Nyola *et al* developed a method at 274nm and 231vnm respectively in distilled water **[45, 46]**. Method for another combination that is Saxagliptin and Dapagliflozin developed at 212 and 223nm in distilled water **[47]**. All of above methods are shown in **Table 4**.

- **High Performance thin layer Chromatography methods of Saxagliptin**

For Saxagliptin drug srividya *et al* produce a method using stationary phase silica gel aluminium plate 60 F_{234} (10X10) and mobile phase is methanol: Chloroform (6:4) v/v at 222 nm **[48]**. Rode et al gave a different method using stationary phase silica gel aluminium plate 60 F234 (10X10) and mobile phase is 1% methanolic ammonium acetate: Toluene (5:5) v/v at 215 nm **[49]**. Ghode *et al* produce a method using same stationary phase as above and mobile phase is Toluene:methanol:Ammonia (6:4:0.2) at 222nm **[50]**. All of above methods are shown in **Table 5**.

- **Liquid chromatography- Mass Spectrometry method for Saxagliptin**

Ghany *et al* developed a method using mobile phase potassium dihydrogen phosphate (pH-4.6): Acetonitrile: Methanol (40:30:30) at 208 nm **[51]**. All of above methods are shown in **Table 6**.

Table 3: Summary of HPLC methods of Saxagliptin

S. No.	Drugs	Techniques	Mobile Phase/Solvent	Column	Wavelength	Flow rate
1	Saxagliptin	RP-HPLC	Acetonitrile: Water (20:80)	C-18	211nm	1mL/min
2	Saxagliptin	RP-HPLC	0.1%ortho phosphoric acid/Methanol (70:30)	C-18	225nm	1mL/min
3	Saxagliptin	RP-HPLC	Methanol: Water (80:20)	C-18	212nm	0.8mL/min
4	Saxagliptin	RP-HPLC	Phosphate buffer/ acetonitrile (80:20)	C-18	210nm	1mL/min
5	Saxagliptin	RP-HPLC	Methanol: phosphate buffer (70:30)	C-18	212nm	0.8mL/min
6	Saxagliptin	RP-HPLC	Acetonitrile:10mM ortho phosphoric Acid	Florescence	378nm	1.3mL/min
7	Saxagliptin/ Metformin	RP-HPLC	Acetonitrile: Phosphate buffer (13:87)	C-18	220nm	1.5mL/min
8	Saxagliptin/ Metformin	RP-HPLC	Potassium dihydrogen phosphate/Acetonitrile/methanol (50:25:25)	C-18	240nm	1mL/min
9	Saxagliptin/ Metformin	RP-HPLC	Methanol: phosphate buffer (70:30)	C-18	228nm	1mL/min
10	Saxagliptin/ Metformin	RP-HPLC	Methanol: phosphate buffer (10:90)	C-18	225nm	1mL/min
11	Saxagliptin/ Metformin	RP-HPLC	Acetate buffer:Acetonitrile:Methanol (70:20:10)	C-18	225nm	1mL/min

12	**Saxagliptin/ Metformin**	**RP-HPLC**	**Ammonium dihydrogen phosphate Buffer/methanol (70:30)**	**C-18**	**210nm**	**1mL/min**
13	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Phosphate buffer/ acetonitrile (50:50)**	**C-18**	**225nm**	**1mL/min**
14	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Potassium dihydrogren phosphate Buffer/ Acetonitrile (45:55)**	**C-18**	**220nm**	**1.5mL/min**
15	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Sodium dihydrogen phosphate buffer/ Acetonitrile (53:47)**	**C-18**	**230nm**	**1.2mL/min**
16	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Methanol/Potassium dihydrogen phosphate buffer (45:55)**	**C-18**	**210nm**	**1mL/min**
17	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Potassium dihydrogren phosphate Buffer/ Acetonitrile (55:45)**	**C-18**	**210nm**	**1mL/min**
18	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Potassium dihydrogren phosphate Buffer/ Acetonitrile (70:30)**	**C-18**	**275nm**	**1mL/min**
19	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Potassium dihydrogren phosphate Buffer/ Acetonitrile (45:55)**	**C-18**	**247nm**	**1mL/min**
20	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Phosphate buffer/ methanol (85:15)**	**C-18**	**222nm**	**1 mL/min**
21	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Potassium phosphate /Acetonitrile (70:30)**	**C-18**	**275nm**	**1mL/min**
22	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Methanol/Water (75:25)**	**C-18**	**270 nm**	**0.9mL/min**
23	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Methanol/ortho phosphoric acid (60:40)**	**C-18**	**220 nm**	**1 mL/min**
24	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Acetonitrile: Water (60:40)**	**C-18**	**248nm**	**1 mL/min**
25	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Acetonitrile/ortho phosphoric acid (50:50)**	**C-18**	**210nm**	**0.98mL/min**
26	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Ammonium dihydrogen phosphate Buffer/methanol (65:35)**	**C-18**	**280nm**	**1.5mL/min**
27	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Phosphate buffer/acetonitrile (40:60)**	**C-18**	**260nm**	**1mL/min**
28	**Saxagliptin/ Dapagliflozin**	**RP-HPLC**	**Ortho phosphoric acid/Acetonitrile (50:50)**	**C-18**	**254nm**	**1mL/min**

Table 4: Summary of Spectrophotometric Methods of Saxagliptin

S. No.	Drugs	Techniques	Solvent	wavelength	Spectrophotometer
1	**Saxagliptin**	**UV-Visble**	**Methanol**	**208nm**	**Double Beam**
2	**Saxagliptin**	**UV-Visble**	**Acetonitrile**	**212nm**	**Double Beam**
3	**Saxagliptin**	**UV-Visble**	**Methanol**	**211nm**	**Double Beam**
4	**Saxagliptin/Metformin**	**UV-Visble**	**Distilled Water**	**274nm/231nm**	**Double Beam**
5	**Saxagliptin/Metformin**	**UV-Visble**	**Distilled Water**	**274nm/231nm**	**Double Beam**
6	**Saxagliptin/Dapagliflozin**	**UV-Visble**	**Distilled Water**	**223nm/212nm**	**Double Beam**

Table 5: Summary of HPTLC Methods of Saxagliptin

S. No.	Drugs	Techniques	Stationary Phase	Mobile Phase	Wavelength
1	**Saxagliptin**	**HPTLC**	**Silica gel aluminium plate 60 F_{254} (10X10)**	**Methanol:Chloroform (6:4)**	**222nm**
2	**Saxagliptin**	**HPTLC**	**Silica gel aluminium plate 60 F254 (10X10)**	**1%Methanolic ammonium acetate:Toluene (5:5)**	**215nm**
3	**Saxagliptin**	**HPTLC**	**Silica gel aluminium plate 60 F254 (10X10)**	**Toluene:Methanol: Ammonia (6:4:0.2)**	**222nm**

Table 6: Summary of LC/MS Method of Saxagliptin

S. No.	Drug	Technique	Mobile phase	Wavelength	Flow rate
1	**Saxagliptin**	**LC/MS**	**Potassium dihydrogen phosphate/acetonitrile/methanol (40:30:30)**	**208nm**	**1mL/min**

CONCLUSION:

Saxagliptin, (DPP-4) inhibitor used in diabetes has many HPLC and Spectrophotometric methods as individually as well as in combination those mentioned in **Table 3-6**. Dihydrogen phosphate buffer and other reagents mostly used in analysis that applicable to industries. These novel analytical techniques are used for quantify and defining Saxagliptin and that is safe for the chemist analyst and the community. There is much need of defining the impurities using LC/MS. Ultra performance liquid chromatography method not develop till now. So there must me develop method for the same.

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