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Review Article

A REVIEW ON ANALYTICAL METHODS FOR ESTIMATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN IN BULK AND IN PHARMACEUTCAL DOSAGE FORMS

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ABSTRACT

Dapagliflozin and Saxagliptin are very effectively used treatment for type II diabetes. They are very potent inhibitors of renal glucose reabsorption and dipeptidyl peptidase protein 4(DPP-4) and sodium glucose transport protein 2 and also they are called as DPP4 & SGLT2 inhibitors. They are generally administered as tablets. Determination of Dapagliflozin and Saxagliptin in pharmaceutical dosage form and bulk form, several analytical methods including UV, HPLC, LC-MS and HPTLC techniques has been developed. Methods indicating human plasma stability and impurity profiling are also described for both drugs. For qualitative and quantitative estimation of Dapagliflozin and Saxagliptin, these analytical methods can be used and it can also be used for its related degradants in bulk formulations and biological fluids. The following study depicts the review on analytical methods which includes estimating the antidiabetic drugs.

Keywords: Dapagliflozin, Saxagliptin, UV-Spectroscopy, RP-HPLC, LC-MS and Gas Chromatography,

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic progressive metabolic disorder characterized by absolute or relative insulin deficiency[1]. Expected rise in prevalence of diabetes is mainly due to increased life span because of better healthcare facilities and increase in diabetic risk factors, especially physical inactivity and obesity due to sedentary life style. Pancreatic β-cell function is gradually deteriorated in patients of Type II DM which is reflected into inadequate glycemic control on a long run[2].

Dapagliflozin is chemically known as (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol. It has a molecular formula of $C_{24}H_{33}ClO_8$ with molecular weight 408.98 g/mol[3]. Dapagliflozin is selective Sodium Glucose Co Transporter 2 inhibitor (SGLT 2). It acts by reducing the re absorption of glucose by the kidney, leading to excretion of excess glucose in the urine, thereby improving glycemic control in patients with type 2 diabetes mellitus[4]. Saxagliptin is chemically known as (1S, 3 S, 5S)-2[(2S)-2-amino-2-(3-hydroxy-1- adamantyl) acetyl]-2 azabicyclo hexane-3-carbonitrile) with molecular formula of $C_{18}H_{25}N_3O_2$ and molecular weight of 315.41 g/mol[5]. Saxagliptin is a selective and potent dipeptidyl peptidase (DPP) - 4 inhibitor, approved as an adjunct to diet and exercise to improve glycemic control in type 2 diabetes mellitus (T2DM). In patients with T2DM, once-daily administration of Saxagliptin before breakfast achieves sustained inhibition of plasma DPP-4 activity and reduction of postprandial hyperglycemia, including after dinner, associated with an increase in plasma glucagon-like peptide-1 levels[6-8].

Combination of Dapagliflozin and Saxagliptin is marketed as a Tablet (Qtern) containing 10 mg of Dapagliflozin, 5 mg of Saxagliptin. Combination of these two drugs is indicated for the treatment of type-2 Diabetes. Using Dapagliflozin leads to heavy glycosuria (glucose excretion in the urine), which can lead to weight loss and tiredness.

Reported methods are categorized depending on the following considerations:

1. Methods for determination of Dapagliflozin Single and combination with other drugs by UV-Spectroscopy and Chromatography and other techniques.
2. Methods for determination of Saxagliptin Single and combination with other drugs by UV-Spectroscopy and Chromatography and other techniques.

Table1: Methods for determination of Dapagliflozin Single and combination with other drugs by UV Spectroscopy, Chromatography and other techniques

S.No	DRUGS	METHOD	DESCRIPTION	REF .NO
1	Dapagliflozin in tablet formulation	UV Spectrophotometric Method	**Detection wavelength :** 224 nm **Mobile phase :** Methanol :Water **Linearity range :**5-40 µg/ml **Correlation coefficient :**< 1	9
2	Dapagliflozin in bulk and pharmaceutical dosage forms	UV Spectrophotometric Method	**Detection wavelength:**233.65nm **Mobile Phase:** Ethanol: Phosphate buffer (1:1) **Linearity range:**10-35 µg/ml **Correlation coefficient :**0.9998 **% Recovery:** 99.7 **LOD:**1.24 µg/ml **LOQ:**3.62 µg/ml	10
3	First derivative for simultaneous estimation of Dapagliflozin and Metformin HCL in synthetic mixture	UV Spectrophotometric Method	**Wavelength :** Dapagliflozin-235 nm Metformin HCl-272 nm **Solvent :** Methanol **Linearity range :** Dapagliflozin-0.5-2.5 µg/ml Metformin-25-125 µg/ml **Correlation co-efficient :** Dapagliflozin-0.980 Metformin HCl-0.982 **LOD :** Dapagliflozin-0.009 µg/ml Metformin HCl-0.013 µg/ml **LOQ :** Dapagliflozin-0.039 µg/ml Metformin HCl-0.041 µg/ml	11
4	Dapagliflozin API	UV Spectrophotometric Method	**Wavelength :** 237nm **Solvent :** Ethanol **Linearity range :**0.5-0.9 µg/ml **Correlation coefficient :** 0.994	12
5	Dapagliflozin in API.	RP-HPLC and UV-Spectroscopy.	**Wavelength :**203 nm **Mobile phase :** Acetonitrile: Ortho phosphoric acid (55:45%) **Linearity range :** In HPLC -25-150 µg/ml In UV-1-5 µg/ml **Correlation co-efficient:**0.999 **LOD :** 0.01µg/ml **LOQ** : 0.05µg/ml	13
6	Metformin and Dapagliflozin in bulk and synthetic mixture	RP-HPLC method	**Wavelength :** 285 nm **Mobile phase :** Acetonitrile: Water (75:25% v/v) **Flow rate :**1ml/min **Injection volume :**10µl **Retention time :** Metformin-3.2 min Dapagliflozin-5.4 min **Linearity range :** Metformin-20-100µg/ml Dapagliflozin-10-50µg/ml **% Recovery** : 99.3-99.6% **LOD :** Metformin-5.0µg/ml Dapagliflozin-3.7µg/ml **LOQ :** Metformin-15.2µg/ml Dapagliflozin -11.42µg/ml	14

7	Dapagliflozin in bulk and tablet dosage form.	RP-HPLC method	**Wavelength :** 237 nm **Mobile phase :** Phosphate buffer : acetonitrile (75:25% v/v) **Flow rate :** 1.0 ml min-1 **Retention time** : 3.461min **Linearity range** : 10-60 µg/ml **LOD** : 0.02 µg/ml **LOQ** : 0.06 µg/ml	15
8	Metformin Hydrochloride and Dapagliflozin in tablet dosage form.	RP-HPLC method	**Wavelength :**240nm **Mobile phase :** Phosphate Buffer (pH 6.5): Methanol: Acetonitrile in the ratio of 50:30:20 v/v/v **Flow rate :**1 ml/min **Retention time :** Metformin HCL-2.475 min Dapagliflozin-3.647 min **Linearity range :** Metformin HCL 85-510µg/ml Dapagliflozin 0.5–3µg/ml **LOD :** Metformin HCL- 2.469 ppm Dapagliflozin-3.650 ppm **LOQ :** Metformin HCl-2.468 ppm Dapagliflozin-3.649 ppm **Correlation co-efficient :** Metformin HCl-0.997 Dapagliflozin-0.9973 **% Recovery :** Metformin HCl-100.67% Dapagliflozin-99.54%	16
9	Metformin and Dapagliflozin in pharmaceutical dosage forms	RP-HPLC method	**Wavelength :** 240 nm **Mobile phase :** Acetonitrile: phosphate buffer (70:30 %v/v) **Flow rate :** 1 ml/min **Retention time :** Metformin-2.463min Dapagliflozin-3.760 min **Linearity range :** Metformin-50-250 µg/ml Dapagliflozin -5-25 µg/ml **% Recovery :** Metformin -97.0-98.0 % Dapagliflozin-100-103 %	17
10	Dapagliflozin in API	RP-HPLC method	**Wavelength :**245nm **Mobile phase :** Ortho phosphoric acid : Acetonitrile (45:55 v/v) **Flow rate :** 1 ml/min **Retention time :** 2.963 min **Linearity range** : 25-150µg/ml **Correlation co-efficient :**0.999 **LOD :** 0.6µg/ml **LOQ**: 1.8µg/ml **% Recovery :** 99.8%	18
11	Dapagliflozin in Bulk and Table Formulation	RP-HPLC Method	**Wavelength :** 210 nm **Mobile phase :** 0.1% Ortho phosphoric acid buffer : Acetonitrile (60:40 % v/v) **Flow rate :** 1 ml/min **Injection volume :** 10 µL **Runtime:** 5min **Retention time :** 2.226 min **Linearity range** : 25 – 150 µg/ml **% Recovery** : 98.95 – 101.72 % **%RSD intraday precision :** 0.6% **%RSD inter day precision :** 0.4%	19
12	Dapagliflozin and Saxagliptin in fixed-dose combination.	RP-HPLC method	**Wavelength :** 230 nm **Mobile phase :** Sodium dihydrogen phosphate: Acetonitrile (53 : 47 v/v) **Flow rate :** 1.2 mL / min **Linearity range** : 2–14 µg/ mL	20

			% Recovery : Dapagliflozin -99.16% Saxagliptin -100.58% **correlation coefficients :** Dapagliflozin -0.997 Saxagliptin - 0.996	
13	Saxagliptin Hydrochloride and Dapagliflozin in bulk and in tablet form	Stability indicating RP-HPLC method	**Wavelength :**220 nm **Mobile phase :** Potassium dihydrogen phosphate Buffer (pH 6.0) : Acetonitrile (45:55 v/v) **Linearity range :** Saxagliptin HCl 56-84 µg/ml Dapagliflozin 112-168 µg/ml	21
14	Saxagliptin and Dapagliflozin in bulk and dosage forms	Stability indicating RP-HPLC method	**Wavelength :**225 nm **Mobile phase :** Phosphate Buffer : Acetonitrile (50:50 v/v) **Flow rate :**1.2 mL/ min **Linearity range :** Saxagliptin 20-60 µg/ml Dapagliflozin 40-120 µg/ml **Retention time :** Saxagliptin 2.1 min Dapagliflozin 2.8 min **Accuracy range :** 99.99-100.50 % **Precision :** Saxagliptin 0.78 % Dapagliflozin 0.44% **LOD :** Saxagliptin 1.63 µg/ml Dapagliflozin 1.94 µg/ml **LOQ :** Saxagliptin 5.39 µg/ml Dapagliflozin6.50 µg/ml **% Assay :**100.24-100.43 %	22
15	Dapagliflozin and Saxagliptin in combined tablet dosage forms.	Stability indicating RP-HPLC method	**Wavelength :** 220 nm **Mobile phase :** Acetonitrile:0.1%Orthophosphoric acid in water(50:50v/v) **Flow rate :** 1.0 mL/ min	23
16	Metformin HCL and Dapagliflozin in bulk drug and tablet dosage form. Asian J Pharm Clin Res 2008; 8(3): 320-326.	Stability indicating High –Performance Liquid Chromatographic method	**Wavelength :** 240 nm **Mobile phase :** Buffer (0.1% Orthophosporic acid) adjusted to pH 6.8 with Triethylamine: Acetonitrile in the ratio of 50:50% /v/v **Flow rate:** 1.0 mL/ min. **Linearity range :** Metformin 85 - 510 µg/ml Dapagliflozin 0.5-3.0 µg/ml **Retention time :** Metformin 2.791 min Dapagliflozin 3.789 min	24

Table 2: Methods for determination of Saxagliptin single and combination with other drugs by UV-Visible Spectroscopy, Chromatography and other techniques

S.No	DRUGS	METHOD	DESCRIPTION	REF .NO
1	Saxagliptin Hydrochloride and Metformin Hydrochloride in API	UV-VIS Spectroscopy method	**Wavelength :** Saxagliptin HCL 274 nm Metformin HCL 231 nm **Linearity range :** Saxagliptin HCL50-90 µg/ml Metformin HCL2-10 µg/ml **Correlation co-efficient :**0.990 **% Recovery :** Saxagliptin HCL 100.1% Metformin HCL 99.98%	25
2	Saxagliptin in Hydrochloride in Bulk and tablet Dosage Form	RP-HPLC method	**Wavelength :** 210 nm **Mobile phase:** Phosphate buffer: Acetonitrile (80:20 v/v) **Flow rate :**1ml/min **Retention time** : 5.43±0.03 min **Linearity range :** 0.10–0.30 mg/ml **Correlation co-efficient :** 0.999 **LOD :**9 µg/ml **LOQ**:27 µg/ml **% Recovery :**100.28% **%RSD for repeatability :** 0.630% **%RSD for intermediate precision:**0.529% **Accuracy:**99.96 %	26
3	Metformin and Saxagliptin in API	RP-HPLC method	**Wavelength** :220 nm **Mobile phase:** 0.05M KH_2PO_4 buffer(pH4.5):Methanol: Acetonitrile (60:20:20%v/v) **Flow rate:**0.6 mL/min **Run time:**10 min **Retention time:** Metformin-4.38 min Saxagliptin-6.92 min **Injection volume:**10µl **LOD:** Metformin-0.112 µg/ml Saxagliptin-0.029 µg/ml **LOQ -:** Metformin-0.373 µg/ml Saxagliptin -0.096 µg/ml	27
4	Metformin and Saxagliptin in Pharmaceutical Dosage Form	RP-HPLC method	**Wavelength :**208 nm **Mobile phase:** Buffer: Methanol (55:45 v/v) **Flow rate :** 1 ml/min **Linearity range:** Metformin60–100 µg/ml Saxagliptin 0.6–1.0 µg/ml **LOD :** Metformin-0.17 µg/ml Saxagliptin-0.064 µg/ml **LOQ :** Metformin-0.08 µg/ml Saxagliptin -0.02 µg/ml **Correlation co-efficient :** 0.999 **Accuracy:** Metformin-101.07% Saxagliptin-101.25%	28
5	Saxagliptin and Methyldopa in a laboratory mixture	Analytical method	**Wavelength** : 211-280 nm **Linearity range :** Saxagliptin-5-30 µg/ml Methyldopa -2-12 µg/ml **% Recovery :** 98-101%	29
6	Saxagliptin in Tablet Dosage Form.	RP-HPLC method	**Wavelength** : 220 nm **Mobile phase :** Acetonitrile : Potassium Di-hydrogen Phosphate Buffer **Flow rate :** 1 ml/min	30

			Retention time : 3.487 min **Linearity range** : 50-150 µg/ml **Correlation co-efficient :** 0.9999	
7	Saxagliptin and Metformin in Bulk and Pharmaceutical Dosage Form,	Stability indicating RP-HPLC method	**Wavelength :** 242 nm **Mobile phase :** 50mM sodium dihydrogen phosphate buffer : methanol (80:20 v/v) **Flow rate :** 0.9 ml/min **Linearity range:** Saxagliptin 0.5-3 µg/mL Metformin 50-300 µg/mL **% Recovery**: Saxagliptin 98.39-101.53% Metformin 100.46 -101.59%	31
8	Saxagliptin in Pharmaceutical Dosage form	Stability indicating RP-HPLC method	**Wavelength** : 225 nm **Mobile phase :** 0.1% phosphoric acid : methanol (70: 30, v/v) **Flow rate :** 1 m/ min **Linearity rang:**15.0 - 100.0 µg / ml **Correlation coefficients :**> 0.999 **Precision :**RSD <1.49% **% Accuracy**: 99.42- 101.59%.	32
9	Metformin hydrochloride and Saxagliptin in bulk and combined tablet dosage form	Stability indicating Quantitative RP-HPLC method	**Wavelength** : 211 nm **Mobile phase :** Phosphate buffer : Acetonitrile : Methanol (25:50:25 % v/v/v) **Flow rate :** 1.0 ml/min **Linearity range :** Metformin HCL125-750 µg/ml Saxagliptin 1.25- 7.5 µg/ml **Retention time :** Metformin 2.246 min Saxagliptin 4.516 min **Correlation coefficients :** 0.999 **% Recovery**: Metformin HCl 99.62-99.93% Saxagliptin 99.66-99.80%	33
10	Saxagliptin and its Forced Degradation Impurities in Bulk Drug and Pharmaceutical Dosage form	Stability Indicating RP-HPLC method	**Wavelength** : 210 nm **Mobile phase :** Sodium dihydrogen phosphate : Acetonitrile **Flow rate :** 1.2 ml/min **Linearity range** : 50-375 µg/ml **Retention time :**7.68 min **Correlation coefficients :** 0.999	34
11	Saxagliptin in Tablet Formulations	Stability Indicating RP-HPLC method	**Wavelength** :230 nm **Mobile phase :** Phosphate buffer: methanol (65:35) **Flow rate :** 1mL/min **Linearity range** : 1 to 10 µg / ml **Retention time :** 5.12 min **Correlation coefficients :** 0.9899 **LOD :** 0.10 µg/ml **LOQ :** 0.28 µg/ml **Intraday precision** : 0.22 – 0.71% **Inter day precision :** 0.28 – 0.76 % **% Recovery** : 99.90 – 101.03%	35
12	TEMPO in Saxagliptin monohydrate drug substance	GC method	**Linearity range :** 6µg/g-450 µg/g **Correlation coefficients :** 0.995 **LOD**: 2 µg /g **LOQ**: 6 µg /g **% Recovery** : 89%	36
13	Saxagliptin levels and its pharmacokinetic application in presence of sucralose in animal's serum	HPLC method	**Wavelength :** 230 nm **Mobile phase :** Phosphate buffer : Methanol (70:30 v/v) **Flow rate :** 1mL/min **Injection volume**: 50 µl	37

			Intra-day precision : CV % values range (0.14-4.03) **Accuracy % range :** 99.5-104 **Inter-day precision**: CV % range (0.15-2.81) **Accuracy % range**: 99.9-116 **Run time :** 10 min **Correlation coefficients :** 0.999	

CONCLUSION

This review depicts the reported Spectroscopic and Chromatographic methods developed and validated for estimation of Dapagliflozin and Saxagliptin. According to this review it was concluded that for Dapagliflozin and Saxagliptin different Spectroscopic and Chromatographic methods are available for single and combination also it was found that the mobile phase containing Acetonitrile, water, and Phosphate buffer were common for most of the chromatographic method to provide more resolution. It was observed that most common combination of Dapagliflozin and Saxagliptin were with Metformin. For chromatographic method flow rate is observed in the range 1.0-1.5 ml/min to get good resolution time. For most of the Spectroscopic methods common solvent is Methanol. Hence this all methods found to be simple, accurate, economic, precise and reproducible in nature. Most of Methods were of RP-HPLC and UV absorbance detection because these methods provided with best available reliability, repeatability, analysis time and sensitivity.

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