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Research Article

DEVOLPMENT AND VALIDATION OF SIMPLE STABILITY INDICATING RP-HPLC METHOD FOR ANALYSIS OF SAXAGLIPTIN AND ITS FORCED DEGRADATION IMPURITIES IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The objective of the present research work is developing a gradient, reversed phase liquid chromatographic method for the determination of saxagliptin hydrochloride in bulk drugs and pharmaceutical dosage form. The chromatographic separation was achieved on a Inertsil C8 (4.6x250) mm, 5 µm column, the gradient LC method employs solution A and B as mobile phase. The solution A contains (1.20g of sodium dihydrogen phosphate in 1000 ml of water) pH 5.0 solution B (acetonitrile) at flow rate 1.2 ml/min. The UV detector was operated at 210 nm and temperature was 25˚c. The retention time was 7.68 min and linearity was observed in the concentration range of 50-375 µg/ml with correlation coefficient of 0.9999. The percentage relative standard deviation in accuracy and precision studies was found to be less than 2%. The method was successfully validated as per International Conference on Harmonization (ICH) guidelines. Saxagliptin hydrochloride undergoes degradation under acidic, basic, oxidation, dry heat and photolytic conditions, degradation impurities did not interfere with the retention time of Saxagliptin hydrochloride. The proposed method was found to be a new, simple, precise, linear, accurate, specific and stability indicating.

Keywords: Saxagliptin.validation.HPLC.Stability indicating.

INTRODUCTION

Saxagliptin hydrochloride is a new oral hypoglycemic (anti-diabetic drug) of the dipeptidyl peptidase-4 inhibitor class of drugs (1-9). Saxagliptin is part of a class of diabetes medications called dipeptidyl peptidase-4 (DPP-4) inhibitors. DPP-4 is an enzyme that breaks down incretin hormones. As a DPP-4 inhibitor [21], Saxagliptin slows down the breakdown of incretin hormones, increasing the level of these hormones in the body. It is this increase in incretin hormones that is responsible for the beneficial actions of Saxagliptin, including increasing insulin production in response to meals and decreasing the amount of glucose that the liver produces. Because in cretin hormones are more active in response to higher blood sugar levels, the risk of dangerously low blood sugar (hypoglycemia) is low with Saxagliptin. Saxagliptin is available as tablets at the dose of 5 mg in the market under the brand name of Onglyza by Bristol-Myers Squibb and AstraZeneca. Saxagliptin 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-carbonitrile monohydrochloride with empirical formula is C18H25N3O2.HCl and molecular weight 351.87[22].

Various methods in the literatures involve determination of Saxagliptin in human plasma by LCMS/MS (10), estimation of saxagliptin by UV-spectroscopy (11, 12), HPLC (13-18).However no method is available for stability indicating

method for assay of Saxagliptin in bulk drug and pharmaceutical dosage form. In the present work we have developed a new, simple precise and stability indicating method for determination of Saxagliptin in bulk drug and pharmaceutical dosage form.

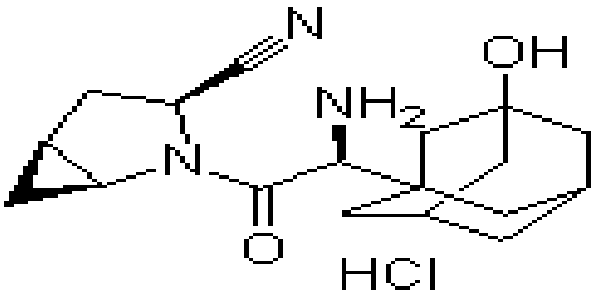


Figure 1: Structure of Saxagliptin hydrochloride

EXPERIMENTAL

Chemicals & Reagents

Saxagliptin is available as tablets with brand name ONGLYZA was purchased from local market, containing Saxagliptin 5mg. HPLC grade acetonitrile, AR grade Sodium dihydrogen phosphate and Phosphoric acid were purchased from Merck, Mumbai. High pure water was prepared by using Millipore Milli-Q plus purification system.

Chromatographic Conditions

A Alliance e2695 separation module (Waters corporation, Milford, MA) equipped with 2998 PDA detector with empower 2 software used for analysis. Buffer consisted of 0.01M sodium dihydrogen phosphate in water (1.20g of sodium dihydrogen phosphate in 1000 ml of water) pH 5.0 with ortho phosphoric acid. Inertcil C8 (4.6x250) mm 5 µm column and gradient mixture of solution A (Buffer) solution B (Acetonitrile) used as stationary and mobile phase respectively. The gradient program (T/%B) was fixed as 0/10, 10/35, 15/50,15.2/10,20/10. Water: Acetonitrile (80:20) %v/v used as diluent. The column oven maintained at 25˚c with 1.2ml flow rate. An injection volume 10µl was used. The elution compounds were monitored at 210 nm.

Preparation of Stock and standard solutions

Accurately 50mg of Saxagliptin standard dissolved in 25ml diluent to get a concentration of 2000µg/ml. Further 5ml of stock solution was taken in 50ml flask and diluted up to the mark with diluent to get concentration of 200µg/ml.

Preparation of Tablets for assay

The formulation tablets of Onglyza were crushed to give finely powdered material. Powder equivalent to 50mg of drug was weighed and transferred to the 25ml flask added 10ml diluent and placed in an ultrasonicator for 10minites made up to the volume with diluent, and filtered through a 0.45µm nylon syringe filter. 5ml of this solution was taken into 50 ml flask and diluted volume with diluent to get concentration 200µg/ml.

FORCED DEGRADATION STUDIES

Acid Degradation studies

Acid decomposition was carried out in 0.4N HCL at concentration of 2000µg/ml Saxagliptin and after refluxation for 24hours at 80˚c, the stressed sample was cooled, neutralized and diluted as per requirement with diluents filtered and injected. The resulting chromatogram is shown in fig.3 (g). The results are tabulated in table 3.

Alkali Degradation studies

Base decomposition was carried out in 0.02N NaOH at concentration of 2000µg/ml Saxagliptin after refluxation for 24hours at 80˚c, the stressed sample was cooled, neutralized and diluted as per requirement with diluents filtered and injected. The resulting chromatogram is shown in fig.3 (i). The results are tabulated in table 2.

Oxidation

Oxidation was conducted by using 4%H2O2 solution at room temperature for 24hours, 5ml of solution was taken in 50ml flask and diluted up to the mark with diluent to get concentration of 200µg/ml filtered and injected. The resulting chromatogram is shown in fig.3 (k). The results are tabulated in table 2.

Temperature Stress studies

1g of Saxagliptin sample was taken into a petridish and kept in oven at 80˚c for 7days. 50mg of sample was taken into 25 ml flask diluted volume with diluent, further 5ml to 50ml made up with diluent. The results are tabulated in table 2.

Photo stability

1g of Saxagliptin was taken in to a petridish and kept in photo stability chamber 200 W.hr/m2 in UV Fluorescent light and 1.2M LUX Fluorescent light. 50mg of sample was taken in 25ml flask, dissolved in diluent, further 5ml in 50ml

flask diluted volume with diluent. The results are tabulated in table 2.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization

To develop a rugged and suitable HPLC assay method for the determination of Saxagliptin, the analytical condition were selected after the consideration of different parameters such as diluents, buffer, organic solvent for mobile phase, column and other chromatographic conditions (20). Initial trails were performed with different composition of buffer (acetate and formate) and organic phase (methanol, teterhydrofuran) with different column like c8, phenyl, cyno, amino and basic but Saxagliptin peak shape was not good. Finally 0.02 M Sodium dihydrogen phosphates in water pH 5.0 with ortho phosphoric acid and acetonitrile with gradient and Inertsil C8 (4.6x250) mm 5 µm column were optimized. Different diluents were tried to dilute sample like water, buffer, methanol, tetrahydrofuran and mixture of water: methanol and water: teterhydrofuran, buffer: methanol and buffer: acetonitrile. Saxagliptin was not dissolved, finally (water: acetonitrile) (80:20) %v/v was optimized. The detection wavelength was chosen as 210nm for Saxagliptin because they have better absorption and sensitivity at this wavelength (fig-2). Hence selected method was best among the all trails by many aspects.

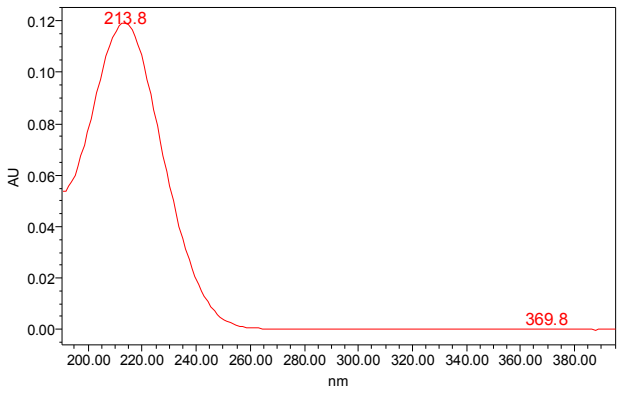


Fig-2 wavelength spectrum of Saxagliptin hydrochloride

Method Validation

The method was validated for the following parameters specificity, linearity, accuracy, limit of detection (LOD), limit of quantitation (LOQ), precision and robustness (19).

Specificity

A study to establish the interference, blank detection was conducted. Diluent was injected as per the test method. Solution of standard and sample were prepared as per test method and injected into the chromatographic system. The chromatograms of blank, standard and sample were shown in the fig a, b, c.

Precision

The precision for assay method was established by evaluating method precision and intermediate precision study. Method precision was determined by analyzing six independent assays were performed and calculated the % RSD for replicate assay determinations. Intermediate precision of the analytical method was determined by conducting method precision on another day and another analyst under same experiment condition. The result obtained for method precision and intermediate precision are shown in table 4. The percentage of RSD was calculated. The %RSD range was obtained as 0.12 and 0.20 for method precision and intermediate precision respectively (Table 4) which is less than 2% indicating that the method is more precise.

Accuracy

The accuracy of the method was estimated by determination of recovery for three concentrations (corresponding to 50,100 and 150% of test solution concentration) covering the range of the method. For each concentration three sets were prepared and injected. The drug concentrations of Saxagliptin were calculated, the results obtained are shown in table 3. The drug concentrations of Saxagliptin were calculated, the percentage recovery was found to be 99.89-100.01% with %RSD 0.05 - 0.23(<2.0%) indicating that the method is more accurate (table 3).

LOD & LOQ

The LOD and LOQ were determined at a signal to noise ratio of 3:1 and 10:1 respectively by injecting a series of test solutions of known concentrations within the linearity range. Precision study was also carried out at the LOQ level by injecting six pharmaceutical preparations. The LOD and LOQ were to be 0.39µg/ml and 1.29µg/ml respectively. The %RSD value was noticed to be less than 2.0% at LOQ concentration level.

Linearity

The linearity plot was prepared with six concentration levels (40, 80, 160,200,240 and 300 µg/ml of Saxagliptin). These concentration levels were respectively corresponding to 20, 40, 80,100,120 and 150 % of test solution concentration. The results obtained are shown in table 1. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve (figure 4).

Robustness

Robustness of method was checked by making slight deliberate changes in chromatographic conditions like flow rate (±0.1 ml/min), PH (±0.1units) and column temperature (±5˚c). In the all above varied conditions, the components of the mobile phase were held constant. The results are tabulated in table 5.

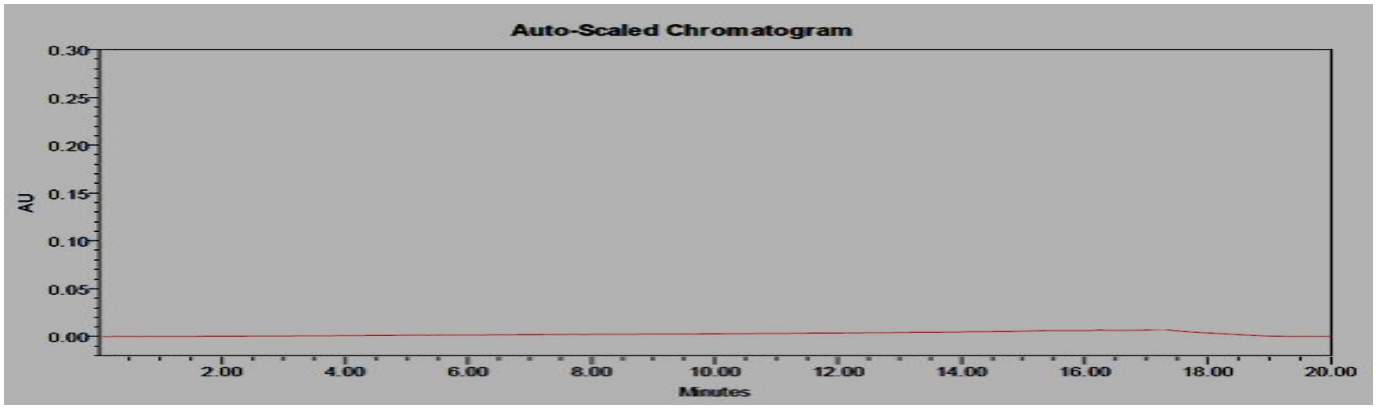
Solution stability and Mobile phase stability

Solution stability checked for stability of standard and sample solutions.

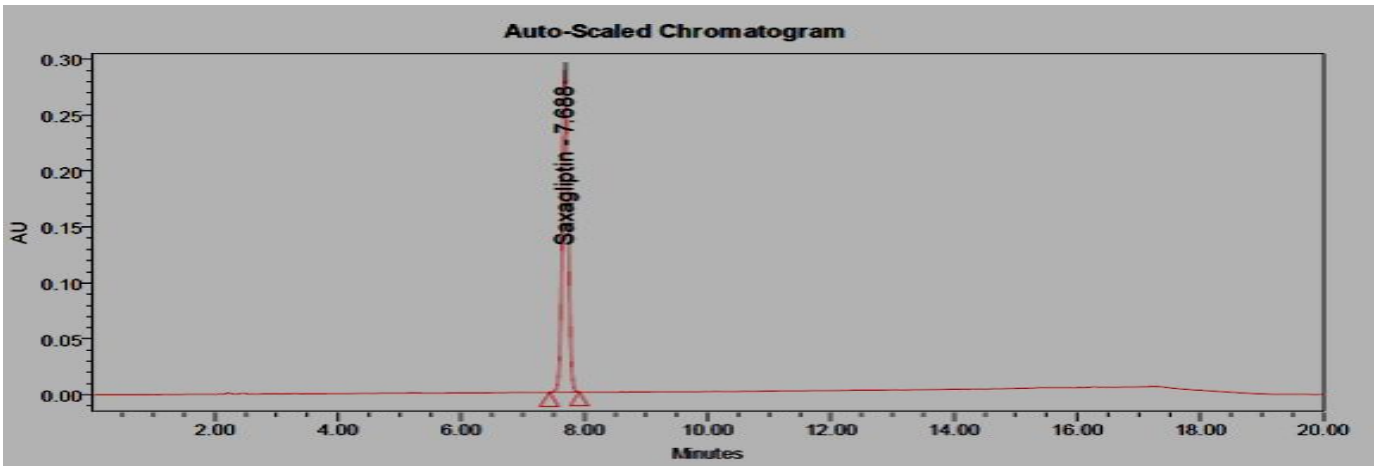
Solution stability checked at each interval initial 2,4,6,8,12,16,20 and 24 hours. For standard solution stability and sample solution stability %assay value calculated at each interval. %RSD (NMT 2.0%) between initial assay value and assay value obtained at predetermined time interval calculated.

Forced Degradation Studies

Stress studies on Saxaglipin were carried out under oxidation, thermal stress, photolysis, acid and alkali hydrolysis conditions. Significant degradation was observed in acid (fig 3g) base (fig 3i), oxidation and Thermal of Saxagliptin. There was no significant degradation of Saxagliptin upon exposure to photolysis total impurity increased to 0.22%, which indicated that the drug was stable against these stress conditions. The developed method revealed that there was no interference from the impurities, degradation products and excipients to determine the assay of drug substance in pure and pharmaceutical formulation.



(a)



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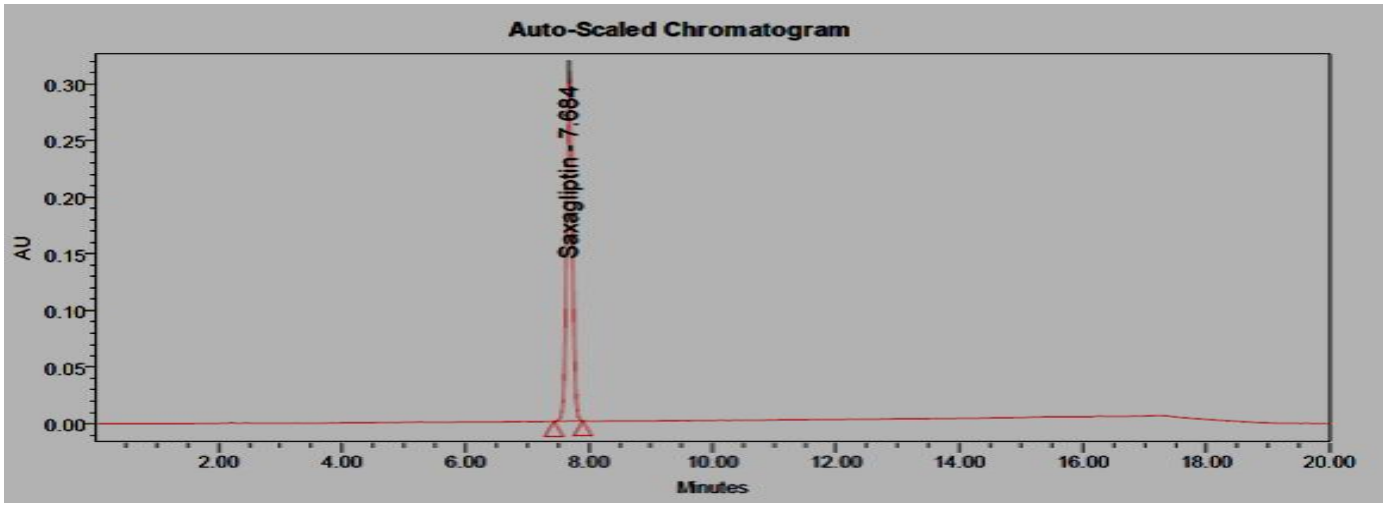

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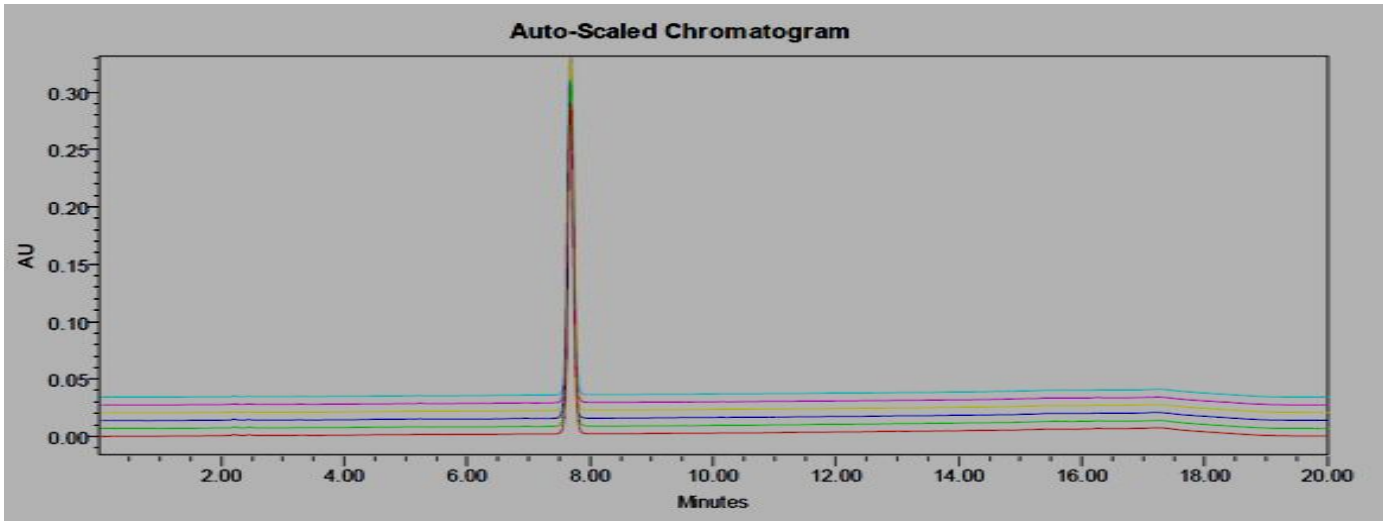

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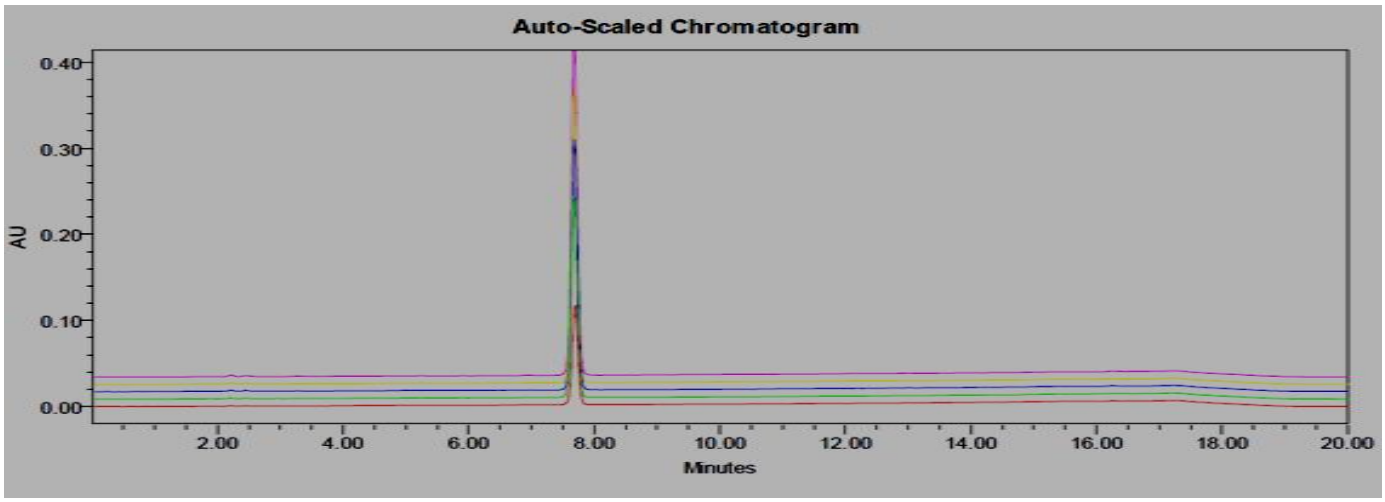

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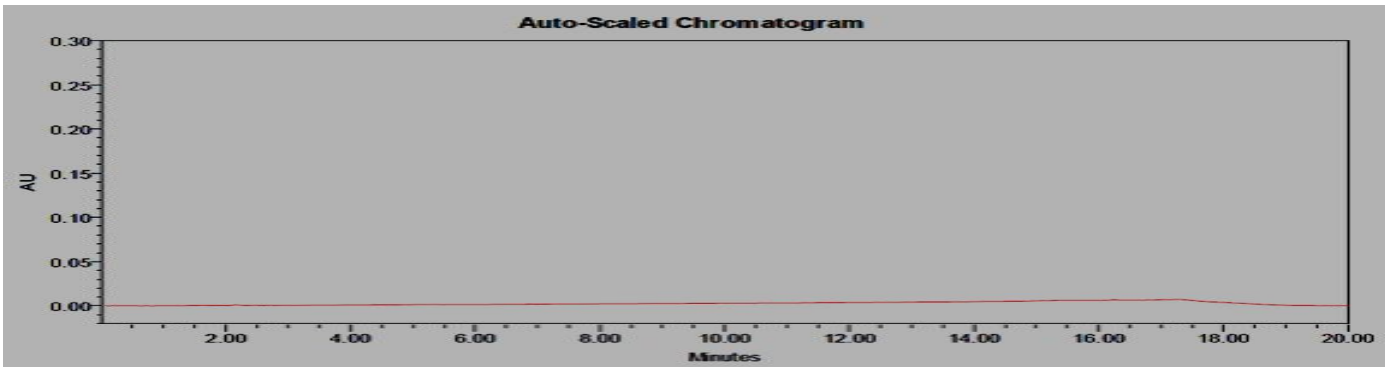

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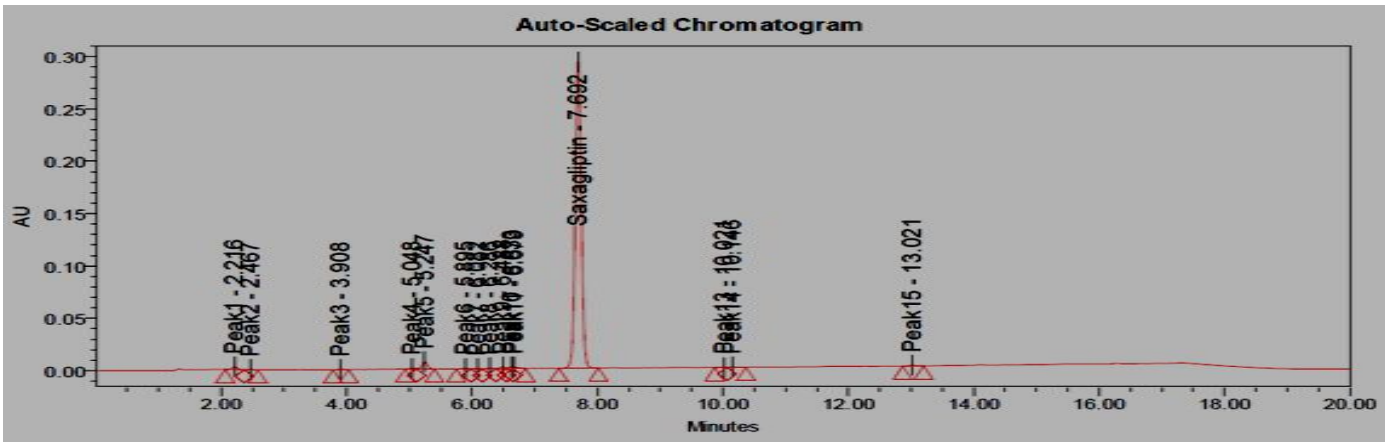

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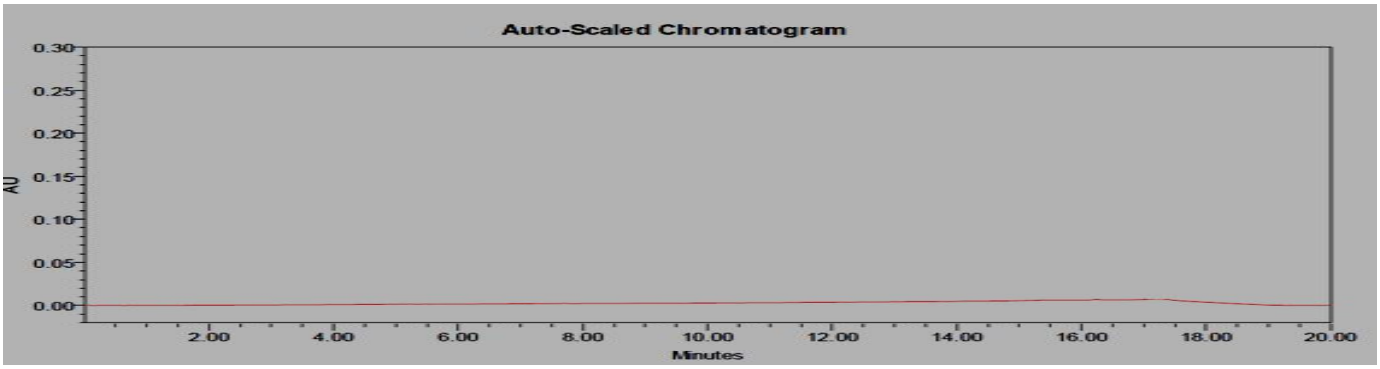

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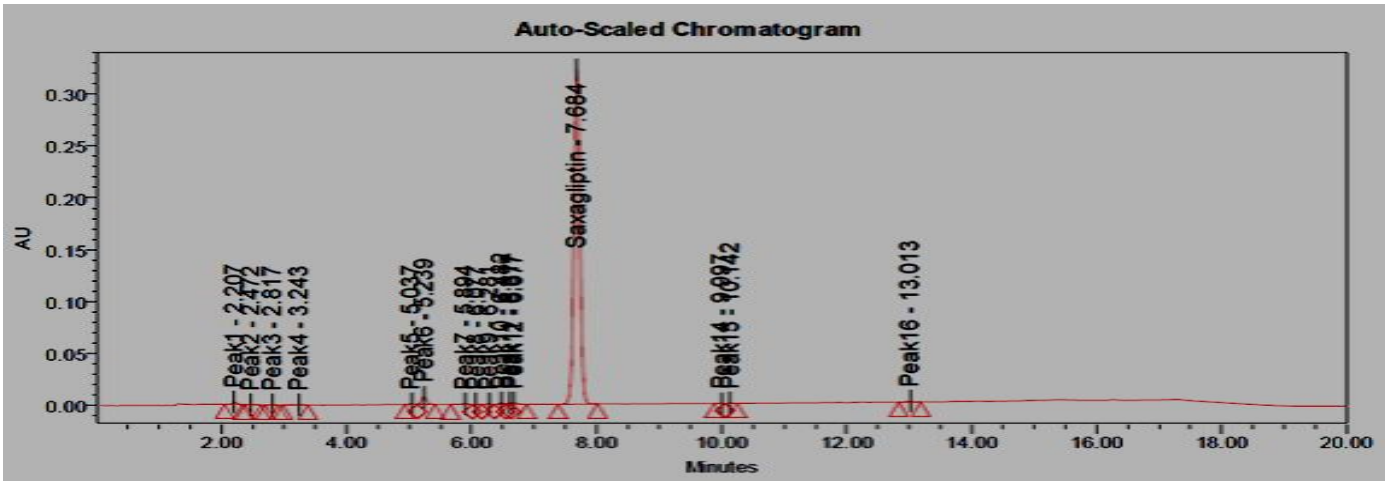
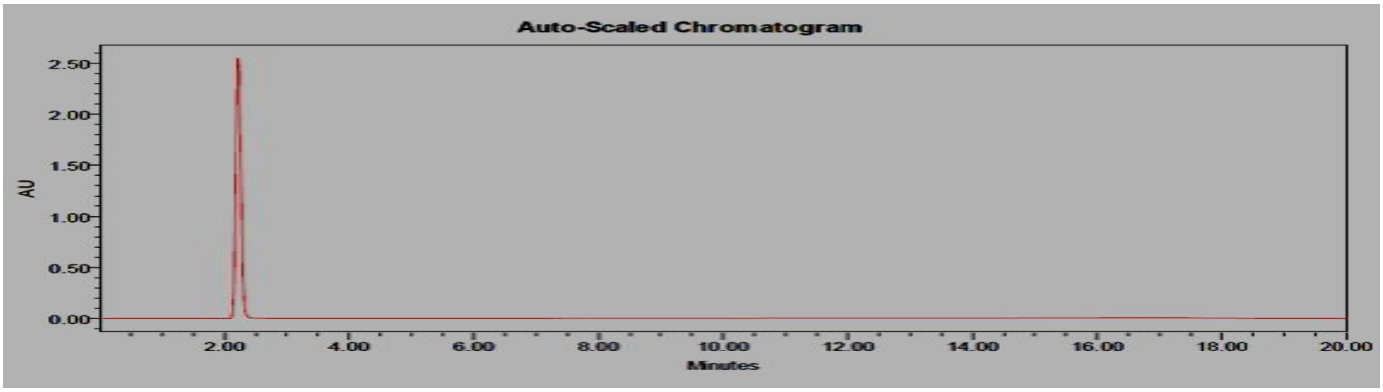


(i)



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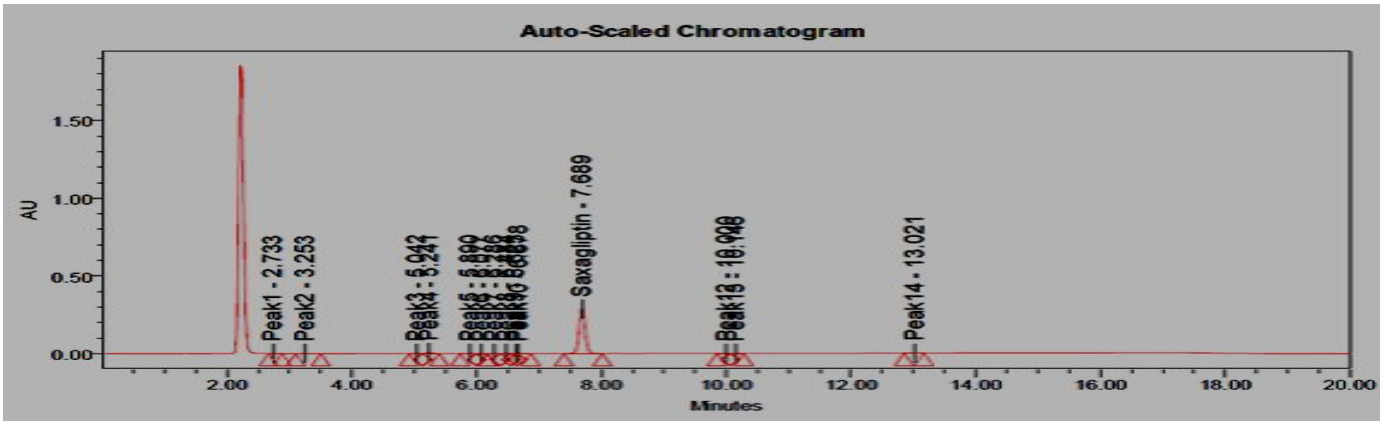
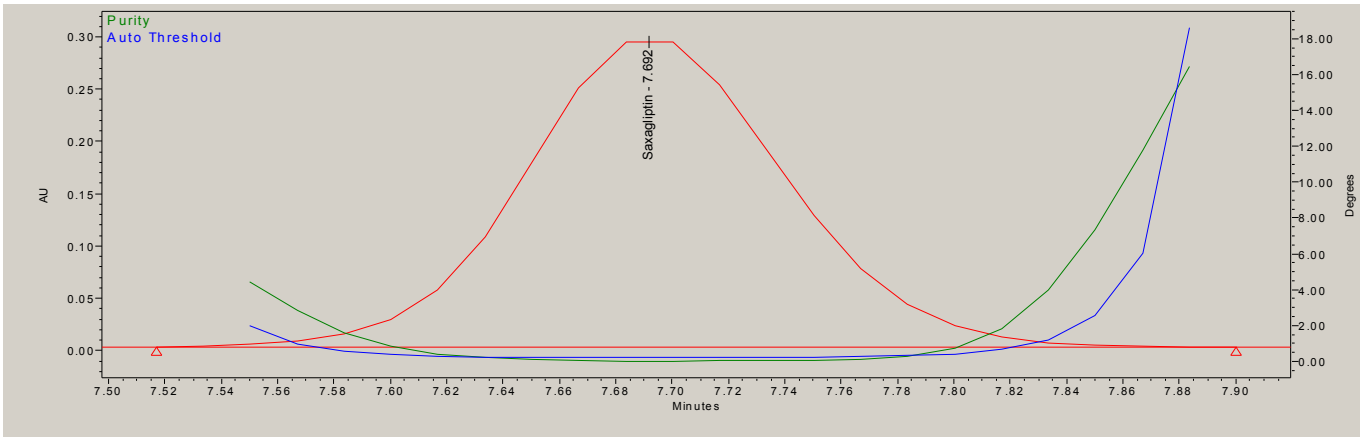


(k)



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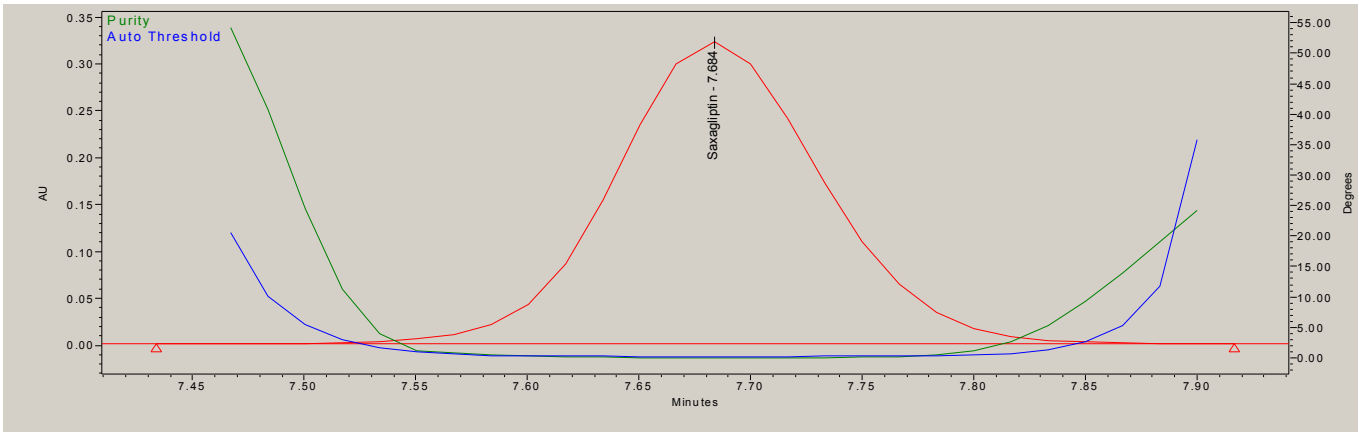

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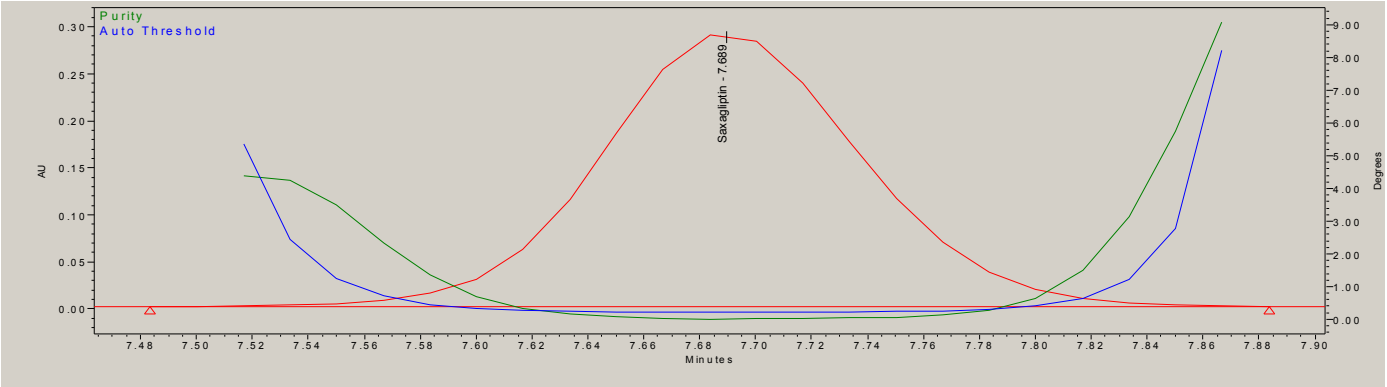

(n)

Fig-3 Typical chromatograms of (a) Blank (b) Standard (c) Sample (d) precision injections (e) Linearity injections (f) Acid blank (g) Acid sample (h) Base blank (i) Base sample (j) Peroxide blank (k) Peroxide sample (l)Purity plot of Acid (m) Purity plot of Base (n) Purity plot of Peroxide

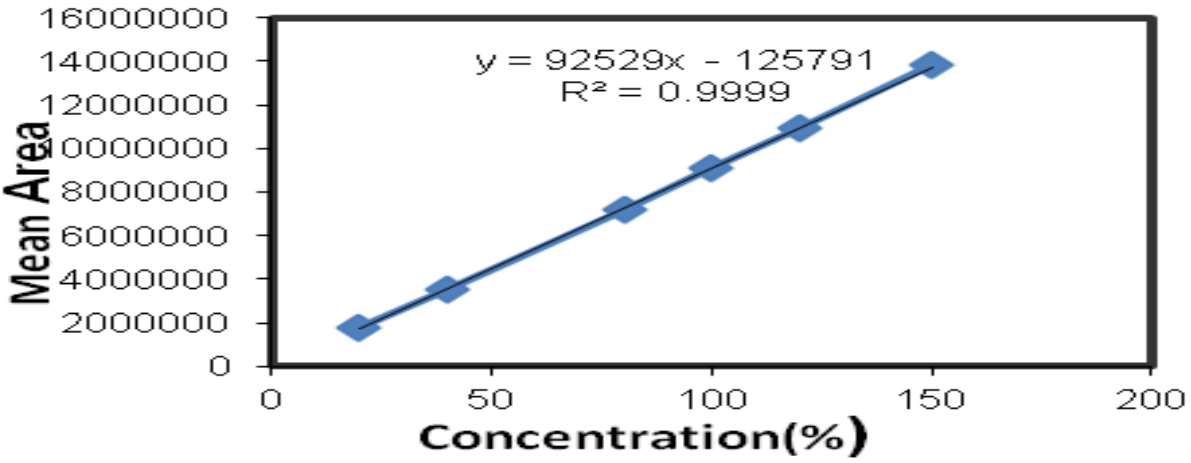

Fig-4 Linearity of Saxagliptin

Table-1 Results for linearity of Saxagliptin

Linearity level	% Level	Area
1	20	380548
2	40	761116
3	80	1544951
4	100	1933417
5	120	2298366
6	150	2900156
Correlation co-efficient		0.999959
	intercept	-8403.36
	slope	19350.93

Table-2 forced degradation results for Saxagliptin

Stress condition	Drug recovered (%)	Drug decomposed (%)
Standard drug	100	-
Acid degradation	94.72	5.28
Alkali degradation	88.70	11.30
Oxidation degradation	95.37	4.63
Thermal degradation	96.65	3.35
Photolytic degradation	99.78	0.22

Table-3 Recoveries study for Saxagliptin

Accuracy (Recovery) study							
Accuracy Level	**Set No**	**Amount Added (µg/ml)**	**Amount Found (µg/ml)**	**Recovery (%)**	**Average recovery**	**Std Dev.**	**% RSD**
50%	1	125.24	125.02	99.82	99.89	0.23	0.23
	2	125.12	124.96	99.7			
	3	124.96	125.14	100.14			
100%	1	250.06	249.75	99.88	100.01	0.12	0.12
	2	250.12	250.36	100.1			
	3	250.18	250.32	100.06			
150%	1	375.2	375.02	99.95	100.01	0.05	0.05
	2	375.08	375.18	100.03			
	3	375.14	375.28	100.04			

Table-4 Precision results for Saxagliptin

Study	Set no	Assay (%)	Mean assay(%)	Stdev	RSD%
Method precision	1	100.22	100.07	0.12	0.12
	2	100.12			
	3	99.98			
	4	99.9			
	5	100.16			
	6	100.02			
Intermediate precision	1	99.86	99.96	0.2	0.2
	2	100.14			
	3	99.65			
	4	100.1			
	5	99.86			
	6	100.16			

Table -5 Robustness results for Saxagliptin

Robust conditions	variation	Retention time(min)	USP Tailing	USP Plate count
Flow	1.1ml	8.35	1.08	30765
	1.2ml	7.68	1.04	31459
	1.3ml	6.9	1.01	33432
Temperature	20°c	7.84	1.07	30012
	25°c	7.68	1.04	31459
	30°c	7.42	1.02	33648
PH	4.9	7.55	1.02	34864
	5.0	7.68	1.04	31459
	5.1	7.8	1.08	30142

CONCLUSIONS

A validated RP-HPLC method has been developed for determination of Saxagliptin in presence of degradation impurities. The proposed method was found to be a new, simple, precise, linear, accurate and specific. Degradation impurities did not interfere with the retention time of Saxagliptin, and assay method is thus stability indicating.

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