

# Method development and validation on antidiabetic drug in its pharmaceutical dosage form by using HPLC (High performance liquid chromatography)

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**Abstract** The paper describes the invention of a simple, precise, and sensitive approach for detecting saxagliptin in bulk medication and marketed formulations using reverse-phase liquid chromatography. The separation was done on Epic C12 (250 mm×4.6 mm×5 μm) using mobile phase (Methanol: buffer) in the ratio of 80:20 (v/v). The run time was 7 minutes, and the wavelength for saxagliptin was taken as 230 nm. A literature review finds that there are very few HPLC techniques available using this composition of mobile phase. As a result, an attempt was undertaken to create an RP-HPLC technique for saxagliptin.

The devised method was validated in terms of accuracy, precision, linearity, system suitability, LOD and LOQ, robustness, and assay. The linearity ranged from 1-3 μg/ml, with a correlation value of 0.999. The designed and validated RP-HPLC method is used to identify the eluted.

**Index Terms**— Saxagliptin, Antidiabetic, HPLC, Method Development, Validation.

## I. INTRODUCTION

Saxagliptin is used as a monotherapy or in combination with other medications to treat type 2 diabetes. It doesn't appear to reduce the risk of heart attacks or strokes.[1] One study found a 3.5% probability of hospitalization for heart failure compared to 2.8% in a placebo-controlled group. It, like other DPP-4 inhibitors, has a small capacity to reduce HbA1c, is associated with a minimal risk of hypoglycemia, and does not promote weight gain.[1,2]

Brand name: Onglyza, Saxagliptin hydrate  
Kombiglyze, Komboglyze, Qtern, Qternmet.

Generic name: Saxagliptin

Drug class: DPP-4 inhibitors

Metabolism: Liver (CYP3A4 and CYP3A5)

IUPAC name: (1*S*,3*S*,5*S*)-2-[(2*S*)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile.

Formula: C<sub>18</sub>H<sub>25</sub>N<sub>3</sub>O<sub>2</sub>

Molar mass: 315.417 g·mol<sup>-1</sup>

Routes of administration: By mouth (tablet)

Background: Saxagliptin (rINN) is an orally active hypoglycemic (antidiabetic) medication from the new class of DPP-4 inhibitors. The FDA authorized on July 31, 2009.

Type: Small Molecule

Pharmacodynamic: Following Saxagliptin administration, GLP-1 and GIP levels increase by two to threefold. There are less systemic side effects since it inhibits DPP-4 with high selectivity. Saxagliptin suppresses DPP-4 enzyme activity for 24 hours. It also reduced glucagon levels and stimulated glucose-dependent insulin production from pancreatic beta cells. The IC<sub>50</sub> (half maximum inhibitory concentration) is 0.5 nmol/L. Saxagliptin did not increase the QTc interval to a clinically meaningful level.

Absorption: After a single oral administration of 5 mg of Saxagliptin in healthy participants, the mean plasma AUC values for Saxagliptin and its active metabolite were 78 and 214 ng·h/mL, respectively. The matching plasma C<sub>max</sub> values were 24 and 47 ng/mL, respectively. Saxagliptin did not accumulate after repeated dosages. The median time to maximum concentration (T<sub>max</sub>) after a 5 mg once-daily dosing was 2 hours for Saxagliptin and 4 hours for its active metabolite. Bioavailability, 2.5 - 50 mg dose: 67%.

Metabolism: Saxagliptin is predominantly metabolized by cytochrome P450 3A4/5 (CYP3A4). 50% of the absorbed dosage will be metabolized in

the liver. The primary metabolite of Saxagliptin, 5-hydroxy Saxagliptin, is a DPP4 inhibitor that is one-half as effective as Saxagliptin.

Mechanism of action: Saxagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor used to treat type 2 diabetes. DPP-4 inhibitors are a type of drug that affects the activity of the body's endogenous hormones known as incretins. Incretins lower blood sugar by boosting the body's sugar consumption, mostly through increased insulin synthesis in the pancreas, and lowering sugar production in the liver. [Bristol-Myers Squibb Press Release]. DPP-4 is a membrane-associated peptidase present in various organs, including lymphocytes and plasma. DPP-4 has two basic methods of action: an enzymatic activity and a mechanism in which it binds to adenosine deaminase, which, when active, transmits intracellular signals via dimerization. Saxagliptin establishes a reversible, histidine-assisted covalent link between the nitrile group and the S630 hydroxyl.

Route of elimination: Saxagliptin is removed through both the renal and hepatic routes. After receiving a single 50 mg dose of <sup>14</sup>C-Saxagliptin, 24%, 36%, and 75% of the dose was eliminated in urine as Saxagliptin, its active metabolite, and total radioactivity, respectively. A total of 22% of the given radioactivity was recovered in feces, indicating the fraction of the Saxagliptin dose discharged in bile and/or unabsorbed from the gastrointestinal tract.

## II MATERIALS AND EQUIPMENTS

Materials:

The drugs, chemicals, reagents, instruments, and filters used during the experiment.

Table No: 01 Active Pharmaceutical Ingredients

| Drug        | Manufacturer/ supplier   |
|-------------|--|
| Saxagliptin | Astrazeneca Pharmaceutical LP, 4601 Highway, 62 East INDIANA-47620 USA |

Solvents And Chemicals:

- ✓ Methanol (gradient grade)
- ✓ Ammonium Dihydrogen orthophosphate
- ✓ Acetonitrile (Gradient Grade)

- ✓ Sodium Acetate
- ✓ Ammonium Formate
- ✓ Water (HPLC grade)

Instruments Used:

Table No: 02 Instruments Used in Method Development

| Name of instrument   | Model   |
|----------------------|---|
| HPLC System          | Younglin-HPLC system                                |
| Detector System      | Detector – UV detector (730D)                       |
| Analytical Column    | Epic C12 (4.6 × 250mm, 5µm)                         |
| Software             | Autochrom 3000                                      |
| Ph Meter             | M Lab   |
| Injector             | Manual  |
| Analytical Balance   | Shimadzu Model-ATX224                               |
| UV Spectrophotometer | Shimadzu UV1800 Spectrophotometer Japan Corporation |

Table No: 03 Optimization of Chromatographic Conditions

|                      |                             |
|----------------------|-----------------------------|
| HPLC system          | Younglin HPLC System        |
| Column               | Epic C12 (4.6 × 250mm, 5µm) |
| Pump                 | Pump – SP930 D              |
| Mobile phase         | MeOH: Buffer (80:20)        |
| Detection wavelength | 230 nm                      |
| Flow rate            | 1.0 ml per minute.          |
| Temperature          | Ambient                     |
| Injection volume     | 20 µl                       |
| Run time             | 7 minutes                   |

Sample preparation:

The method of sample preparation is a crucial consideration for biological samples. For the current investigation, the protein precipitation method was chosen for sample preparation.

## III PREPARATION OF STANDARD STOCK SOLUTION

Weighed precisely 30 mg of saxagliptine std and transferred to a 100 ml volumetric flask, dissolved and diluted to the mark with diluent, shake well, and sonicate for 5 minutes.

Standard stock solution:

Pipette 2ml from the stock solution into a 20ml volumetric flask, dilute with diluent, shake well, sonicate for 2 minutes, then filter the solution with a 0.2µm syringe filter.

Preparation of Mobile Phase:

Various mobile phase combinations were used on a trial-and-error basis. The appropriate mobile phase

solvent used was Methanol (80): Buffer (20)

Selection of Wavelength for Saxagliptin:

After baseline correction, the UV spectrophotometer scanned with 10 µg/mL working standard solution between 400 to 200 nm against methanol as a blank. The UV-analyst software displayed a maximum wavelength of 230nm.

Marketed Tablet Test Preparation:

$$\% \text{ Accuracy} = \frac{\text{Mean measured concentration}}{\text{Nominal Concentration}} \times 100$$

Weighed 10 tablets separately, crush all tablets in mortar and pestle. Weighed tablet powder (API) equivalent to standard concentration and dissolve to 100 ml with the help of diluent and shake well. Sonicate for 2 mins, filter through 0.2 µm membrane syringe filter.

1. HPLC Method Optimization:

For method optimization various mobile phases were tried in different ratios, such as

- 1) Solvent A- Ammonium dihydrogen orthophosphate (50%); Solvent B- Water (50%)
- 2) Solvent A- ammonium formate (55%); Solvent B- Buffer (45%)
- 3) Solvent A- Methanol (65%); Solvent B- Buffer (35%)
- 4) Solvent A- Methanol (65%); Solvent B- Water (25%)
- 5) Solvent A- Methanol (80%); Solvent B- Buffer (20%)

2. All these mobile phases were unacceptable due to tailing, fronting and no sharpness in the peak. After various trials mobile phase consisting of Methanol: Buffer in ratio (80:20) was selected which gave sharp peaks with no tailing and fronting. The chromatogram of standard Saxagliptin was shown in Fig 01.

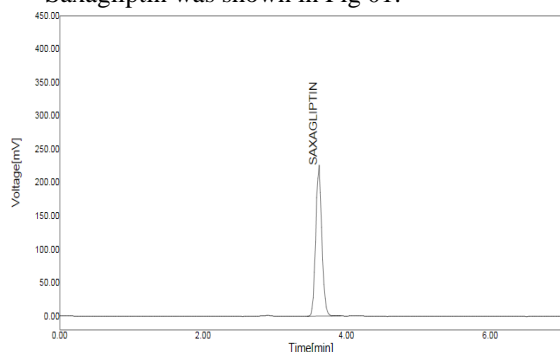


Fig. No: 01 Chromatogram of Final Trail.

IV RESULT AND DISCUSSION

ACCURACY:

The concentrations used were 80, 100, and 120% to analyze the recovery studies using the standard method. The procedure involved combining 0.8, 1.0, and 1.2 mL of standard solution with 0.2 mL of tab solution having 10 µg/mL concentration. The % accuracy was determined by using the following formula:

Table No: 04 – Saxagliptin Accuracy Data.

|                   | MEAN% recovery | SD     | %RSD (NMT 2) |
|-------------------|----------------|--------|--------------|
| Accuracy at 80 %  | 100.76         | 0.6169 | 0.61         |
| Accuracy at 100 % | 100.67         | 0.9561 | 0.95         |
| Accuracy at 120 % | 100.91         | 1.0676 | 1.06         |

The mean % recovery of 100.76 to 100.91 was observed and within %RSD between 0.61 to 1.06. All the obtained results were within the range of acceptable limits. [3]

PRECISION:

The system precision was demonstrated by preparing the standard solution at test concentration and injected repeatedly six times. [4]

Acceptance Criteria:

%RSD of assay results should be NMT 2.0%. Assay should be in the range of test method. [8]

Precision studies were carried out by injecting six replicate injections of the standard drug mixture on one day. This process is called intraday precision. The results were calculated in terms of %RSD.

Table No: 05- Intraday Precision

| Name            | Preparation | % ASSAY |
|-----------------|-------------|---------|
| Set-1           | prep-01     | 98.77   |
|                 | prep-02     | 99.45   |
| Set-2           | prep-01     | 98.18   |
|                 | prep-02     | 98.39   |
| Mean            |             | 98.95   |
| SD              |             | 0.7808  |
| % RSD (NMT 2.0) |             | 0.79    |

Precision studies were also carried out by injecting six replicate injections of the standard drug mixture on six different days. This process is called interday precision. The results were calculated in terms of % RSD. [9]

|              |           |        |
|--------------|-----------|--------|
| Ph 2.8       | Test prep | 98.14  |
| Ph 3.2       | Test prep | 98.91  |
| Mean         |           | 98.89  |
| SD           |           | 0.6863 |
| %RSD (NMT 2) |           | 0.69   |

Table No: 06- Interday Precision

|                 |  |        |
|-----------------|--|--------|
| Mean            |  | 100.59 |
| SD              |  | 1.1420 |
| % RSD (NMT 2.0) |  | 1.14   |

**LINEARITY:**

Table No: 07 Linearity study of Saxagliptin

| Level | Con. (ppm or µg/ml) | Area      |
|-------|---------------------|-----------|
| 1     | 15.00               | 640.5532  |
| 2     | 22.50               | 1013.5779 |
| 3     | 30.00               | 1334.7620 |
| 4     | 37.50               | 1650.7721 |
| 5     | 45.00               | 1987.4371 |

**SYSTEM SUITABILITY:**

System suitability tests were performed using saxagliptin standard and test solutions to check for compliance with specified parameters [10].

Table No: 08- System Suitability Parameters

| Name            | Area      | RT (min) | TP (NLT 2000) | TF (NMT 2.0) |
|-----------------|-----------|----------|---------------|--------------|
| Standard_Inj_01 | 1351.9138 | 3.600    | 15060         | 1.15         |
| Standard_Inj_02 | 1328.6306 | 3.583    | 14925         | 1.15         |
| Standard_Inj_03 | 1337.6561 | 3.600    | 15058         | 0.99         |
| Standard_Inj_04 | 1322.3206 | 3.600    | 10166         | 1.04         |
| Standard_Inj_05 | 1292.8695 | 3.617    | 15172         | 1.02         |
| Mean            | 1326.6781 | 3.600    |               |              |
| SD              | 21.9270   | 0.0120   |               |              |
| %RSD (NMT 2.0)  | 1.65      | 0.33     |               |              |

The plate count and tailing factor results were found to be within the limits.

**LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ):**

**LIMIT OF DETECTION (LOD):**

The limit of detection is the lowest concentration of an analyte that can be detected in a sample but not necessarily quantitated, under the given experimental conditions.

**LIMIT OF QUANTIFICATION (LOQ):**

It is the lowest concentration of analyte in a sample that can be accurately and precisely identified under the given experimental conditions.

LOD and LOQ were determined using the following formulas.  $LOD = 3.3 \times (SD)/S$

$$LOQ = 10 \times (SD)/S$$

Where, SD = Standard deviation

S= slope

% Recovery [11]

Table No: 09 LOD and LOQ Data

| Name  | Preparation                 | % ASSAY   |
|-------|-----------------------------|-----------|
| Day-1 | prep-01                     | 99.77     |
|       | prep-02                     | 99.45     |
| Day-2 | prep-01                     | 101.47    |
|       | prep-02                     | 101.67    |
| Level | Con. (ppm or µg/ml)         | Area      |
| 1     | 15.00                       | 640.5532  |
| 2     | 22.50                       | 1013.5779 |
| 3     | 30.00                       | 1334.7620 |
| 4     | 37.50                       | 1650.7721 |
| 5     | 45.00                       | 1987.4371 |
|       | Correlation coefficient (r) | 0.9996    |
|       | STEYX                       | 17.9364   |
|       | SLOPE                       | 44.413    |
|       | LOD (µg/ml)                 | 1.33      |
|       | LOQ (µg/ml)                 | 4.04      |

LOD and LOQ observed 1.33 µg/ml and 4.04 µg/ml respectively.

**ROBUSTNESS:**

For the parameters like Flow rate, wavelength and the chosen solution was used for a robustness assessment.

Table No: 10 Robustness

| Name                                    | Preparations | % Assay |
|---|--------------|---------|
| Robustness changes in method parameters |              |         |
| Original method parameters              | Test prep 1  | 99.77   |
| Original method parameters              | Test prep 2  | 99.45   |
| Flow rate 1.08 ml/min                   | Test prep    | 98.47   |
| Flow rate 1.32 ml/min                   | Test prep    | 98.38   |
| Wavelength 228 nm                       | Test prep    | 99.77   |
| Wavelength 232 nm                       | Test prep    | 98.23   |

Robustness examines the effect of operational parameters on the analytical method. [12]

**ASSAY:**

Assay % =

$$\frac{AT \times WS \times DT \times P}{S \times WT \times 100} \times 100$$

Where:

AT = Peak Area of medication acquired with test readiness

AS = Peak Area of medication acquired with standard readiness

WS = Weight of working standard taken in mg

WT = Weight of test taken in mg

DS = Dilution of Standard arrangement

DT = Dilution of test arrangement

P = Percentage virtue of working standard

Table No:11- Preparation of Standard Solution for saxagliptin

| Test Preparation | Saxagliptin Wt. of test (mg) | Diluted to (ml) | ml taken | Diluted to (ml) |
|------------------|------------------------------|-----------------|----------|-----------------|
| Preparation-1    | 30.1                         | 100             | 2        | 20              |
| Preparation-2    | 30.1                         | 100             | 2        | 20              |

Table No:12- Marketed Preparation

| Name             | Area      | RT (min) | % ASSAY |
|------------------|-----------|----------|---------|
| Test solutions 1 | 1302.2242 | 3.633    | 97.49   |
| Test solutions 2 | 1338.3309 | 3.633    | 98.83   |

## CONCLUSION

The method has been demonstrated to be specific for determining the percentage assay of saxagliptin in saxagliptin Film Coated Tablet 5 mg. A quick, user-friendly, and precise method for determining saxagliptin in pharmaceutical dose form was devised and validated. The linearity, accuracy, precision, LOD, LOQ, robustness, and percentage recovery were all within the parameters indicated by the ICH recommendations. This approach demonstrated remarkable sensitivity and quickness. This approach is suitable for estimating saxagliptin.

## REFERENCE

- [1] Scirica, B.M., Bhatt, D.L., Braunwald, E., Steg, P.G., Davidson, J., Hirshberg, B., Ohman, P., Frederich, R., Wiviott, S.D., Hoffman, E.B. and Cavender, M.A., 2013. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *New England Journal of Medicine*, 369(14), pp.1317-1326.
- [2] Ali, S. and Fonseca, V., 2013. Saxagliptin overview: special focus on safety and adverse effects. *Expert opinion on drug safety*, 12(1),

pp.103-109.

- [3] Boulton, D.W., Goyal, A., Li, L., Korn-Hauser, D.M. and Frevert, U., 2008, June. The effects of age and gender on the single-dose pharmacokinetics and safety of saxagliptin in healthy subjects. In *Diabetes* (Vol. 57, pp. A164-A164). 1701 N BEAUREGARD ST, ALEXANDRIA, VA 22311-1717 USA: AMER DIABETES ASSOC.
- [4] Dave, D.J., 2011. Saxagliptin: A dipeptidyl peptidase-4 inhibitor in the treatment of type 2 diabetes mellitus. *Journal of Pharmacology and Therapeutics*, 2(4), pp.230-235.
- [5] Sarat, M., Krishna, P.M. and Rambabu, C., 2012. RP-HPLC method for estimation of saxagliptin and pioglitazone in tablets. *Int. Res. J. Pharm*, 3, pp.399-402.
- [6] Pharmacopoeia, I., 2010. Published by the Indian pharmacopoeia commission. *Ghaziabad*, 2, pp.1573-1574.