



SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF ANTI-DIABETIC DRUG IN BULK AND PHARMACEUTICAL DOSAGE FORMS USING SULPHONPHTHALEIN ACID DYES

Suma Latha, Vinutha Kommineni*, Sneha Avija, R Pavani, B Ramya, P Sreedevi, M Bhagavan Raju

Department of Pharmaceutical Analysis, Sri Venkateshwara College of Pharmacy, Madhapur, Hyderabad, India

*Corresponding author E-mail: vinutha08.ch@gmail.com

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ABSTRACT

Key Words

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



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.

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 little UV spectrophotometric method [7-14] HPLC method [15-29]. The above reported chromatographic methods employed sophisticated and expensive instrumentation

that are generally not available in most of the quality control laboratories of under developed and developing countries. As a result, the applications of these methods for the quantification of Saxagliptine in biological samples, bulk and pharmaceutical formulations are limited.

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 PvtLtd., Hyderabad, India. Standard stock solution of Saxagliptine was 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 flask and 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 passing over anhydrous 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.

Results and discussion

Method development: Saxagliptine forms ion-pair complexes with bromothymol blue and bromocresolgreen. 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).

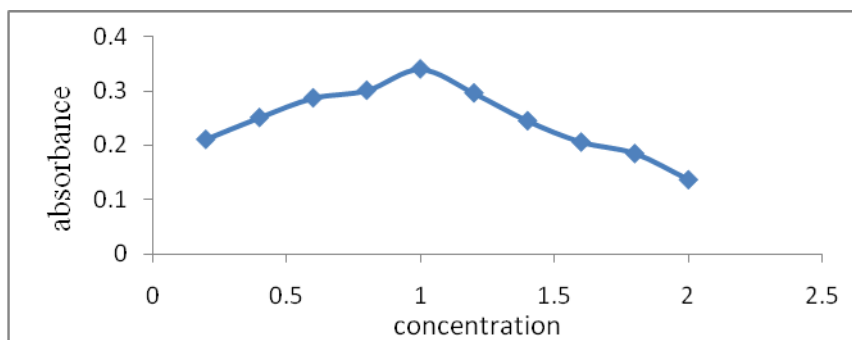


Figure 1: Effect of volume of Bromothymol blue

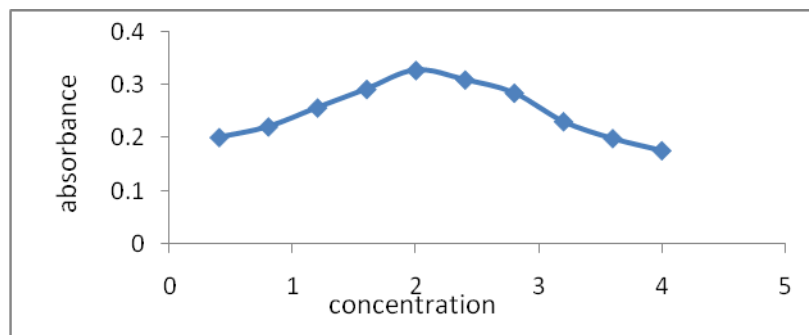


Figure 2: Effect of volume of Bromocresol green

Table1: Thermodynamic studies, linearity and Sensitivity of the proposed methods

Parameter	BTB	BCG
Beer's law limit ($\mu\text{g/mL}$)	6-24	6-24
Regression Equation ($y=mx+c$)	$Y = 0.0368x + 0.0027$	$Y = 0.0332x + 0.0027$
Slope (m)	0.0368	0.0332
Intercept (x)	0.0056	0.0057
Molar Absorptivity	1.36×10^4	1.22×10^4
Regression coefficient (r^2)	0.9994	0.9998
Sandell's sensitivity	0.027322	0.030326
LOD ($\mu\text{g/mL}$)	0.9	0.06
LOQ ($\mu\text{g/mL}$)	2.7	0.18

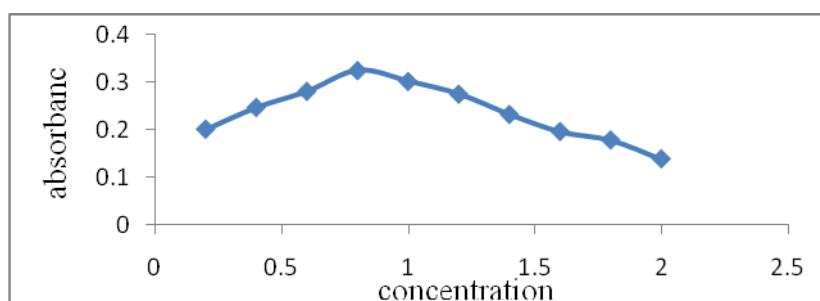


Figure 3: Effect of volume of 0.1N HCl

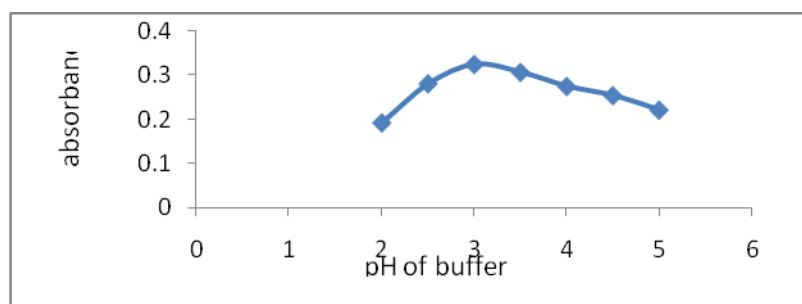


Figure 4: Effect of pH

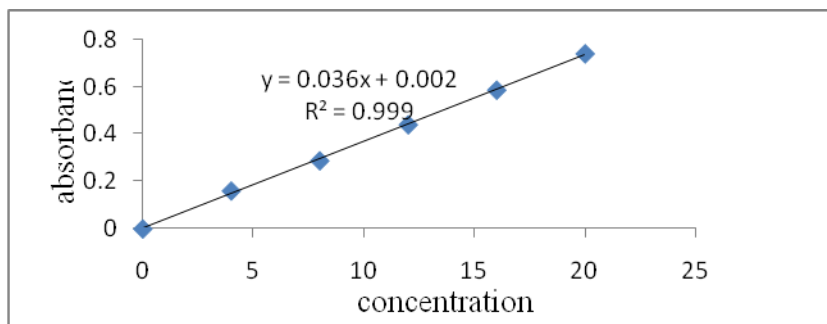


Figure 5:Linearity graph of Saxagliptine (Method A)

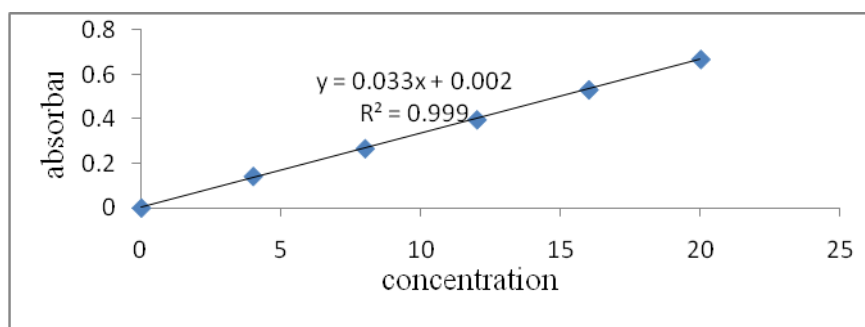


Figure 6: Linearity graph of Saxagliptine (Method B)

Table 2: Accuracy and Precision of the proposed methods

Methods	Type of Assay	Concentration(μ g/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
B (BCG)	Inter day	6	5.991	0.016	0.402	99.8	0.2
		14	14.010	0.017	0.153	100.1	-0.1
		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

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

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	0.205	99.52	1.29	0.881	99.55	1.09
		1.0	0.202	97.58	0.75	0.887	100.23	0.45
		1.1	0.207	100	0.39	0.884	99.89	0.79
2	Hydrochloric acid	0.7	0.206	99.52	0.37	0.883	99.77	1.21
		0.8	0.203	98.07	0.40	0.885	100	0.51
		0.9	0.205	99.03	0.71	0.884	99.89	0.62
3	Bromocresol green	1.9	0.143	100	0.40	0.667	99.9	0.15
		2.0	0.142	99.3	0.51	0.668	100	0.10
		2.1	0.143	100.0	0.40	0.668	100	0.10

Table 5: Results of analysis in tablet formulation

Method	Formulation	Labeled claim(mg)	Found ± SD (n=5)	RSD(%)	Recovery(%)
A (BTB)	ONGLYZA	5	4.86 ± 0.094	0.953	99.52
B (BCG)	ONGLYZA	5	4.79 ± 0.091	0.948	99.49

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).

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)

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.

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

Sensitivity: According to the ICH guidelines, the sensitivity parameters like molar absorptivity, Sandell's sensitivity, Limit of Detection and Limit of Quantification were calculated and summarized in (Table 1).

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

Precision: The repeatability (intra-day precision) of the proposed method was determined by replicate analysis (n=5) of standard solutions at three concentration levels (6 μ g/ml, 14 μ g/ml and 24 μ g/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.

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.

Robustness: The robustness of the proposed methods was checked for each operational parameter and 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 robustness of the methods was assessed by analyzing the Saxagliptine at two different concentration levels (6 and 24 μ g/mL). The percent recovery and % RSD of the method (Table 4) was found to be satisfactory, indicating that the method is robust.

Application of the methods: The developed spectrophotometric methods were applied to the pharmaceutical formulation containing Saxagliptine through complex formation by BTB and BCG in chloroform. The percent recovery and %RSD (Table 5) clearly showed no interference of any excipients of formulation, thus proving accuracy & precision in the quantification of Saxagliptine

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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REFERENCES

1. Abdulfatai B. Olokoba, Olusegun A. Obateru and Lateefat B. Olokoba. Type 2 Diabetes Mellitus: A Review of Current Trends. *Oman Med J* 2012 : 27(4),269–273.

2. Bassam Abdul Rasool Hassan. Overview on Diabetes Mellitus (Type 2). , J Chromat Separation Techniq 2013:4(3).
3. Augeri DJ, Robl JA, Betebenner DA, Magnin DR and Khanna A .Discovery and preclinical profile of Saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. J Med Chem2005 ; 48, 5025-5037.
4. Kristen Kulasa and Steven Edelman.Saxagliptin: The evidence for its place in the treatment of type 2 diabetes mellitus. Core Evid 2010; 5,23–37.
5. Darshan J. Dave .Saxagliptin: A dipeptidyl peptidase-4 inhibitor in the treatment of type 2 diabetes mellitus J PharmacolPharmacother 2011; 2(4), 230–235.
6. Rose Anderson, Jennifer Hayes and Jeffrey W. Stephens. Pharmacokinetic, pharmacodynamic and clinical evaluation of saxagliptin in type 2 diabetes. Journal Expert opinion on Drug Metabolism & Toxicology 2016 ; 12(4) , 467-473.
7. Pravin cholke, Dr.Mrunalshirsath, Yogita temak, Aditee kagde, Rutujalagad. Development of uv - visible spectroscopy method for simultaneous estimation of saxagliptin hydrochloride in tablet dosage form, international journal of research in pharmacy and pharmaceutical sciences, 3(4) ; 31-34, 2018.
8. Sonalinarsing koli1, Adaviraovenkatraobelvotagi, Rekha pritishkumarmudke, Somnath Ashok patil. UV spectrophotometric method development and validation for estimation of saxagliptin in API and in pharmaceutical dosage form,international journal of pharmacy & pharmaceutical research, 14(4); 2019.
9. Asim M suthar, Laxman M Prajapati, Amit K Joshi R patel, Mohammadali L kharodiya. Estimation of saxagliptin hydrochloride and Dapagliflozin propendiol monohydrate in combined dosage form, Journal of innovations in applied pharmaceutical science,3(2); 2018.
10. Mr.Mohd.Zameeruddin1, Miss.sandhya S. Bundell, Mr.Vishvanath B. Bharkad , Miss.HajeraN.Khan, Mr.Thokesandip T. Development and validation of UV spectroscopic method for simultaneous estimation of dapagliflozin and saxagliptin in synthetic mixture, International journal of pharmacy and analytical research, 8(1): 2019.
11. Sanjeev V.Deshpande, Madhumita A. Roy, shubhangi C. Daswadkar,development and validation of UV spectrophotometric method for estimation of saxagliptin in bulk and pharmaceutical dosage form,International journal of pharmaceuticals & drug analysis, 4(1); 30-34, 2016.
12. R.Kalaichelvi, E. Jayachandran.Validated spectroscopic method for estimation of saxagliptin in pure and from tablet formulation,international journal of pharmacy and pharmaceutical sciences,3(3); 2011.
13. R.Aswini, MM.Eswarudu and P. SrinivasaBabu .A review on analytical methods for estimation of dapagliflozin and saxagliptin bulk and in pharmaceutical dosage forms, International journal of research in pharmacy and chemistry, 8(8): 2018.
14. Pawanjeet.j.chhabda,M.Balaji,Srinivasa sarao, V.K.Ramakrishnaand K.M.Ch.Apparao. Development and Validation of Simplest Stability Indicating RP-HPLC method for Analysis of Saxagliptin and its Forced Degradation Impurities in Bulk Drug and Pharmaceutical Dosage form, International journal of Research and Development in Pharmacy and Life Sciences ,3(3):993-1003;2014.
15. R.PravinCumar,M.Vasudevan and Deecaraman.A Validated RP-HPLC Method for Simultaneous estimation of Metformin and Saxagliptinin

- Tablets. *RasayanJ.Chem*, 5(2),137-141,2012.
16. P.B.N.Prasad,K.Satyanarayana and G.Krishnamohan.Development and Validation of a Method for Simultaneous determination of Metformin and Saxagliptin in formulation by RP-HPLC. *American Journal of Analytical chemistry*, 6,841-850,2015.
 17. SenaCaglar and AliRahmiAlp.A Validated High Performance Liquid Chromatography Method for the Determination of Saxagliptin and Metformin in Bulk,a Stability Indicating Study.*Journal of Analytical & Bioanalytical Techniques*,(12),1-5:2014.
 18. Shaban A Abdalla.Validation of Stability Indicating RP-HPLC method for the estimation for Saxagliptin in Tablet Formulations.*Indo American Journal of Pharmaceutical Research*,3550-3558,2014.
 19. Narendra Kumar Nyola and JeyabalanGovindasamy.Simultaneous estimation of Saxagliptin Hydrochloride and Metformin Hydrochloride in active Pharmaceutical ingredient .*AJPRHC*, 4(3),70-77,2012.
 20. ACK.Prasanna and Kanuri Priyanka.Method Development and validation of Simultaneous determination of Metformin and Saxagliptin in Pharmaceutical Dosage Form by RP-HPLC. *International journal of pharmaceutical,chemical and biological sciences*, 5(1),381-387,2015.
 21. Mohammad Yunoos and D.GowriSankar.Stability indicating Quantitative RP-HPLC method development and validation for simultaneous determination of Metformin hydrochloride and Saxagliptin in bulk and combined tablet dosage form. *J. Chem. Pharm. Res*, 7(4),346-355,2015.
 22. ShubhangiC.Daswadkar, MadhumitaA.Roy, Sanjay G.Walode and Mahendrakumar CB. Quality by Design approach for the development and validation of Saxagliptin by RP-HPLC with application to formulated forms *IJPSR*,7(4),1670-1677,2016 .
 23. Ramesh.J and Senthil Kumar.N. A validated High Performance Liquid Chromatography Method for the determination of Saxagliptin and Metformin in Bulk and Pharmaceutical Dosage Form,a Stability Indicating Study. *IOSR Journal of Pharmacy and Biological Sciences*,11(6):92-100,2016.
 24. LaisEngroffScheeren,Ana Isa PedrosMarcolino,Andrea Ines Horn Adams and Clarice Madalena Bueno Rolim.Stability indicating RP-LC-PDA method for the quantitative analysis of Saxagliptin in Pharmaceutical Dosage form. *Brazilian Journal of Pharmaceutical Sciences*,51(2),461-466,2015.
 25. Md.Saiful Islam,Md.Taleb Hossain,Sukalyan Kumar Kundu,Md.abdul Halim and Md.Rafiquzzaman. Development and Validation of RP-HPLC method for determination of Saxagliptin in Hydrochloride in Bulk and tablet Dosage Form. *World journal of pharmacy and pharmaceutical sciences*, 5(5),107-119,2016.
 26. Jeyabaskaran.M,Prof.Rambabu.C and Dhanalakshmi.B.RP-HPLC Method Development and Validation of Dapagliflozin in Bulk and Table Formulation. *IJPART* 2(4),221-226,2013.
 27. Rafaela Zielinski Cavalheiro de Meira, Aline BiggiMaciel,Fabio Seigi Murakami, Paulo Renato de Oliveira and Larissa SakisBernardi.In Vitro Dissolution Profile of

Dapagliflozin:Development,Method Validation and analysis of Commercial Tablets. International Journal of Analytical Chemistry,1-6:2017.

28. Shyamsundar, Development and Validation of RP-HPLC method for simultaneous estimation of Dapagliflozin and Metformin in bulk and in synthetic Mixture. World journal of pharmacy and pharmaceutical sciences ,6(7),2139-2150,2017.
29. Mohammad Yunoos and Gowri sankar.D.A validated Stability indicating High –Performance Liquid Chromatographic method for simultaneous Determination of Metformin HCL and Dapagliflozin in bulk drug and tablet dosage form. Asian J Pharm Clin Res, 8(3),320-326,2008.