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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF SAXAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract: Three simple, precise and economical UV spectrophotometric methods have been developed for the estimation of Saxagliptin in bulk and pharmaceutical formulations. Saxagliptin is an antidiabetic drug belonging the chemical class is dipeptidyl peptidase-4 enzyme (DPP-4) inhibitor. Saxagliptin has absorbance maxima at 211 nm in zero order spectrum method (Method A), and in the first order derivative spectra, showed sharp peak at 204 nm when n = 1 (Method B). The drug followed the Beer- Lambert's law in the concentration range of 5-50 µg/ml in all two methods. Results of the analysis, validated statistically and by recovery studies were found to be satisfactory.

Key words: Saxagliptin (onglyza); Ultraviolet spectrophotometry; Zero order spectrum; First order derivative & spectroscopy

INTRODUCTION:

Saxagliptin previously identified as BMS-477118, is an oral hypoglycemic (anti-diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Chemically, Saxagliptin is (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. DPP-4 is an enzyme that breaks down incretin hormones. As a DPP-4 inhibitor, 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 rate of gluconeogenesis in the liver Dipeptidyl peptidase-4's role in blood glucose regulation is thought to be through degradation of GIP and the degradation of GLP-1.Because incretin hormones are more active in response to higher blood sugar levels (and are less active in response to low blood sugar), the risk of dangerously low blood sugar (hypoglycemia) is low with saxagliptin monotherapy.Saxagliptin is not official in any of the pharmacopoeias and Merck Index & Martindale,The Complete Drug Reference. Literature survey has indicated that there are reported few analytical methods for determination of Lornoxicam in plasma by UV spectroscopy, HPLC, and other few methods like RP-HPLC has been reported for analysis of combination formulation of saxagliptin. Hence the objective of the work is to develop simple, precise, accurate, sensitive, rapid and economical UV Visible Spectrophotometric methods for the estimation of Saxagliptin (SXG) in bulk and pharmaceutical formulations.

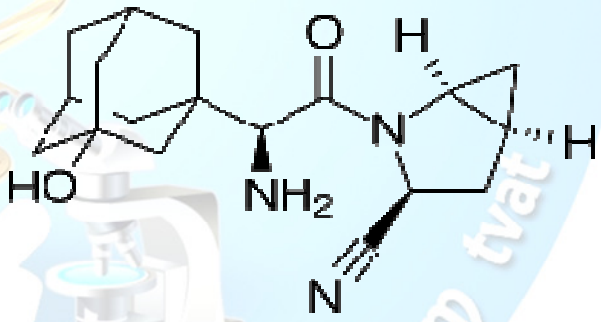

Figure 1: Chemical structure of Saxagliptin

MATERIALS AND METHODS:

Materials

Saxagliptin (SXG) bulk drug was obtained from Neuland laboratories ltd, Hyderabad, Telangana India. The commercially tablets of SXG are available in Indian market as Onglyza tablet containing 5mg Saxagliptin. Other chemicals used were analytical or HPLC-grade and glassware used were Class A grade.

Instruments

Shimadzu UV - 1700 UV/VISIBLE spectrophotometer

with UV probe 2.10 software and 1 cm matched quartz cells were used for absorbance measurements. Analytical balance used for weighing standard and sample was SHIMADZV AUX 220 Uni Bloc PAT 1987.

Preparation of standard stock solution

Accurately weighed 10 mg of SXG working standard was transferred into a 100 mL volumetric flask it was dissolved with Methanol which further sonicated for10 min. The volume was made up to 100 mL with distilled water to give the solution containing 100 µg/mL of SXG.

Selection of λmax

The standard stock solution was further diluted with water to get a 10 µg/mL of concentration. The solution was scanned between 200 and 400 nm using water as blank. The UV spectrum of SXG in water had shown λmax at 211 nm in zero order spectrum (method A) and 204 nm in first order derivative method (method B). Hence, it was selected for the analysis of SXG (Figure 1 and 3).

Preparation of the calibration curve

Aliquots of standard stock solution were further diluted with water to get the solutions of concentration 5–50 µg/mL. The absorbances were measured at 211 nm and 204 nm against water as blank. All measurements were repeated three times for each concentration. The calibration curve was constructed by plotting mean of absorbance against corresponding concentration.

Preparation of the sample solution :

Method A: Aliquots of standard stock solution were pipetted out and suitably diluted with distilled water to get the final concentration of 5, 10,15,20, upto 50 µg/ml of standard solutions. The solutions were scanned in the spectrum mode from 400 nm to 200 nm wavelength range and the zero order derivative spectra was obtained (Fig.2). The maximum absorbance of Saxagliptin was observed at 211 nm. The drug followed the Beer-Lambert's law in the concentration range of 5-50 µg/ml. The calibration curve was plotted as absorbance against concentration of Saxagliptin. The coefficient of correlation (r), slope and intercept values of this method are given in Table III. The concentrations of sample solutions were determined from calibration curve.

Method B: The first order derivative spectra at n=1 showed a sharp peak at 204.0 nm (Fig.4). The absorbance difference at n=1 (dA/dl) was calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solution. The standard drug solutions were scanned in the first order derivative spectra. A calibration curve was plotted taking the absorbance difference (dA/dl) against the concentration of Saxagliptin. The coefficient of correlation (r), slope and intercept values of this method are given in Table III. The method was applied for determination of concentration of sample solution.

METHOD VALIDATION:

The developed method was validated as per ICH guidelines' for following parameters [5].

Linearity

The linearity was studied in the concentration range of 5–50 µg/mL at 211 nm and 204 nm.

Specificity and selectivity

The spectra obtained from tablet solutions were identical with that obtained from standard solution containing an equivalent concentration of SXG. This showed that there was no any interference from excipients. Therefore, it could be said that developed method is highly selective.

Recovery studies

To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100%, and 120% level to preanalyzed samples and subsequent solutions were reanalyzed. At each level, three determinations were performed.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and interday precisions.

Repeatability

Repeatability of the method was determined by analyzing six samples of same concentrations of drug. Graphs were recorded, and the area of each graph was measured.

Intraday and interday precision

Intraday precision was determined by analyzing the drugs at three different concentrations and each concentration for three times, on the same day. Interday precision was determined similarly, but the analysis being carried out daily, for three consecutive days.

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ±2 nm. For changes of conditions, the sample was assayed in tripli-

cate.

Solution stability

The stability of the standard solution was tested at intervals of 1, 6, 12 and 24 h. The stability of solutions was determined by comparing absorbance of SXG.

RESULTS AND DISCUSSION:

All methods A, and B for the estimation of Saxagliptin (SXG) in tablet dosage form were found to be simple, accurate, specific and reproducible. Beer-Lambert's law was obeyed in the concentration range of 5-50 µg/ml in all the methods. The values of standard deviation were satisfactory low and the recovery studies were close to 100%. Saxagliptin showed a broad spectrum the derivative spectroscopy method applied has the advantage that it locates the hidden peaks in the normal spectrum when the spectrum is not sharp and it also eliminates the interference caused by the excipients present in the formulation. Hence these methods can be useful in the routine analysis of Saxagliptin in bulk drug and formulations.

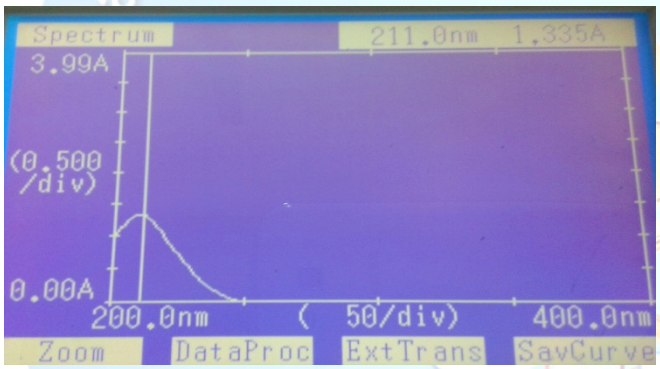

Fig. 2: Zero order spectrum of Saxagliptin

Table I: Standard calibration table for saxagliptin Zero Order Derivative Spectrum

Sr.No.	Conc. (µg/ml)	Absorbance
1	5	0.125
2	10	0.248
3	15	0.375
4	20	0.512
5	25	0.623
6	30	0.746
7	35	0.858
8	40	0.972
9	50	1.247

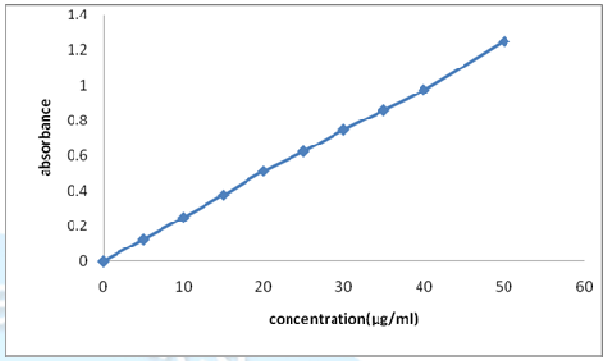

Fig 3: Calibration curve of Saxagliptin in zero order derivative spectrum

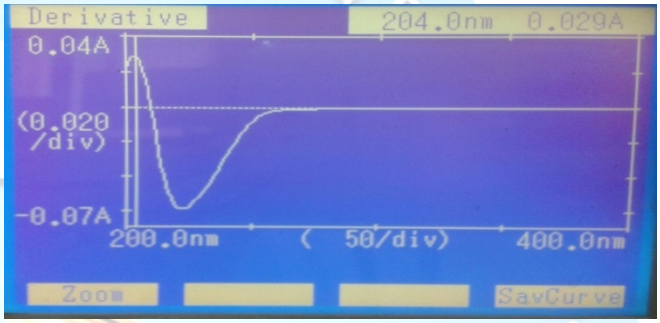

Fig. 4: first order derivative spectrum of Saxagliptin

Table II: Standard calibration table for Saxagliptin First order derivative spectrum

Sr.No.	Conc (µg/ml)	Absorbance
1	05	0.003
2	10	0.006
3	15	0.009
4	20	0.012
5	25	0.015
6	30	0.018
7	35	0.021
8	40	0.024
9	45	0.026
10	50	0.029

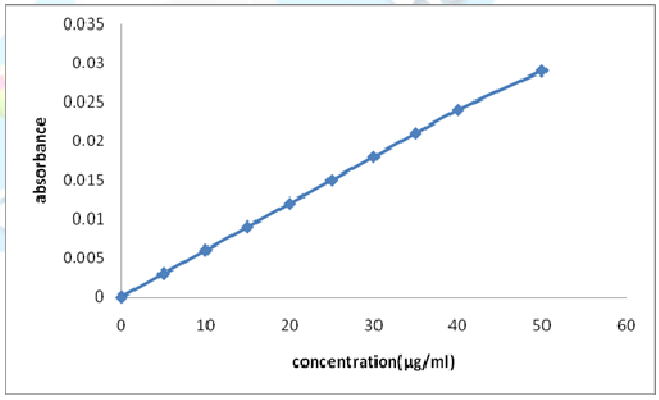

Fig. 5: Calibration curve of Saxagliptin in first order derivative spectrum.

Table III : Optical characteristic and other parameter of Saxagliptin

Parameter	Method A	Method B
λMax (nm) / wavelength range (nm)	211	204
Beer's-lambert's range (µg/ml)	5-50	5-50
Coefficient of correlation (r2)	0.9991	0.9981
Regression equation Y = mx + c a. Slope (m); b. Intercept (c)	 0.0247 -	 0.0006 -
LOD(µg/ml)	0.040	0.836
LOQ(µg/ml)	0.121	2.533

Where, x is concentration in µg/ml and Y is absorbance unit.
A is Zero order derivative spectrum method with n = 0.
B is First order Derivative spectrum method with n = 1.

Table IV : Estimation of Saxagliptin in tablet formulation

Method	Tablet formulation	Label claim(mg)	Amount found (mg)	% mean	S.D.	C.O.V.	S.E.
A	T_1	5	5.98	99.76	0.5296	0.5308	0.2162
B	T_1	5	5.99	99.89	0.5829	0.5835	0.2380

Where, T1 (onglyza) is brand of tablet formulation.
* Mean of six estimations (n=6).

Tabel V: Recovery study data

Method	Tablet	Level of recovery (%)	Amount present (mg/tab)	Amount of drug added(mg)	Amount recovered (mg)	% recovery	S.D.	C.O.V.	S.E.
A	T_1	80	10	8	17.88	99.35	0.1955	0.1967	0.1129
		100	10	10	19.89	99.47	0.2255	0.2267	0.1302
		120	10	12	21.89	99.50	0.2084	0.2094	0.1203
B	T_1	80	10	8	17.95	99.74	0.2829	0.2836	0.1633
		100	10	10	19.98	99.91	0.1607	0.1608	0.0928
		120	10	12	22.06	100.25	0.4732	0.4720	0.2731

* Mean of six estimations (n=6).

Table VI: Result of Repeatability, Intraday, and Interday precision studies; Data for repeatability

Sample conc. (µg/mL)	No. of Measurement	Absorbance	Statistical Analysis
Method A 10 µg/mL	1	0.544	Mean= 0.543 S.D= 0.002966 %RSD= 0.54
	2	0.542	
	3	0.543	
	4	0.542	
	5	0.548	
	6	0.539	
Method B 10 µg/mL	1	0.021	Mean= 0.023 S.D= 0.002732 %RSD= 0.02
	2	0.024	
	3	0.026	
	4	0.020	
	5	0.021	
	6	0.028	

Method A:

Sr.No.	Component	Mean *	S.D.	C.O.V.	S.E.
1	Intra-Day	99.86	0.0674	0.0674	0.0275
2	Inter-Day	99.81	0.0871	0.0872	0.0355

Method B:

Sr.No.	Component	Mean *	S.D.*	C.O.V.*	S.E.
1	Intra-Day	99.82	0.2264	0.2268	0.0924
2	Inter-Day	99.77	0.1797	0.1801	0.0733

*Mean of three determinations

Table VII: Result of robustness studies

Method wavelength (nm)	Condition (nm)	% Assay *	% RSD
211 (method A)	212	98.16	0.77
	208	99.44	0.80
204 (method B)	205.5	99.20	0.84
	202	97.66	0.76

*Mean of three determinations.

CONCLUSION

The developed UV spectrophotometric method for the determination of SXG has the advantage of being fast, simple, reproducible, and applicable over a wide concentration range with high precision and accuracy. The method was validated as per the ICH guidelines. The results of the validation tests were found to be satisfactory and therefore those methods can be applied successfully to analyze drug tablet formulations.

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