Original Article

DEVELOPMENT OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF SAXAGLIPTIN PHARMACEUTICAL DOSAGE FORMS

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Abstract

A simple, accurate, precise spectrophotometric method was developed for the estimation of saxagliptin (SGN).The optimum condition for the analysis of the SGN was studied. SGN was subjected to stress degradation under different conditions like acidic, alkali, neutral, oxidation, photolytic, and thermal degradation as per recommended by the International Conference on Harmonization (ICH). The samples thus prepared were used for degradation studies by with the developed method. The lambda max i.e. absorption maxima found at 211 nm and calibration curve linear over the range of 0-40µg /ml. The standard regression equation and correlation coefficient fond to be y = 0.03x + 0.047 R^2 = 0.998 respectively. % RSD found to be less than one. The accepted limits of accuracy (recovery) were found to be 99.79 % and all observed data are within required range which indicates good recovery value. LOD and LOQ found to be 0.0523 µg /ml and 0.2146 µg /ml respectively by developed UV spectroscopic method.

Keywords: Saxagliptin, UV Spectrophotometric Method, Accuracy, Precision, Recovery and Stress Degradation studies etc.

Introduction

Saxagliptin (SGN) is chemically (1S, 3S, 5S)-2- [(2S)-2-Amino-2-(3 hydroxytricyclo [3.3.1.13, 7] dec1-yl) acetyl]-2-azabicyclo hexane-3-carbonitrile previously[1-2].This is new oral hypoglycemic agent of the new dipeptidyl peptidase- 4 (DPP-4) inhibitor class of drugs. The empirical formula is $C_{18}H_{25}N_3O_2,H_2O$ and the molecular weight is 333.43[3-4] Saxagliptin (Fig. 1) was a dipeptidyl peptidase-4 (DPP-4) inhibitor approved by FDA in 2009 and launched its journey in China since 2011; it has been widely accepted because of low dosage and low risk of weight gain; also it was used as an adjunct to other anti-diabetic agents and lifestyle modifications to target glycemic control. It is worthwhile to note that saxaglipitn was mainly metabolized by cytochrome P450 (CYP) 3A4 in human; in that way, whether cooked or wine processed rhubarb modified the activity of CYP3A4 would need to be specified if they were intended to combined use. Normally SGN was determined and validated by HPLC and RP-HPLC[5-6] which was costly and consume ample of time on the other hand UV method is rapid and coast effective along with we have demonstrated degradation studies. The present work aimed to develop a simple, rapid, and accurate method for the estimation of saxagliptin in bulk and pharmaceutical dosage forms as per ICH guidelines along with degradation studies.

Material and methods:

Chemicals and Reagents:

Standard saxagliptin obtained as gift sample from Mylan Pharma Hydrabad, India. Methanol supplied by OZONE International. Pvt. Ltd. Mumbai, Maharashtra and Sodium hydroxide, double distilled water obtained from Unique Chemical Kolhapur. All other chemicals & reagents used in this study were of analytical grade.

Preparation of standard stock solutions:

Accurately weighted 10 mg of pure saxagliptin In 10ml volumetric flask containing 5 ml of methanol and water in 1:1 ratio and then sonicated for 15 minutes and final make up the volume up to 10 ml with solvent which having the final strength of 1000µg/ml.

Preparation of Working Standard Solution:

From standard stock solution 10 ml was withdrawn and diluted up to 10 ml to get the solution of 100 µg/ml concentration and filtered through Whatman filter paper before analyzing.

Selection of Wavelength for Analysis of Saxagliptin:

Appropriate volume 1 ml of working stock solution of saxagliptin was transferred into 10 ml volumetric flask, diluted with solvent up to the mark to give a concentration 10 µg/ml. The resulting solution was scanned between 200-400 nm. Absorbance was recorded against methanol and water (1:1) as blank using UV-visible spectrophotometer (Shimadzu UV-1900 Japan) [7-8]

Validation of UV Spectroscopic method:

The method developed was validated for the following parameters according to the ICH Guidelines Q2 (R1): Validation of Analytical Procedures: Text and Methodology.

Procedure for calibration curve

Primary stock solution was diluted suitably with methanol and water (1:1) ratio to get standard solution to obtain working standard at room temperature. The stock solutions scanned in the UV range 200-400 nm (Shemadzu UV-1900) by using an appropriate blank. For linearity study, dilutions were made for the drugs in the range of 0-100 µg/ml concentrations were prepared by diluting the stock solution with working solvent methanol and water. [9-10]

Precision:

The intra-day and inter-day precisions of developed methods was measured by estimating thrice corresponding response on present day and on three different days, over a period of one week and the results were reported in terms of relative standard deviation

Repeatability:

By analyzing six samples of same drug concentrations (10µg/ml) the repeatability was determined. From the resulting absorbance SD and RSD were calculated [11]

Accuracy:

The accuracy of the developed method is calculated by comparing closeness of the observed value to the standard value for the sample. Recovery study was performed by addition level of 75, 100 and 125 % for test solution and absorbance of each measured in triplicate [12]

Limit of Detection and Limit of Quantification ((LOD & LOQ) :

It is the lowest concentration of analyte in the sample that can be detected but not necessarily quantified. LOD and LOQ were calculated with help of response along with its standard deviation as per ICH guidelines. [13-14]

LOD and LOQ were calculated using formula

LOD = 3.3 *σ/s The smallest possible quantity of analyte that can be measured quantitatively.

LOQ = 10 *σ/s Where **σ** is the standard deviation and **S** is the slope

Degradation Studies:

The ICH guidelines allowed stability testing of new drug substances and products that required stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this study was to perform the stress degradation studies on saxagliptin using the method developed. [15-16]

Stress Degradation by Hydrolysis under Acidic Condition:

A stock solution of saxagliptin was prepared by dissolving 10 mg of the drug in 10 ml of methanol and water (50:50) to produce 1000 µg/ml of the solution. To 1 ml of the stock solution, 1 ml of 1 N HCL was added in a 10 ml volumetric flask and the volume was make up to the mark using solvent. Three sample prepared and these volumetric flask was kept under normal conditions for 1hr.2hr,& 4hr and then make the solution neutral by adding 1m NaOH solution and absorbance were checked .Sample were tested in triplicate. [17]

Stress Degradation by Hydrolysis under Alkaline Condition:

A stock solution of saxagliptin was prepared by dissolving 10 mg of the drug in 10 ml of methanol and water (50:50) to produce 1000 µg/ml of the solution. To 1 ml of the stock solution, 1 ml of 1 N NaOH was added in a 10 ml volumetric flask and the volume was make upto the mark using solvent. Three sample prepared and these volumetric flask was kept under normal conditions for 1hr.2hr,& 4hr and then make the solution neutral by adding 1m HCl solution and absorbance were checked . Sample were tested in triplicate [18]

Dry Heat -Induced Degradation:

To three amber colored 10 ml volumetric flasks sample solution containing 1 ml aliquot of methanol and water (50:50) was transferred and flasks were kept on water bath for 1 hr, 2 hr, 4 hr at 60°C ± 2°C then diluted with solvent. All samples were tested in triplicate [19]

Oxidative Degradation:

To 1.5 ml of the stock solution of methanol and water (50:50) (1000µg/ml), 1 ml of 30% w/v of hydrogen peroxide was added in a 10 ml volumetric flask and the volume was made up to the mark with methanol and water (50:50). The volumetric flask was kept at room temperature for 1hr.,2hr.& 4hr.dilutions were made from the stock solution to achieve the required concentration (6µg/ml). The solution was further analyzed with the help of a UV-Visible spectrophotometer [16] All samples were then tested in triplicate.

Photolytic Degradation:

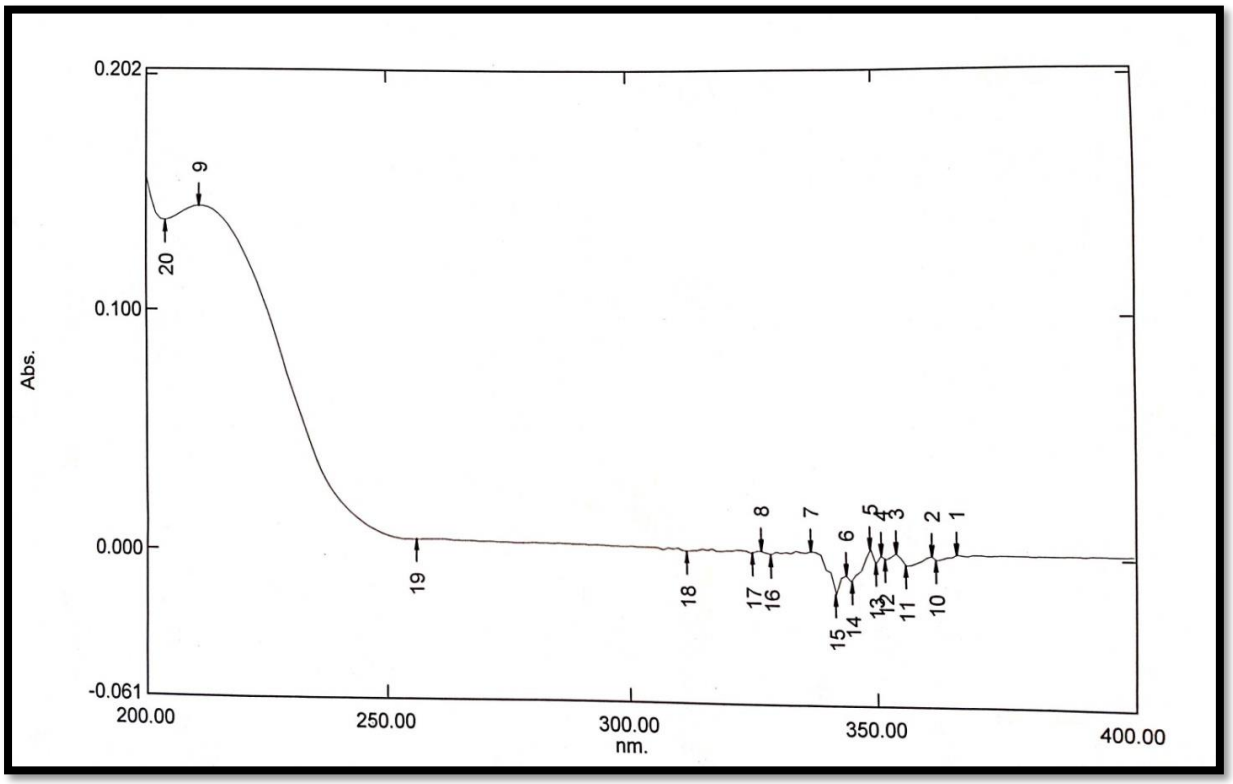
To a clear volumetric flasks, 1ml sample was added and then exposed to direct UV light for 1, 2 and 4 Hour. All samples were then tested in triplicate. [20-21]

Results and discussion:

Determination of absorption maxima (λ max):

The standard stock solution of saxagliptin having the concentration 1000 µg/ml was further diluted to 100µg/ml with methanol and water (50:50). The absorbance of solution was scanned in the range of 200-400 nm. The λmax was found to be 211 nm as shown in figure 1.

Fig. 1: Lambda Max of Saxagliptin

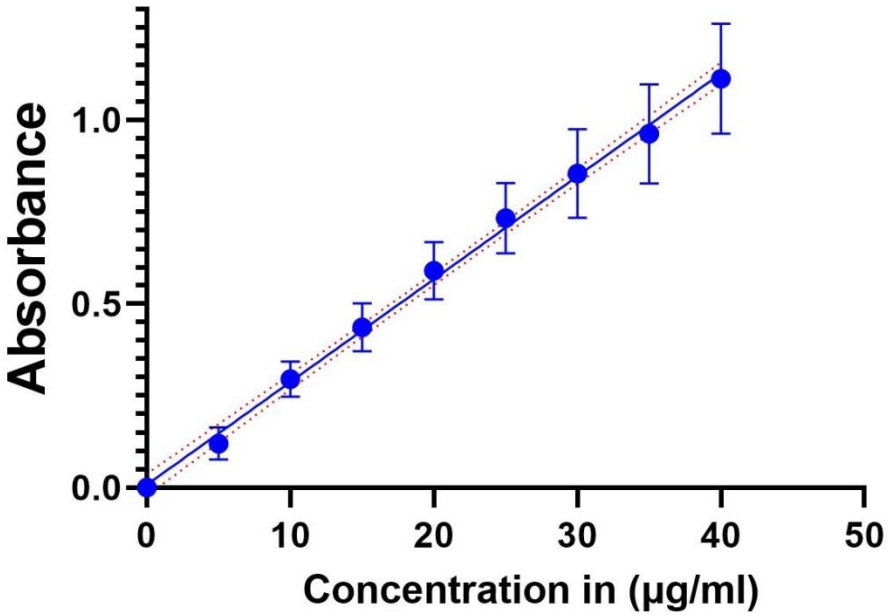


Linearity:

The linearity study of the saxagliptin was performed by plotting different concentrations of standard solution against their respective absorbance as shown in and figure 2 and table 1.The calibration curve was found to be linear having R^2 value 0.997 in the concentration range of 0-40µg/ml.

Sr .No.	Concentration (µg/ml)	Absorbance at 211 nm ± standard deviation
1	0	00 ± 00
2	5	0.1843±0.0025
3	10	0.2955±0.0047
4	15	0.4363±0.0064
5	20	0.5908±0.0078
6	25	0.7335±0.0090
7	30	0.8454±0.0133
8	35	0.9628±0.0121
9	40	1.2488±0.0134

Fig. 2: Standard Calibration Curve of Saxagliptin



Precision:

Intraday precision

The intraday precision was determined by analyzing the drug in particular concentration for three times on the same day taking the time intervals of 4 h at 8:00 am, 12:00 noon and 4:00 pm respectively.

Inter-day precision

Precision was determined by measuring values of precision for 03 consecutive days. The values of relative standard deviation (%RSD) were in the range of 0.254-0.425% respectively. This indicates the reproducibility of the method. Results were shown in table 2. Results of precision shows that the current method is reliable and repeatable. Thus, the methodology can be applied for the determination of saxagliptin in bulk and pharmaceutical dosage forms in treatment of diabetes malitus.[18]

Table 01: Results for Intra-day and Inter-day precision of Saxagliptin

Drug	**Conc. (µg/ml)**	**Intra-day Mean Abs.**	**Absorbance ± S.D.**	**%RSD**	**Inter-day Mean Abs.**	**Absorbance ± S.D.**	**%RSD**
Saxagliptin	10	0.2943	±0.0053	0.425	0.2938	± 0.0064	0.354
	20	0.5934	±0.0083	0.364	0.5986	± 0.0073	0.373
	40	1.2345	±0.0118	0.254	0.660	± 0.0126	0.278
Mean %RSD				**0.348**			**0.335**

*** Each value represents mean ± S.D. of three observations**

Repeatability

The repeatability of the developed method was validated by taking the absorbance of six samples of the same concentration (10 µg/ml) The SD and %RSD was in the given limits as shown in table 3. The Repeatability of the methodology is significant for routine and frequent result analysis of drugs in API.Result Conforms that results remains unchanged on repetition of developed methods.[19]

Table 3: Data showing Repeatability of Absorbance

Sr. No.	**Conc. (µg/ml)**	**Absorbance**	**Mean± S.D.**	**%R.S.D**
1	10	0.2940	0.2944±0.0042	0.487
2		0.2932		
3		0.2954		
4		0.2935		
5		0.2958		

*** S.D. = Standard Deviation, R.S.D. = Relative Standard Deviation**

Accuracy:

To analyze the accuracy of developed method, it was applied to analyze marketed available saxagliptin tablet (Onglyza 5mg Astrazeneca). 20 tablets were weighed and powdered. The amount of tablet powder equivalent to 50 mg of saxagliptin was weighed accurately and transfer to 100 ml volumetric flask then 10 ml of methanol and water (1:1) ratio as a solvent was added and kept for 15-20 min with frequent shaking and volume was made up to mark with given solvent. The solution was then filtered through Whattman filter paper. This filtrate was diluted suitably with solvent to get the solution of 05 µg/ml concentration. The absorbance was measured against blank solution. The recovery experiment was performed at three different levels that are 75%, 100%, 125%. To the preanalyzed sample solution, a known amount of standard drug solution was added at three different levels and absorbance was recorded. The drug content of the preparation was calculated using standard calibration curve. Amount of drug estimated by this method is given in (Table no. 4).

Table 3: Accuracy Study of Saxagliptin

SGN (µg/ml)	**Level of addition (%)**	**Standard SGN added (µg/ml)**	**Amount recovered (µg/ml)**	**% Recovery**	**Average % Recovery**
05	75	7.5	12.46	99.68	99.79
05	100	10	14.98	99.87	
05	125	12.5	17.47	99.83	

Ruggedness:

Ruggedness was determined by carrying out analysis by two different analysts and the respective percentage recovery was noted and the results were indicated as % RSD found as follows

Analyst 1: Percentage recovery of saxagliptin at 211 nm is 99.78 ± 0.543

Analyst 2: Percentage recovery of saxagliptin at 211nm is 99.83± 0.565

Summary of validation parameters

Table 4: Summary of validation parameters

Sr. No.	**Parameters**	**Results**
1	Absorption maxima (nm)	211 nm
2	Linearity range (µg/ml)	0-40 µg/ml
3	Standard Regression Equation	y = 0.04x + 0.056
4	Correlation coefficient (R^2)	R^2 = 0.997
5	Specificity	A 10 µg /ml solution of saxagliptin

		in methanol and water (50:50) as a solvent at UV detection of 211 nm will show an absorbance value of 0.2944±0.0042
6	% RSD Repeatability (n=5) Intra-day(n=3) Inter-day(n=3)	0.487 0.348 0.335
9	LOD	0.0523 µg /ml
10	LOQ	0.2146 µg /ml
11	Molar Absorptivity	$6.5842*10^4$ L/mol

Summary of stress degration study

The effect of Acid/Base hydrolysis, Oxidation, Photo Degradation, Heat-induced degradation on the spectra was observed. On acid hydrolysis saxagliptin do not showed any significant degradation or no additional peak of sample after 1hr, 2hr and 4hr at different process. In other cases there was minor shift in peak that was not significant

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Table 5: Summary of Stress Degradation Study

Degradation condition	**Observed peak-211nm**	
	Time (Hr)	**Reported peak (nm)**
1 N HCL 1ml.	1	211
	2	211
	4	211
0.1 N NAOH 1ml	1	211
	2	211
	4	212
30% w/v of hydrogen peroxide 1ml	1	211
	2	210
	4	211
Dry heat 70° C	1	211
	2	209
	4	210
photostability chamber	1	211
	2	210
	4	209

Conclusion:

A simple, accurate, precise and cost effective UV-spectroscopic method has been developed for the estimation of saxagliptin. The proposed method is successfully applied for estimation of SGN in marketed formulations. The method can be used for the routine quality control analysis of saxagliptin. In force degradation studies as Acid/Base hydrolysis, Oxidation, Photo Degradation, Heat-induced degradation the spectra for acid degradation of saxagliptin do not showed any significant degradation or no additional peak of sample after 1hr, 2hr and 4hr at different process.

Conflict of interest

The authors declare that they don't have any competing interests.

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Authors' contributions

MD research scholar who contributed in development of UV spectroscopic method and, force degradation studies of SGN and MD has major contributors in writing the manuscript, EB supervisors who contributed in research guidance and has major contribution in monitoring anticancer studies and discussion. All authors read and approved the final manuscript.

Abbreviations

1. UV-Ultraviolet
2. nm-Nanometer
3. µg-Microgram
4. ICH-International Conference of Harmonization.
5. % RSD-Percent Relative Standard Deviation
6. λ max-Lambda Maximum
7. S.D-Standard Deviation
8. RSD-Relative standard deviation.
9. SGN- Saxagliptin

References

1. Onglyza Product information. Bristol-Meyers-Squibb. Princeton New Jersey, USA. 1st July 2009. Available at: http://packageinserts.bms.com/pi/pi_onglyza.pdf (cited 1st August 2010).
2. Australian Drug Evaluation Committee Recommendations. Australian Government Department of Health and Aging, January 2010. Available at: http://www.tga.gov.au/docs/ html/ adec/adec0267.htm (cited 30th Sept 2010)

3. Deacon CF, Holst JJ. Saxagliptin: a new dipeptidyl peptidase-4 inhibitor for the treatment of type 2 diabetes. Adv Ther 2009; 26(5):488-499.

4. Robertson JG. 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 Chem 2005; 48(15): 5025- 37.

5. Deepan, Thiyagarajan and Dhanaraju, Magharla Dasaratha. "Stability indicating HPLC method for the simultaneous determination of dapagliflozin and saxagliptin in bulk and tablet dosage form" Current Issues in Pharmacy and Medical Sciences, vol.31, no.1, 2018, pp.39-43.

6. N. Singh, P. Bansal, M. Maithani. Development and validation of a stability-indicating RP-HPLC method for simultaneous determination of dapagliflozin and saxagliptin in fixed-dose combination. New J. Chem., 2018,42, 2459-2466.

7. Patil SM, Galatage ST, Patil AB. Development Of Validated Uv Spectrophotometric Method For Estimation Of Fosfestrol In Bulk and Pharmaceutical Dosage Form. World journal of pharmaceutical research.2013;6(3):2345-2353.

8. Hiremath R. et.al. Development and Validation of UV Spectrophotometric Area Under Curve (AUC) Method for Sildenafil Citrate in Pharmaceutical Formulation. Research Journal of Pharmacy and Technology. 2016;9(3):235-237.

9. Sumithra M, Yuvanesh P, Mistry A. Analytical method development and validation of ambroxol hydrochloride by UV spectroscopy and forced degradation study and detection of stability. Research Journal of Pharmacy and Technology. 2016; 9(7):794-800.

10. Patil SM,Galatage ST,Choudhari AU.Evelopment Of Uv Spectrophotometric Method For Estimation Of Letrozole In Pure And Pharmaceutical Dosage Form. Indo American Journal of Pharmaceutical Research.2018 May;8(04):1080-1085.

11. Galatage ST, Trivedi R, Bhagwat DA. Characterization of camptothecin by analytical methods and determination of anticancer potential against prostate cancer. Future Journal of Pharmaceutical science.2021May7;104:1-9. doi.org/10.1186/s 43094- 021- 00236-0.

12. Pravallika KE et.al. Development and validation of UV spectrophotometric methods for the estimation of vortioxetinehydrobromide in bulk and pharmaceutical dosage forms. Research Journal of Pharmacy and Technology.(2017); 10(11): 3928-3932.

13. Sushma G.et.al New first derivative spectrophotometric methods for the determination of Sitagliptin - An antidiabetic agent. Research Journal of Pharmacy and Technology.2020;13(6): 2838-2842.

14. Galatage ST .Determination Of Amphotericin B In Bulk And Pharmaceutical Formulation By Spectrophotometer. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2020May;11(3):31-37

15. Bandgar SA, Jadhav NR. Validated UV Spectrophotometric method for Estimation of Simvastatin in Bulk and Pharmaceutical Formulation. Research Journal of Pharmacy and Technology. 2019; 12(12): 5745-5748.

16. Manideep G. et.al. Development and Validation of A UV Spectroscopic Method to Estimate Etoricoxib in Bulk and Tablet Formulation. Research Journal of Pharmacy and Technology 2018; 11(2):758-760.

17. Kumar S. et.al. UV Spectrophotometric Method Development and Validation for the Estimation of Gliclazide in Bulk and Pharmaceutical Dosage Form. Asian Journal of Pharma Analysis 2016; 6(3): 143-146.

18. Raut ID, Doijad RC, Mohite SK. Validated Spectrophotometric method for estimation of Paclitaxel in Bulk and Pharmaceutical Formulation. International Journal of Pharmacy. 2017; 7(1): 68-72.

19. Sreejith KR, Rajagopal PL, Premaletha K. Analytical Method Development and Validation of Anastrozole in Pure and Tablet Dosage Form by UV Spectroscopy Research Journal of Pharmacy and Technology 2017;10(4), 1015-1019.

20. Mulsa N.et. al. Development of the UV Spectrophotometric Method of Phenytoin Sodium in API and Stress DegradationStudies. Inventi Rapid: Pharm Analysis &QualityAssurance. 2013; (1): 1-5.

21. Hussain QS, Bukhari K. Method Development and Stress Degradation Profile of 5-Benzyl-1,3,4-Oxadiazole-2-Thiol Studied by UV Spectroscopy. Pharmaceutical Chemestry Journal. 2018; (52):278–283