ISSN: 0975-2633
Research Article
Arch Pharm Sci& Res Vol 6 No 1 No 6- 12 June 2014

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SAXAGLIPTIN IN FORMULATION AND BULK DRUG

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In the present work, simple and sensitive RP-HPLC method has been developed for the quantitative estimation of Saxagliptinin bulk and in Pharmaceutical formulations. Isocratic elution at a flow rate of 1.0ml/min was employed on a waters X- Bridge C18 column 5µm 4.6x250mm at ambient temperature. The mobile phase consisted of acetonitrile: ammonium acetate 25:75 (V/V/). In the range of 0.4-12.8µg/ml, the linearity of saxagliptin shows a correlation co-efficient of 0.999. The UV detection wavelength was 220nm and 20µl sample was injected. The retention time for Saxagliptin was 5.483 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%.In the pharmaceutical formulations,(LOQ) of 1.247 µg /ml and (LOD) of 0.156 µg/ ml. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of saxagliptin in tablet dosage form.

Keywords: Saxagliptin, RP-HPLC, UV detection, recovery, precise.

INTRODUCTION

Saxagliptin hydrochloride[1,2]is a new oral hypoglycemic (antidiabetic drug) of the dipeptidyl peptidase-4 inhibitor class of drugs. Saxagliptin is part of a class of diabetesmedications called dipeptidylpeptidase-4 (DPP-4) inhibitorsSaxagliptin is chemically(1S,3S,5S)-2-[(2S)-Amino(3-hydroxytricyclo [3.3.1. 13,7] dec-1-yl)acetyl]-2-azabicyclo[3.1.0] hexane-3-carbonitrilemonohydrochloride with empirical formula is C18H25N3O2.HCl and molecular weight 351.87. Various methods in the literatures involve determination of Saxagliptin in human plasma by LCMS/MS[20], estimation of saxagliptin by HPLC[4-16,23,26] and UV-spectroscopy[18,19,21].Since very few method are available for assay of Saxagliptin in bulk drug and pharmaceutical dosage form, hence an attempt has been made to develop a new, simple precise and new analytical method for determination of Saxagliptin in bulk drug and pharmaceutical dosage form.

Methods

Materials and reagents

Saxagliptin (99.8% purity) was received as gift sample from Bristol – Hetero labs, Hyderabad, India. HPLC grade Acetonitrile, and Analytical grade ammonium acetate (Merck, Mumbai, India). Pharmaceutical formulation ONGLYZA tablet (Bristol – Myers Squibb Company, Mumbai, Maharashtra, India)(label claim 5 mg Saxagliptin) was used in HPLC analysis. HPLC grade water obtained in-house by using Direct-Q water purification system was used in HPLC study.

Chromatographic conditions and equipment:

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC-10AT vp pump) (4MPa or 40barr), rheodyne injector, UV variable wavelength detector, Standard cell and agilent syringe was used. The separations were achieved on a waters X- Bridge C18 column 5µm 4.6x250mm with UV detection at 220nm. Analytical weighing balance (Shimadzu AUX 220) was used for weighing. Double beam UV Visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software-single channel was used for acquisition, evaluation and storage of chromatographic data. Mobile phase used was (25:75) acetonitrile and ammonium acetate (pH 8.8)

Instrumental parameters

After several trials with the different combination and ratio of solvents, the mobile phase Acetonitrile: Ammonium acetate (25:75 v/v, pH 8.81±0.1) was selected, because it was found that it ideal with retention time (R_t) 5.483 min and the same is shown in Fig.4. Wavelength was selected by scanning the standard drug over a wide range of wavelength 200 nm to 400 nm. The component show reasonably good response at 220 nm (fig 2).

Preparation of Stock Solution

Accurately weighed 100 mg of Saxagliptin in 100 ml volumetric flask and dissolved in methanol

and the volume was made up to the mark with the same solvent. From the above 10 mL solution was pipette out into separate 100 ml volumetric flask and volume was made up to the mark with the same solvent. This gave the concentration of 100 µg mL^{-1} of Saxagliptin, from this six dilutions of Saxagliptin were prepared in between 0.4-12.8 µg mL^{-1} with acetonitrile and used in HPLC estimation.

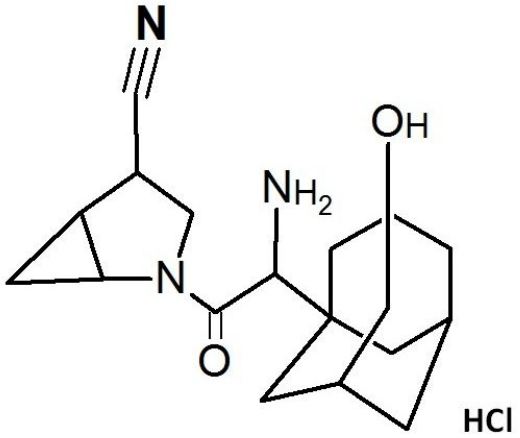

Figure 1: Structure of Saxagliptin hydrochloride

Procedure for Preparation of Calibration Curve

Calibration study was carried out individually at six different concentration levels. All stock and working solutions were sonicated for 10 min then filtered through the nylon membrane filter (0.45µ) prior to use. Triplicate of 20µL injections were made for each concentration and chromatographed under specified condition at ambient temperature (24^0C).

Preparation of Tablet Extracts and Assay Procedure

Twenty tablets each containing 5 mg of Saxagliptin were weighed and powdered for further study. The powder equivalent to 50mg of saxagliptin was accurately weighed and transferred to 50ml volumetric flask containing 25ml of methanolandsonicatedfor10min. The above solution was carefully filtered through Whatmann filter paper (No.41) and the residue was washed with 3 portions of 5 ml of solvent. The volume was made up to the mark with acetonitrile. From this solution, required dilutions for HPLC method were prepared by using acetonitrile as a solvent.

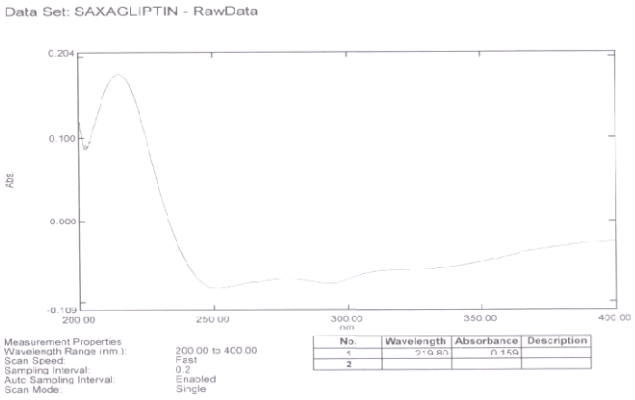

Fig. 2 UV Spectrum of Saxagliptin at 220nm

Procedure for Method Validation

Specificity: blank interference

A study to establish the interference of blank was conducted. Mobile phase was injected as per the test method. Chromatogram of blank (fig-3) should not show any peak at the retention time of analyte peak.

S.NO	Concentration (µg/ml)	Peak Area
1.	0.4	376007
2	0.8	538594
3.	1.6	830519
4.	3.2	1677153
5.	6.4	3667715
6.	12.8	6608394

Table.1. Linearity data for Saxagliptin

Linearity:

Linearity of the proposed HPLC method for determination of Saxagliptinwas evaluated by analyzing a series of different concentrations of standard drug. In this study six concentrations were chosen ranging between 0.4–12.8µg mL^{-1} for Saxagliptin fig.6. Each concentration was injected three times and obtained information on variation in the peak area response of pure analyte was plotted against corresponding concentrations and result was shown in Table 1.

The linearity of the calibration graph was validated by the high value of correlation coefficient, slope and the intercept value was shown in Fig.5. The optimized method parameters are given in the Table1.

Precision:

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. It was demonstrated by repeatability and intermediate precision measurements of peak area and peak symmetry parameters of HPLC method for the title ingredient. The repeatability (fig.7 and 8) (within-day) and intermediate precision (fig.9 and 10) (for 2 days) were carried out at one concentration levels and six replicates for compound. The obtained results were within and between the acceptable range. The precision expressed as % RSD is given in Table 2 and 3.

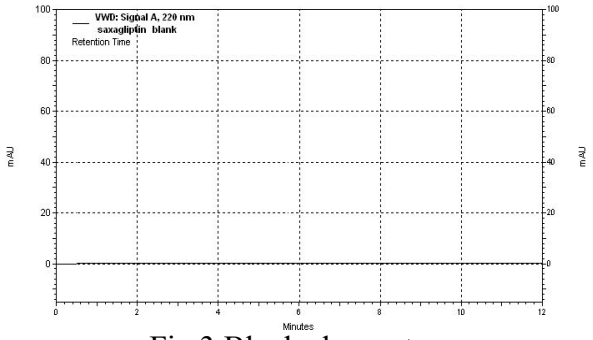

Fig.3 Blank chromatogram

Accuracy:

Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its linearity range. Accuracy was performed in three different levels, each level in triplicate for saxagliptinusing standards at 80%, 100% and 120% (Fig 11,12 and 13).Each sample was analysed in triplicate for each level. From the results, % recovery is calculated and shown in Table 4 and 5.

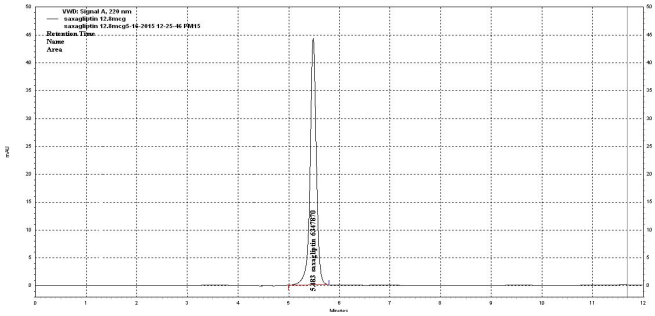

Fig. 4 Chromatogram of Saxagliptin with optimized method

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

It is calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively. The values of LOD and LOQ were given in Table 6.

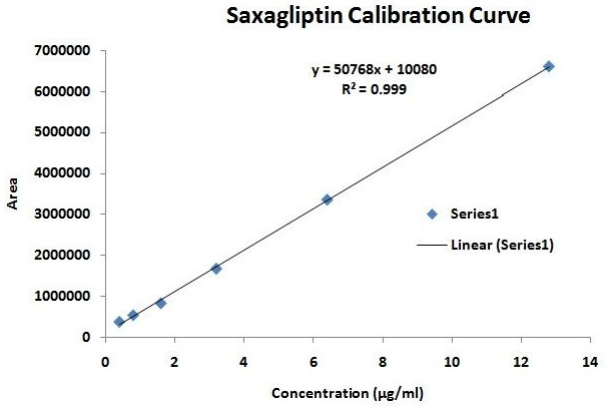

Fig.5.Linearity curve for Saxagliptin at 220nm

6. RESULT AND DISCUSSION

The objective of the proposed work was to develop a method for the determination of Saxagliptin to validate the methods according to USP and ICH guidelines and applying the same for its estimation in laboratory prepared mixtures. HPLC method developed was found to be rapid, simple, precise, accurate and economic for routine estimation of saxaglipitn in laboratory prepared mixtures.

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compound. Initially, various mobile phase compositions were tried, to separate title

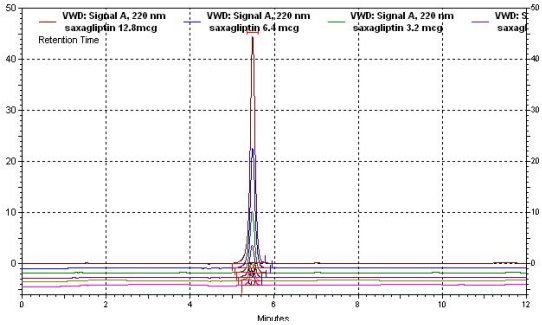

FIG.6 Overlay chromatograms of Saxagliptin of linearity

Precision	Conc (6.4µg/ml)	Area	
		Morning	Afternoon
Nateglinide	Injection 1	3680329	3633615
	Injection 2	3680328	3646720
	Injection 3	3672390	3654720
	Injection 4	3668902	3662802
	Injection 5	3664933	3620591
	Injection 6	3660711	3628902
	Average	3671265	3641225
	STDEV	8032.924	16207.45
	RSD %	0.2188	0.445

Table .2. Precision (intraday) study results of prepared sample

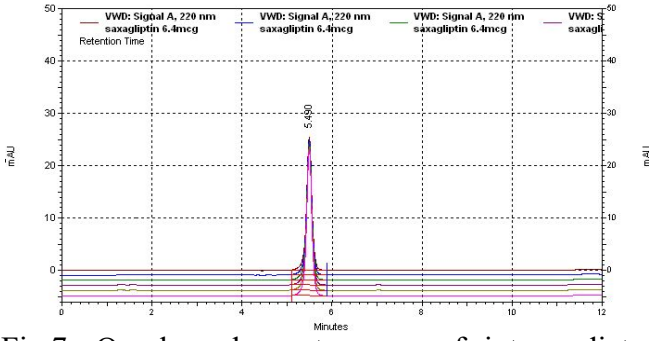

Fig.7. Overlay chromatograms of intermediate precision-Morning

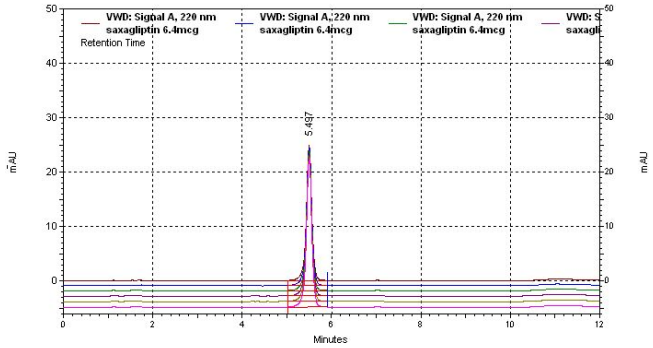

Fig. 8. Overlay chromatograms of intermediate precision-afternoon

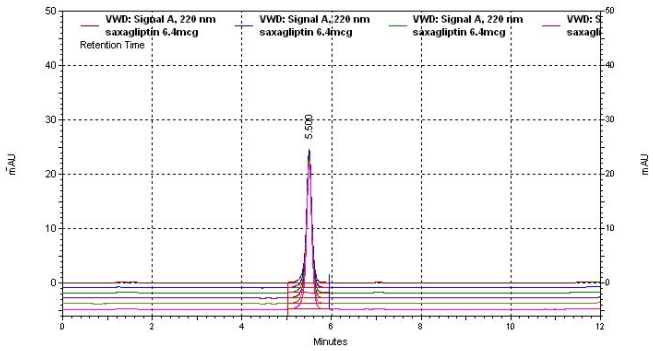

Fig. 9. Overlay chromatograms of intermediate precision day-1

Precision	Conc (6.4µg/ml)	Area	
Nateglinide		Morning	Afternoon
	Injection 1	3680329	3633615
	Injection 2	3680328	3646720
	Injection 3	3672390	3654720
	Injection 4	3668902	3662802
	Injection 5	3664933	3620591
	Injection 6	3660711	3628902
	Average	3671265	3641225
	STDEV	8032.924	16207.45
	RSD %	0.2188	0.445

Table.3. Precision (Inter day) study results of prepared sample

S.No.	Level in %	Area Response Saxagliptin	Mean % recovery Saxagliptin
1	80	3607780	98.26
2		3602264	
3		3602342	
1	100	3622710	98.67
2		3607720	
3		3627520	
1	120	3656723	99.82
2		3652342	
3		3675320	

Table .4. Mean % recovery of Saxagliptin

ingredient. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time, and resolution. The system with

acetonitrile: ammonium acetate (pH 8.8) (25:75 v/v) with 1ml.min^{-1} flow rate is quite robust.

The optimum wavelength for detection was 220 nm at which better detector response for the title drug was obtained. The retention time for Saxagliptin was found to be 5.483 min respectively. The calibration was linear in concentration range of 0.4-12.8µg mL^{-1} withregression 0.999, intercept 10080 and slope 50768x for Saxagliptin. The low values of % R.S.D indicate the method is precise and accurate. The mean recoveries were found in the range of 100.62-101 %.

Precision	Concentration (µg/ml)	Area	
Nateglinide		Day 1	Day 2
	Injection 1	3690113	3656723
	Injection 2	3682461	3656213
	Injection 3	3672809	3624432
	Injection 4	3644802	3632263
	Injection 5	3624220	3677123
	Injection 6	3634278	3600320
	Average	3658114	3641179
	STDEV	27301.37	27528.25
	RSD %	0.746	0.756

Table .5 Assay results of Saxagliptin

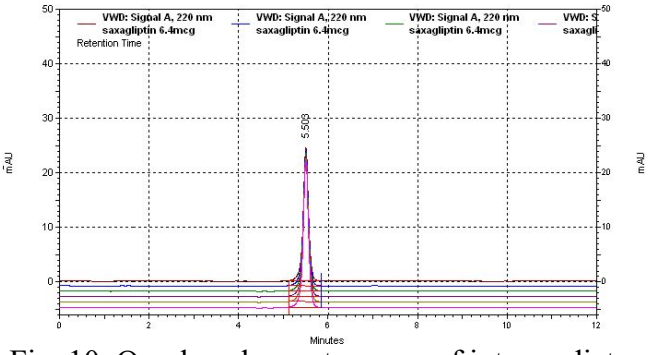

Fig. 10. Overlay chromatograms of intermediate precision-day-2

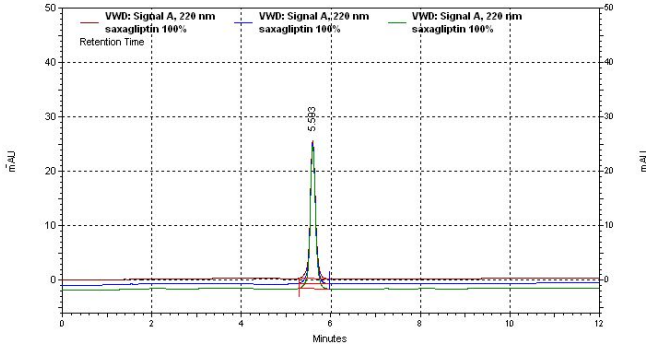

Fig11 Overlay chromatograms of 80% recovery

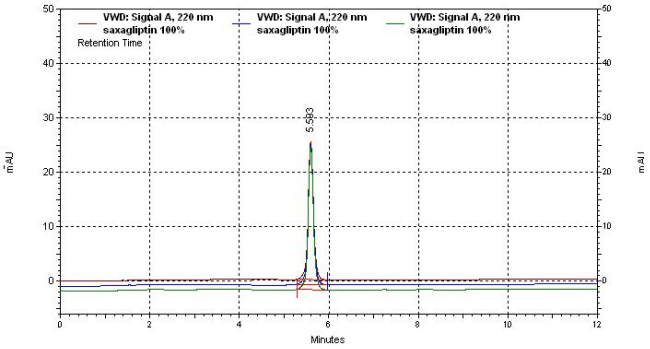

Fig.12 Overlay chromatograms of 100% recovery

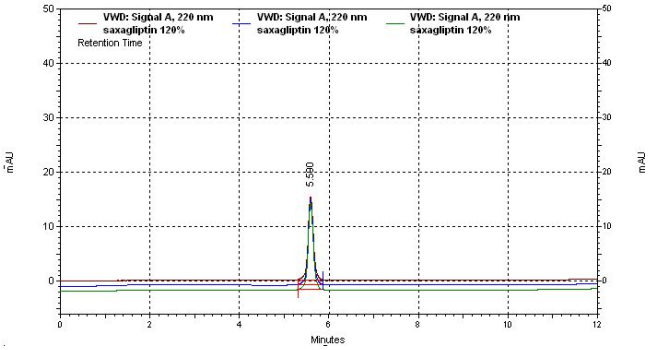

Fig 12 Overlay chromatograms of 100% recovery

Parameters	Nateglinide
LOD(µg/ml)	0.156
LOQ(µg/ml)	1.247

Table .6. Result of LOD and LOQ

Sample to sample precision and accuracy were evaluated using three samples of five different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days over a period of three days. These results show the accuracy and reproducibility of the assay. The % R.S.D. reported was found to be less than 2 %.The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in laboratory prepared mixtures.

The proposed methods are accurate, simple, rapid and selective for the estimation of Saxagliptin in laboratory prepared mixtures. Hence, these methods can be conveniently adopted for the routine analysis of Saxagliptin in quality control laboratories.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): It is calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively. The values of LOD and LOQ were given in Table 5.7

SUMMERY AND CONCLUSION

On the basis of the experiments, we can conclude that the RP-HPLC & method developed for the Estimation of Saxagliptin can be used for routine analysis Q.C. Samples. Saxagliptin was determined by reverse phase HPLC using ammonium acetate (pH 8.8): Acetonitrile (75:25v/v) as mobile phase, and Waters X Bridge C 18Column, 5µ 250×4.6mm as a stationary phase. Detection was carried out using UV detector at 220 nm. After development of the method, it was validated for system suitability, specificity and linearity, limit of detection and limit of quantification, precision, and accuracy.

Summary of the present study (RP-HPLC)

Validation Parameters	**Nateglinide**
Mobile phase	Acetonitrile: ammonium acetate(25:75v/v)
Flow rate	1.0ml/min
Detection Wavelength	220
Rt	5.483
Run Time	12min
Theoretical Plates	10350
LOD	0.156µg/ml
LOQ	1.247µg/ml
Linearity	0.4-12.8µg/ml
Precision	% RSD < 2

- The system suitability was found to be within the limits. The limit was Not more than RSD <2. The retention time of Saxagliptin is 5.483mins. The data regarding the system suitability is shown in table 5.1.
- The Specificity of Saxagliptin is shown in Chromatogram there was no interference. In this method it means no impurity was interfered and also reveals that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods.
- The precision was found to be within the limits. The limit were not more than RSD <2. This indicates that the method is precise. The data regarding the precision are shown in table 2 and 3.
- From the linearity table 1, it was found that, the drug obeys Beer's Law. For HPLC the calibration plot of Saxagliptin was observed as linear in the range 0.4-12.8µg/ml and the correlation coefficients were found to be 0.999 respectively.
- From the results shown in the accuracy table 4 and 5, it was found that Recovery value of pure drug from the solution were between 100.62-101 %.This indicates that the method is accurate.

ACKNOWLEDGEMENT

Authors thank Hetero labs, Hyderabad, India for gifting pure saxagliptin. Authors thank the authorities of the Karnataka College of Pharmacy for permission to use the facilities.

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