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## VALIDATED NOVEL LC DETERMINATION OF SAXAGLIPTIN IN PURE BULK AND PHARMACEUTICAL DOSAGE FORMS

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### ABSTRACT

A simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of Saxagliptin in pharmaceutical dosage forms. The mobile phase consist of buffer (0.02M sodium dihydrogen phosphate, pH-3 adjusted with ortho phosphoric acid) : methanol : acetonitrile in the ratio of 45:20:35 v/v delivered at a flow rate of 1.0 ml / min and wavelength of detection at 220 nm. The retention times of Saxagliptin was 8.20 min. The developed method was validated according to ICH guidelines. The result indicates that the method was found to be simple, rapid, and accurate and can be adopted in routine analysis of Saxagliptin in Pharmaceutical dosage forms.

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### Key Words

Saxagliptin, Validation, Liquid chromatography.

## INTRODUCTION

Saxagliptin is chemically (1S,3S,5S)-2-[(2S)-2-Amino-2-(3hydroxytricyclo[3.3.1.1<sup>3,7</sup>]dec-1-yl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile previously identified as BMS-477118, is a new oral hypoglycemic agent (anti-diabetic drug) of the new peptidyl peptidase-4 (DPP-4) inhibitor class of drugs<sup>1-8</sup>. Saxagliptin recently approved for the treatment of type-2 diabetes mellitus<sup>9-17</sup>. Literature survey reveals that the drug can be estimated only by LC-MS/MS<sup>18</sup>, Spectrophotometric method<sup>19</sup> and no HPLC method have been reported. The present study describes a simple, sensitive, accurate and precise HPLC method for the estimation of Saxagliptin in bulk and pharmaceutical dosage forms.

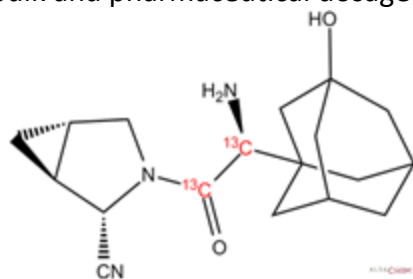


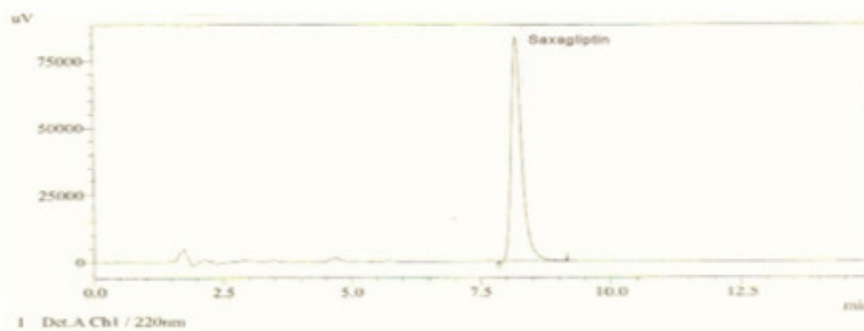
Fig. 1. Structure of Saxagliptin

## EXPERIMENTAL SECTION:

### Reagents and Chemicals:

Saxagliptin API was obtained as gift sample from Matrix laboratories limited, Hyderabad. The branded formulations (tablets) (Onglyza tablets containing 5mg of Saxagliptin) was procured from the local market. Acetonitrile, Methanol, Sodium dihydrogen phosphate, Water and ortho phosphoric acid used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.

**Figure 1:** A Representative Chromatogram of Saxagliptin (Standard)



### Instrumentation:

Chromatographic separation was performed on a Shimadzu chromatographic system (class VP series) equipped with two LC-10AT VP pumps; variable wavelength programmable UV/VIS detector, SPD-20A and Rheodyne injector (7725i) with 20µl fixed loop.

### Chromatographic conditions:

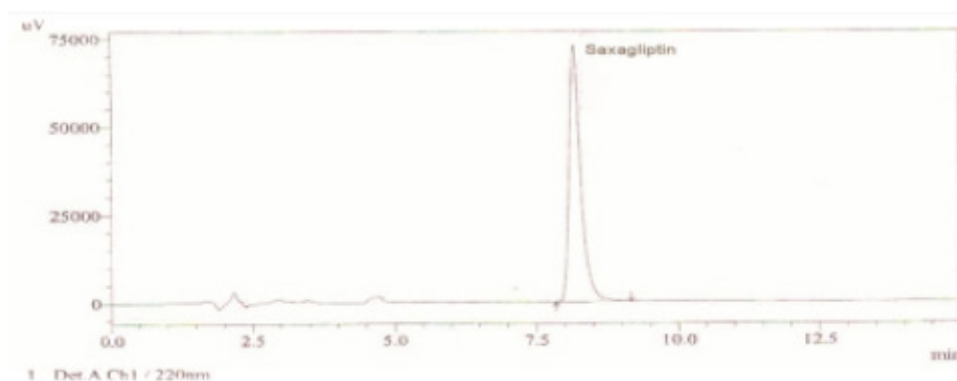
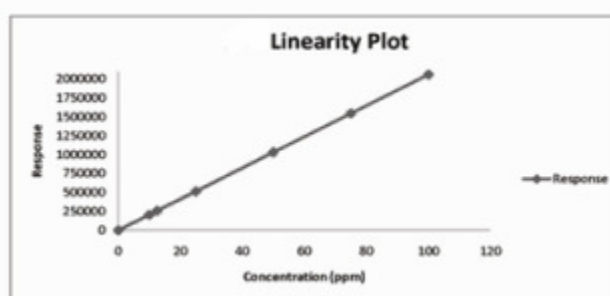
Grace Smart C18 (150 x 4.6mm, 5µ) was the column used for separation. Mobile phase consisting of a mixture of Buffer (0.02M sodium dihydrogen phosphate, pH-3 was adjusted with ortho phosphoric acid ), methanol and acetonitrile in the ratio 45:20:35 v/v was delivered at a flow rate of 1.0 ml/min with detection at 220 nm. The mobile phase was filtered through a 0.45 nylon filter and sonicated for 15 min. Analysis was performed at ambient temperature.

### Pharmaceutical dosage form:

Commercial tablets (Onglyza) were procured randomly from the local market.

### Method development:

Buffer (0.02M sodium dihydrogen phosphate pH-3 was adjusted with ortho phosphoric acid ) and Methanol in different proportions were tried and finally Buffer (0.02M sodium dihydrogen phosphate, pH-3 was adjusted with ortho phosphoric acid) , methanol and acetonitrile (45: 20: 35 v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The chromatogram of working standard solution is shown in fig 1.

**Figure 2:** A Representative Chromatogram of Saxagliptin**Figure 3:** Linearity plot of Saxagliptin**PROCEDURE:****Preparation of standard solution:**

Weigh and transfer accurately about 50.0 mg of Saxagliptin standard into a 100 ml clean dry volumetric flask, add about 60 ml of diluent, sonicate for 5 minutes, and dilute to volume with diluent. Transfer 5ml of this of this solution into a 50ml volumetric flask and dilute to the volume with diluent. Filter the solution through 0.45 $\mu$ m nylon membrane filter, inject 10 $\mu$ l of the solution into chromatograph and record the chromatogram.

**Procedure for analysis of tablets:**

Weigh and powder not less than twenty tablets. Accurately weigh and transfer tablet powder equivalent to 50.0mg of Saxagliptin into 100ml volumetric flask, add about 60ml of diluents and sonicate for 5 mins. Make up the volume with diluent. Transfer 5.0 ml of the solution to 50 ml of volumetric flask and dilute to the

volume with diluent. Filter the solution through 0.45 $\mu$ m nylon filter with the optimized chromatographic conditions, a steady baseline was recorded, the working standard solution was injected into the chromatograph and the chromatogram was recorded. The retention time of Saxagliptin was found to be 8.20 mins . The proposed method was found to be specific and no interference from common tablet excipients. was observed. The response factors of the standard solutions and sample solutions were calculated. The assay was calculated from the equation of regression line for each drug. The assay procedure was repeated for 6 times and the percentage of individual drug in the formulation was calculated. The results of analysis shows that the amount of drug was in good agreement with the label claim of formulation (Table 1).

**Table 1** Analysis of tablet formulation

Formulation	Analyte	Label claim (mg)	%label claim estimated*
Tablet	Saxagliptin	4.89	97.8

\*mean of six determinations

**Calibration curve:**

Accurately measured volume of working standard solution of Saxagliptin was transferred into a series of 100ml volumetric flasks and diluted appropriately with mobile phase. 20 $\mu$ l of each solution was injected under operating chromatographic conditions described above. Calibration curves were obtained by plotting the response (area of drug peak) versus concentration of drug. Regression equations were calculated. The method was found linear over a concentration range of 10 $\mu$ g/ml to 100 $\mu$ g/ml

## METHOD VALIDATION

### Precision:

The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, solutions of standard and sample were repeated thrice in a day and percent relative standard deviation (%RSD) for response factor was calculated. The intraday %RSD of Saxagliptin was found to be 0.04. In the interday variation studies, injections of standard

and sample solutions were made on three consecutive days and %RSD was calculated. The interday %RSD for Saxagliptin was found to be 1.04. From the data obtained the developed RP-HPLC method was found to be precise.

### Accuracy:

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated. Percent recovery was within the range of 98.7 to 100.3 for Saxagliptin which indicates that the method was accurate. The Accuracy data was given in Table 2.

**Table 2:** Accuracy or Recovery of Saxagliptin

Concentration % of spiked level	Amount added(ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	25.36	25.03	98.7	MEAN	99.5
50% Sample 2	25.82	25.85	100.1	SD	0.71
50% Sample 3	25.01	24.91	99.6	%RSD	0.71
100 % Sample 1	50.52	50.67	100.3	MEAN	99.7
100% Sample 2	51.08	50.62	99.1	SD	0.60
100% Sample 3	50.11	50.01	99.8	%RSD	0.60
150% Sample 1	75.20	74.37	98.9	MEAN	99.4
150% Sample 2	74.69	74.24	99.4	SD	0.55
150% Sample 3	76.11	76.11	100	%RSD	0.55

### Linearity:

The method was linear in the range of 10  $\mu$ g/ml to 100  $\mu$ g/ml for Saxagliptin. Linear regression data was given in Table 3.

**Table 3:** Linear regression data for calibration curves

Parameter	Saxagliptin
Linearity range ( $\mu$ g/ml)	10-100
Correlation coefficient	0.9990
Slope	20611
% of y-Intercept	0.04

### Robustness:

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase composition, pH of buffer, flow rate and temperature variation. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust. The result was shown in Table 4.

**Table 4:** Robustness of Saxagliptin

Parameters	Optimum range	Conditions in procedure	Remarks
Mobile phase composition (% of organic phase)	10% variations in organic phase	Isocratic	Beyond the optimum range of % of Organic phase, the resolution factor and relative retention and asymmetry factor were decreased
Flow rate (ml/min)	0.8-1.2	1.0	At lower flow rates the asymmetry factor was increased and at higher flow rates the relative retentions was decreased
Temperature	30-35°C	Ambient	Beyond the optimum range peak shape and symmetry was lost
pH of mobile phase	2.8-3.2	3.0	Beyond the optimum range of pH of the mobile phase, better resolution was not found. When it is reduced or increased beyond optimum range asymmetry factor was increased.

**Effect of variation in mobile phase composition:**

A study was conducted to determine the effect of variation in organic phase composition in mobile phase. Standard solution prepared as per the test method was injected into the HPLC system using two mobile phases. The system suitability parameters were evaluated and found to be within the limits for mobile phase having 90% and 110% of method highest organic phase. Saxagliptin blend solution at target concentration was chromatographed using mobile phase having 90% and 110% of the method organic phase. Saxagliptin was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having 100% of the organic phase. From the study it was established that the allowable variation in mobile phase composition is 90% to 110% of the method highest organic phase of mobile phase.

**Effect of variation of flow rate:**

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 0.8ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 0.8ml/min and 1.2ml/min flow. Saxagliptin was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min. From the

above study it was established that the allowable variation in flow rates is 0.8ml/min and 1.2ml/min.

**Effect of variation of temperature:**

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 30°C temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 30°C. Similarly sample solution was chromatographed at 35°C temperature. Saxagliptin was resolved from all other peaks and the retention times were comparable with those

**iv) Effect of variation of pH:**

A study was conducted to determine the effect of variation in pH. Standard and sample solutions were prepared as per the test method and injected into the HPLC system using pH 2.8 and 3.2. The system suitability parameters were evaluated and found to be within the limits for pH 2.8 and 3.2. Saxagliptin were resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having pH 3. From the above study it was established that the allowable variation in pH 2.4 and 2.8.

Hence the method is robust.

**Specificity:**

A study was conducted to demonstrate the effective separation of degradants from Saxagliptin. Separate

portions of Drug product exposed to following stress conditions to induce degradation. The result was shown

in Table 5.

**Table 5:** Specificity of Saxagliptin

Degradation mechanism / condition	Observation
Protected sample	No interference at RT of analyte peak
Water / Reflux – 30.0 min	No interference at RT of analyte peak
Acid degradation (0.1 N HCl Reflux – 30.0 min)	No interference at RT of analyte peak
Base degradation (0.01 N NaOH Reflux 30.0min)	No interference at RT of analyte peak
Peroxide degradation (3.0% H <sub>2</sub> O <sub>2</sub> Reflux – 30.0min)	No interference at RT of analyte peak
Thermal degradation (At 105°C - 48 Hrs)	No interference at RT of analyte peak
Photolytic degradation (At 254nm - 24 Hrs)	No interference at RT of analyte peak
Accelerated degradation (At 40°C/75% RH - 168 Hrs)	No interference at RT of analyte peak

## RESULTS AND DISCUSSION

The proposed method was found to be linear in the concentration range of 10 to 100 µg/ml for Saxagliptin. The method was specific since excipients in the formulation did not interfere in the estimation of

**Table 6:** Summary of validation parameters

Parameter	Saxagliptin
Mean % recovery	98.7 to 100.3
Precision	
a) Intraday precision	0.04
b) Interday precision	1.04
Robustness	Robust
Retention time (min)	8.20
Theoretical plates	8297
Tailing factor	1.4

Saxagliptin. Accuracy of the method was indicated by recovery values from 98.7 to 100.3 % for Saxagliptin. Precision is reflected by %RSD values less than 2. These low values suggest sensitivity of the developed method. Validation parameters were summarized in Table 6.

## CONCLUSION

The developed RP-HPLC method was simple, sensitive, precise and accurate and hence can be used in routine for the determination of Saxagliptin in bulk as well as in pharmaceutical preparations.

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