



DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF SAXAGLIPTIN IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Saxagliptin in tablet dosage form. **Methods:** The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Saxagliptin in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in analytical grade methanol, Sparingly soluble in water and slightly soluble in ethyl acetate. So, the analytical grade methanol is used as a diluent in method. The melting point of Saxagliptin was found to be 108-109°C (uncorrected). It showed absorption maxima 213 nm in analytical grade methanol. On the basis of absorption spectrum the working concentration was set on 50 µg/ml (PPM). The linearity was observed between 10 - 90 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 99.00 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.88% and for Ruggedness is 1.16%. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Saxagliptin in tablet dosage form. The method could be considered for the determination of Saxagliptin in quality control laboratories.

KEYWORDS: Saxagliptin, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Saxagliptin chemically is (1S, 3S, 5S,-)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclohexane-3-carbonitrile.^[1] Molecular formula is C₁₈H₂₅N₃O₂, H₂O and the molecular weight is 333.43. This is new oral hypoglycemic agent of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Saxagliptin (Fig. 1) was a dipeptidyl peptidase-4 (DPP-4) inhibitor approved by FDA in 2009 and launched its journey in China since 2011.^[2] It has been widely accepted because of low dosage and low risk of weight gain, also it was used as an adjunct to other anti-diabetic agents and lifestyle modifications to target glycemic control.^[3] Increasing insulin production in response to meals and gives the result of decreasing the amount of glucose that is liver produces. Saxagliptin is available in the form of tablets. Saxagliptin it is a white powder and freely soluble in

methanol, acetonitrile, acetone, polyethylene glycol, ethanol.^[4] It is worthwhile to note that saxagliptin was mainly metabolized by cytochrome P450 (CYP) 3A4 in human; in that way, whether cooked or wine processed rhubarb modified the activity of CYP3A4 would need to be specified if they were intended to combined use.^[5]

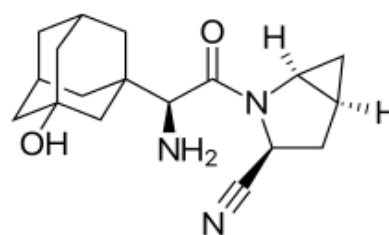


Fig. 1: Chemical structure of saxagliptin.

The available literature studies describe utilization of few analytical methods for the evaluation of Saxagliptin in tablet dosage form. These methods are based on various techniques such as RP-HPLC,^[6-9] Ultra Violet spectroscopy,^[10-11] HPLC with Tandem mass spectroscopy^[12] and on Human plasma.^[13] But, some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Saxagliptin in tablet dosage form. So, the aim of work was to develop and validate an analytical method by UV-Visible Spectrophotometer for the estimation of Saxagliptin.

MATERIALS AND METHODS

• Instruments

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair.

Method development

A. Determination of λ max (80 PPM)^[15,16,17]

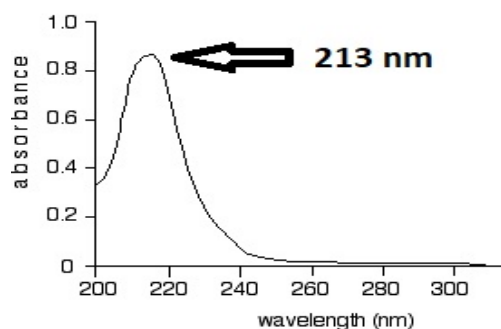


Fig. 2: Calibration curve.

50 mg weighed amount of Saxagliptin was dissolved into 100 ml of volumetric flask with analytical grade methanol. Pipette out 8 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

B. Preparation of working concentration

Preparation of standard stock solution

Standard stock was prepared by dissolving 50 mg of Saxagliptin in 100 ml of analytical grade methanol to get concentration of 500 μ g/ml (PPM).

Preparation of standard solution

Pipette out 5 ml from standard stock solution and diluted up to 50 ml with analytical grade methanol to get concentration of 50 μ g/ml (PPM).

C. Procedure for UV reading

Blank solution: (For Auto zero)

Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.

Melting point apparatus of Equiptronics were used.

• Materials

Saxagliptin was obtained as a gift sample. Saxagliptin tablets were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

Standard solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations^[15,16,17]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Saxagliptin was accurately weighed and transferred into the 100 ml of volumetric flask, added 70 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade methanol. Filtered the solution through nylon syringe filter 0.45 μ . Pipette out 5 ml of the filtered solution and diluted up to 50 ml with analytical grade methanol. The absorbance was measured at 213 nm. The absorbance was recorded.

Table 1: Absorbance of dosage form.

Astra zenca pharma Pvt. Ltd. (25 mg)		
Sr. no.	Sample	Absorbance
1	Blank	0.0000
2	Standard	0.5563
3	Sample	0.5458

Table 2: Dosage form specifications.

No.	Brand / Company	M.D.	E.D.	Batch No.	Avg wt (g)	Assay (%)
1	ONGLYZA [®] - 25 AstraZenca Pharma (25mg)	08/2023	05/2026	D01874S	0.108	98.11

E. Method of validation^[14,16]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 10 - 90 PPM at 213 nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample concentration	Absorbance
1	10 PPM	0.2098
2	30 PPM	0.4154
3	50 PPM	0.6428
4	70 PPM	0.8447
5	90 PPM	1.0526
Correlation coefficient		0.9997 ~ 0.999

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a

known amount of active drug to each sample solution and dissolved in 100 ml of volumetric flask with analytical grade methanol and measuring the absorbance at 213nm.

Table 4: Accuracy studies.

Spectrophotometric method			
Accuracy (%)	Qty weighed (mg)	Qty found (mg)	Recovery (98-102%)
80	0.8	0.78	98.75
100	1	0.99	99.00
120	1.2	1.19	99.17

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.5414
2	Sample Solution 2	0.5364
3	Sample Solution 3	0.5421
4	Sample Solution 4	0.5436
5	Sample Solution 5	0.5322
MEAN		0.5391
SD		0.0047
% RSD		0.8770 ~ 0.88

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for ruggedness studies

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.5421	0.5425	98.22	1.1592 ~ 1.16
		0.5428			
2	Analyst 2	0.5447	0.5467	99.84	
		0.5486			

RESULTS**1. Solubility of saxagliptin**

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

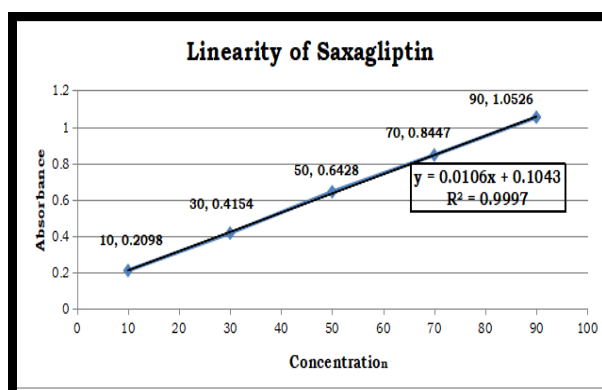
Sr. no.	Title	Result
1	Ethanol, Methanol	Freely Soluble
2	Water	Sparingly Soluble
3	Ethyl Acetate	Slightly Soluble

2. Melting point of saxagliptin

The melting point of Saxagliptin was found to be 108 - 109°C (uncorrected).

3. Results for linearity for assay method of saxagliptin

The linearity of method was determined at concentration level ranging from 10 to 90 µg/ml (PPM). The correlation coefficient value was found to be (R^2) **0.9997** ~ **0.999**.

**Fig. 3: Saxagliptin standard curve.****4. Results for accuracy for assay method of saxagliptin**

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of saxagliptin

The % RSD for different sample of precision was found to be 1.4410 ~ 1.44 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of saxagliptin

The % RSD for different sample of ruggedness was found to be 0.9744 ~ 0.97 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Saxagliptin in tablet form has been developed. From the spectrum of Saxagliptin, it was found that the maximum absorbance was 213 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 10 - 90 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Saxagliptin in solid dosage forms. Hence, the method could be considered for the determination of Saxagliptin in quality control laboratories.

ABBREVIATIONS

1. PPM - Parts per Million
2. nm – Nanometer
3. DPP - Dipeptidyl Peptidase
4. FDA - Food and Drug Administration
5. HPLC - High Performance Liquid Chromatography
6. UV - Ultra violet

7. MS - Mass Spectroscopy
8. LC - Liquid Chromatography
9. ICH - International Council for Harmonization
10. RSD - Relative Standard Deviation
11. SD - Standard Deviation
12. Qty - Quantity
13. °C - Degree Celsius
14. M.D. - Manufacturing Date
15. E.D. - Expiry Date
16. µg/ml - Microgram per milliliter
17. Avg - Average
18. Wt - Weight
19. g - gm

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