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Spectrophotometric Method for Determination Ofsaxagliptine in Bulk and Pharmaceutical Dosage Forms Using Ion Pair Complexation Method

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ABSTRACT
Two simple, accurate, precise and sensitive spectrophotometric methods have been developed and validated for determination of saxagliptine in bulk and pharmaceutical dosage form. Method A and B involves the formation of a colored chloroform extractable ion pair complex of drug with bromothymol blue and Bromocresol green absorbing maximally at 425nm and 415nm. Beer's law is obeyed in the concentration range of 6-24µg/ml for methods A and B. Molar absorptivity, Sandell's sensitivity, association constant, Limit of Quantification and Limit of Detection were calculated. The proposed methods were successfully applied for the determination of saxagliptine in pharmaceutical formulation.

KEYWORDS: Saxagliptine, Spectrophotometry, Bromothymol blue, Bromocresol green, ion-pair complex, Validation.

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic progressive metabolic disorder characterized by absolute or relative insulin deficiency(1). Expected rise in prevalence of diabetes is mainly due to increased life span because of better healthcare facilities and increase in diabetic risk factors, especially physical inactivity and obesity due to sedentary life style. Pancreatic β-cell function is gradually deteriorated in patients of T2DM which is reflected into inadequate glycemic control on a long run(2).

Saxagliptin is chemically known as (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2 azabicyclo hexane-3-carbonitrile) with molecular formula of C18H25N3O2 and molecular weight of 315.41g/mol(3). Saxagliptin is a selective and potent dipeptidyl peptidase (DPP)-4 inhibitor, approved as an adjunct to diet and exercise to improve glycemic control in type 2 diabetes mellitus (T2DM). In patients with T2DM, once-daily administration of saxagliptin before breakfast achieves sustained inhibition of plasma DPP-4 activity and reduction of postprandial hyperglycemia, including after dinner, associated with an increase in plasma glucagon-like peptide-1 levels(4,5,6).

Literature review indicates that few UV spectrophotometric method [7-14] HPLC method [15-29]. Theabove reportedchromatographic methodsemployedsophisticated and expensive instrumentationthatare generally not availableinmostofthequality controllaboratories ofunderdeveloped anddeveloping countries. As aresult, theapplicationsof thesemethodsfor thequantificationof Saxagliptine inbiologicalsamples, bulkand pharmaceutical formulations are limited.

II. MATERIALS AND METHODS:

APPARATUS

A double beam UV- Visible Spectrophotometer, (LAB INDIA-3000) with UV WIN software and 1cm quartz cell in the wavelength range of 200-400nm was used for spectrophotometric measurements. Drug and the reagents were weighed using Sartorius weighing balance. A calibrated digital pH (Systronics, model-361) was used for pH measurement.

Preparation of reagents and solutions:

STANDARD SOLUTION OF SAXAGLIPTINE

Saxagliptine working standard was procured from HiQ Pharma Labs Pvt Ltd., Hyderabad, India. Standard stock solution of Saxagliptine was

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prepared by dissolving accurately weighed 100mg of drug in 100ml volumetric flask and diluted up to the mark with distilled water.

Standard solution of bromothymol blue

0.1% w/v of Bromothymol blue was prepared by dissolving 0.100g in distilled water in 100ml volumetric flaskand diluted up to the mark with water.

Standard solution of bromocresol green

0.1% w/v of Bromocresol green was prepared by dissolving 0.100g in distilled water in 100ml volumetric flask by adding 2ml of 0.1M NaOH for better solubility and diluted up to the mark with water.

Preparation of phosphate buffer ph 3.0

1.78g of Sodium dihydrogen phosphate buffer was accurately weighed and dissolved in 1000ml distilled water and pH was adjusted to 3.0.

STANDARD SOLUTION OF 0.1N HCL

0.1 N HCl was prepared by dissolving 0.85ml in distilled water in 100ml volumetric flask and diluted up to the mark with water.

General procedure for sample preparation

METHOD A (BTB)

Aliquots (0.2-1.0ml) of Saxagliptine standard solution were transferred in to 10ml volumetric flask. To each flask 1ml of Bromothymol blue, 0.8ml of 0.1% Hydrochloric acid was added. The volume was adjusted to 5ml with water and then extracted with 5ml chloroform. Absorbance of each solution was measured at 425nm.

METHOD B (BCG)

Aliquots (0.2-1.0ml) of Saxagliptine standard solution were transferred in to 10ml volumetric flask. To each flask 2ml of Bromocresol green, 2ml of buffer solution was added. The mixture was extracted with 10ml chloroform. The organic phase was extracted and dehydrated by passingoveranhydrous sodium sulphate and volume was made up to the mark with chloroform. Absorbance of each solution was measured at 415nm against a reagent blank.

PROCEDURE FOR THE ASSAY OF DOSAGE FORMS

The tablet formulation of Saxagliptine labeled to contain 5mg was purchased. Twenty tablets were accurately weighed and finely powdered in a mortar. A portion of tablet powder equivalent to 10mg was weighed and transferred into 100ml volumetric flask and the mixture was sonicated for 15mins. The mixture was filtered through Whatman No.1 filter paper. The solution was made up to the mark with distilled and contents were analyzed by the proposed methods.

III. RESULTS AND DISCUSSION

METHOD DEVELOPMENT

Saxagliptine forms ion-pair complexes with bromothymol blue and bromocresol green. This property of drug was followed for development of sensitive colorimetric methods for analysis of drug. The complex of Saxagliptine with BTB and BCG showed maximum absorbance at 425nm and 415nm respectively.

EFFECT OF BTB

The effect of the volume of 0.1% w/v Bromothymol blue on the absorbance of the yellow colored complex was studied in the range of 0.2-2.0ml. The absorbance increases with the increase in the volume of Bromothymol blue up to 1ml. Further addition of Bromothymol blue showed decrease in the absorbance. Therefore, 1ml of 0.1% w/v Bromothymol blue was chosen as an optimum value (Figure 1).

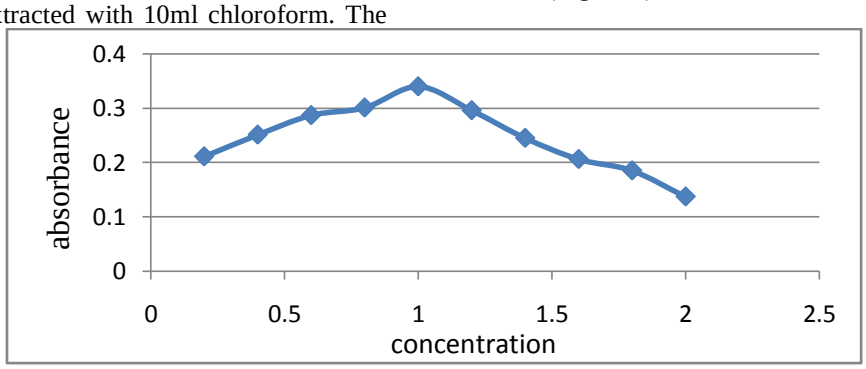

Figure 1: Effect of volume of Bromothymol blue

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EFFECT OF BCG

The effect of the volume of 0.1% w/v Bromocresol green on the absorbance of the yellow colored complex was studied in the range of 0.4-4.0ml. The absorbance increases with the increase in the volume of Bromocresol green up to 2ml. Further addition of Bromothymol blue showed decrease in the absorbance. Therefore, 2ml of 0.1% w/v Bromocresol green was chosen as an optimum value (Figure 2).

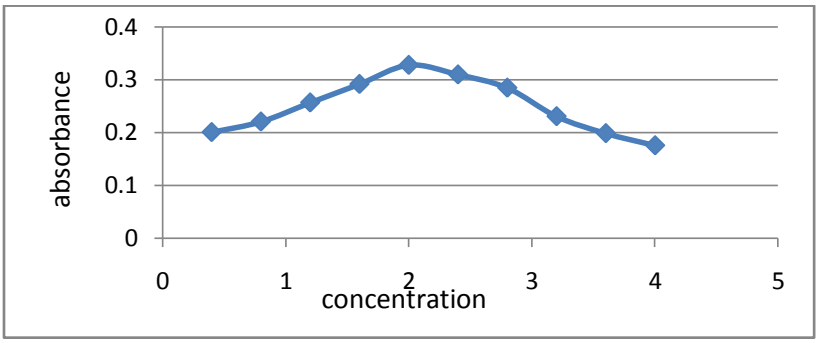

Figure 2: Effect of volume of Bromocresol green

EFFECT OF VOLUME OF 0.1N HCL

The effect of volume of 0.1N HCl on the absorbance of yellow colored complex was studied in the range 0.2-2.0ml. The absorbance increases with increase in the volume of Sodium carbonate and becomes constant at 0.8ml. Further addition of HCl showed decrease in the absorbance. Hence 0.8ml of 0.1N HCl was selected as an optimum value (Figure 3).

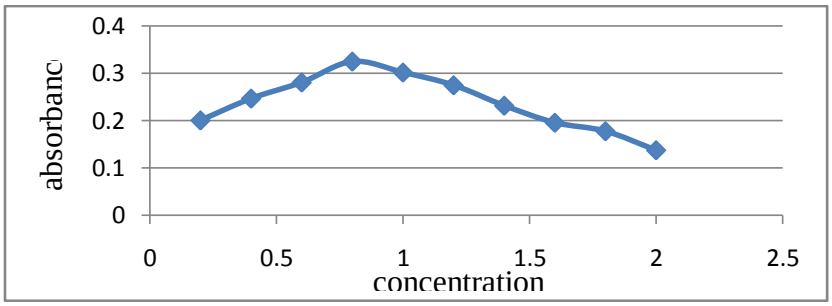

Figure 3: Effect of volume of 0.1N HCl

EFFECT OF PH

The influence on pH on the ion-pair formation between Saxagliptine and BCG was studied using sodium dihydrogen phosphate buffer in the range of 2-5. The maximum absorbance value was obtained at pH 3. It was also observed that addition of 2ml of buffer showed maximum absorbance.

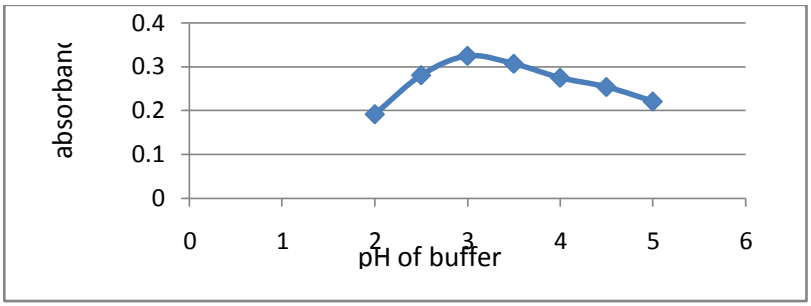

Figure 4: Effect of pH

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EFFECT OF EXTRACTING SOLVENTS

Different solvents (methanol, dichloromethane, chloroform, ethyl acetate and 1,2-dichloromethane) were tested. Maximum absorbance and higher selective extraction of the ion-pair complex were achieved using chloroform as an extracting solvent.

METHOD VALIDATION

LINEARITY: The relation between the absorbance and final concentration ofSaxagliptine was found to be linear over the concentration range of 6-24µg/ml for methods A and B. Results are shown in (Figure 5,6).Linearity overlay graphs are shown in Figure 7,8.

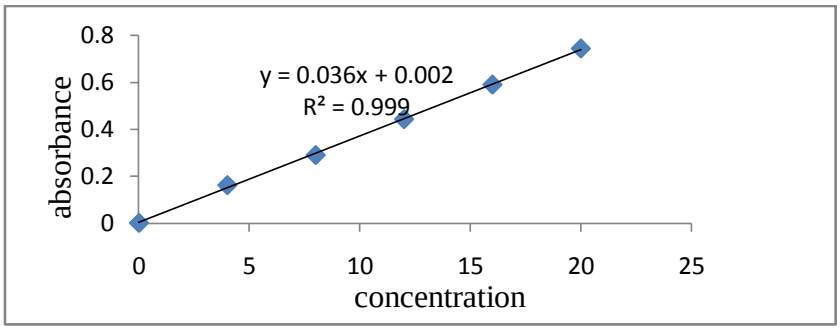

Figure 5: Linearity graph of Saxagliptine (Method A)

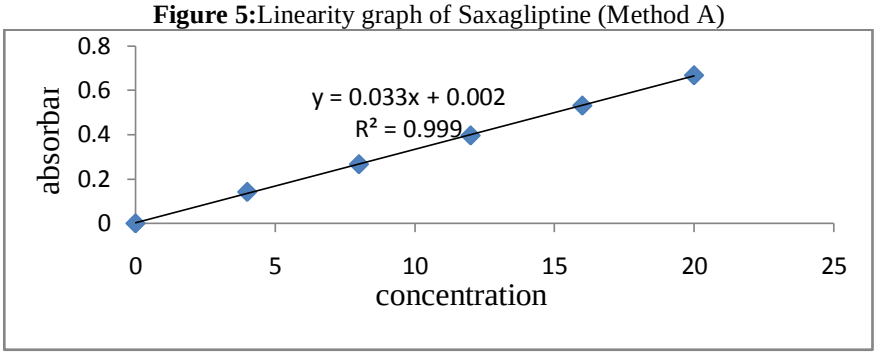

Figure 6: Linearity graph of Saxagliptine (Method B)

PRECISION:

The repeatability (intra-day precision) of the proposed method was determined by replicate analysis (n=5) of standard solutions at three concentration levels (6µg/ml, 14µg/ml and 24µg/ml). The intermediate precision (inter-day precision) was conducted by repeating the analysis over a period of three consecutive days.

The precision of the methods was expressed as standard deviation (SD) and percentage relative standard deviation (%RSD). The results are summarized in (Table 2).The SD and % RSD obtained by both methods are found to be in the acceptable range. Therefore, it can be considered to be satisfactory.

Table 2: Accuracy and Precision of the proposed methods

Methods	Type of Assay	Concentration(µg/mL) Taken	Found	SD	%RSD	%Recovery	%Error
A (BTB)	Inter day	6	6.008	0.016	0.396	100.2	-0.2
		14	13.989	0.031	0.258	99.9	0.1
		24	24.02	0.03	0.15	100.08	-0.08
	Intra day	6	5.981	0.029	0.726	99.5	0.5
		14	13.33	0.083	0.455	99.3	0.7
		24	24.01	0.143	0.714	100	0.0
	Inter day	6	5.991	0.016	0.402	99.8	0.2
		14	14.010	0.017	0.153	100.1	-0.1

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B (BCG)		24	24.080	0.017	0.086	100.4	-0.4
	Intra day	6	6.011	0.032	0.799	100.3	-0.3
		14	14.002	0.052	0.434	100.05	-0.1
		24	23.99	0.03	0.13	99.97	0.0

ACCURACY:

The accuracy of the proposed method was established by performing intra-day and inter-day assays by determining at different levels of drug concentrations [lower concentration (50%), intermediate concentration (100%) and higher concentration (150%)] within 1 day and 3 consecutive days, respectively. The accuracy of the methods is expressed as percentage recoveries and percentage error. The results obtained by both the methods are found to be in the acceptable range. Therefore, we can say it can be considered as satisfactory.

In addition, accuracy and validity of the proposed methods were determined by standard addition technique. The pre analyzed samples were spiked with additional 50,100 and 150% were once again analyzed by the proposed methods. The accuracy of the methods was evaluated by percentage recovery of the Saxagliptine. The average recovery and percentage standard deviation values (Table 3) of the methods lying in the acceptable range show that the methods are accurate.

Table 3: Results of standard addition technique of proposed method

Method	Tablet Concentration(mg)	Spiked	Found	SD	%RSD	%Recovery
A (BTB)	5	10	9.990	0.041	0.408	99.9
	5	20	19.84	0.08	0.39	99.19
	5	30	29.66	0.22	0.75	98.87
B (BCG)	5	10	9.953	0.040	0.406	99.5
	5	20	20.02	0.03	0.150	100.08
	5	30	29.985	0.03	0.09	99.95

ROBUSTNESS:

The robustnessofthepproposedmethodswas checkedforeach operationalparameterand investigated. The operational parameters were:

Volume of 0.1% Bromothymol blue: 1.0 ± 0.1 mL

Volume of 0.1N HCl: 0.8± 0.1 mL

Volume of 0.1% w/v Bromocresol green: 2.0 ± 0.1 ml

The robustnessof themethodswas assessed byanalyzing the Saxagliptine attwo different concentrationlevels (6 and24µg/mL).The percent recovery and % RSD of the method (Table4)was foundtobe satisfactory, indicatingthat the method is robust.

Table 4: Robustness of proposed method

S.No	Parameter	vol	(6µg/ml) Absorbance	% Recovery	% RSD	(24µg/ml) Absorbance	% Recovery	% RSD
1	Bromothymol blue	0.9 1.0 1.1	0.205 0.202 0.207	99.52 97.58 100	1.29 0.75 0.39	0.881 0.887 0.884	99.55 100.23 99.89	1.09 0.45 0.79
2	Hydrochloric acid	0.7 0.8	0.206 0.203	99.52 98.07	0.37 0.40	0.883 0.885	99.77 100	1.21 0.51

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		0.9	0.205	99.03	0.71	0.884	99.89	0.62
3	Bromocr esol green	1.9 2.0 2.1	0.143 0.142 0.143	100 99.3 100.0	0.40 0.51 0.40	0.667 0.668 0.668	99.9 100 100	0.15 0.10 0.10

IV. CONCLUSION:

The proposed methods don't require any expensive sophisticated apparatus. The methods are simple, rapid and robust and have high precision and accuracy. The BTB and BCG are inexpensive reagents and are available in any analytical laboratory. Hence, these methods are valuable for its routine application in quality control laboratories for the analysis of Saxagliptine.

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