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Method Development and Validation of RP-HPLC Method for the Estimation of Sitagliptin Phosphate in Tablet Dosage Form

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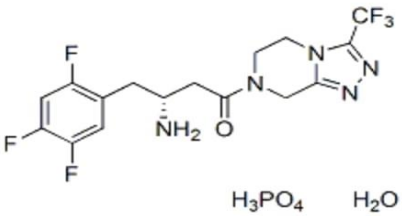
Abstract:

Method Development and Validation of RP-HPLC for the Estimation of Sitagliptin Phosphate in Tablet Dosage Form was developed using Qualisil C18 BDS Column(150×4.5mm,5µ) as stationary phase and Potassium dihydrogen phosphate and Acetonitrile in a ratio 60:40% v/v at pH 4.5 as mobile phase was maintained at a flow rate of 1.0ml/min, the retention time of Sitagliptin Phosphate monohytrate were found to be 2.70min and detection was carried out a 228nm. The significant recovery and minimal coefficients of variation validate the method's appropriateness for concurrent examination of Sitagliptin Phosphate in tablet form. The verified procedure worked well for the tablet's quantitative analysis.

1. Introduction

An oral antihyperglycemic medication belonging to the dipeptidyl peptidase-4 (DPP-4) inhibitor class is sitagliptin phosphate. This medication inhibits an enzyme and is used to treat type 2 diabetes either on its own or in conjunction with other oral anti-hyperglycemic medications (such metformin or thiazolidinedione). Sitagliptin inhibits the DPP-4 enzyme in a competitive manner. The gastrointestinal hormones known as incretins, or GLP-1 and GIP, are broken down by this enzyme in response to a meal. They are able to decrease the pancreas' production of glucagon and boost insulin secretion by blocking GLP-1 and GIP inactivation. As a result, blood glucose levels approach normal.



7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl) butyl]-5,6,7,8 tetrahydro-3 (trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine phosphate (1:1) monohydrate.

2. MATERIALS AND METHODS

The Sitagliptin Phosphate were obtain from Sun Pharma Laboratories and the tablets strip (Brand name: were purchased from local pharmacy.(Label Claim-50mg).

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2.1 Chemicals and reagents Used

S.NO	Chemicals / Reagents	Grade	Company
1	Acetonitrile	HPLC	Purechem
2	Water	HPLC	HPLC-Grade
3	Potassium dihydrogen phosphate	Analytical	Rankem
4	Ortho phosphoric acid	Anaytical	Qualigens

2.2 Instruments Used

Methods development was carried out using Agilent HPLC(model 1100 Series) equipped with UV-Agilent detector and Qualisil C18 BDS Column(150×4.5mm,5µ) was used.Mobile phase comprising of Photassium dihydrogen phosphate:Acetonitrile in a ratio 60:40% v/v at pH 4.5.Orthophosphoric acid was used to adjust the pH and flow rate of 1.0 ml/min and the effluent was detected at 228nm.The volume of injection is 10µl.

2.3 Mobile phase preparation

For the mobile phase 600ml of Potassium dihydrogen phosphate and 400ml of Acetonitrile(60:40) were mixed together and its pH was adjusted to 4.5 with orthophosphoric acid. After 15 minutes of sonication, the mobile phase was transferred to a 1000 ml bottle of mobile phase in order to eliminate any remaining contaminants and undissolved gases that may have caused undesired peaks in the chromatogram.

2.4 Preparation of Standard Solution

64.25 mg of sitagliptin phosphate monohydrate, precisely weighed, were divided into individual 100 ml volumetric flasks and dissolved in the mobile phase using a sonicator. Following a shake of the flasks, the volumes were adjusted with mobile phase to yield solutions with a concentration of 500 µg/ml for each.

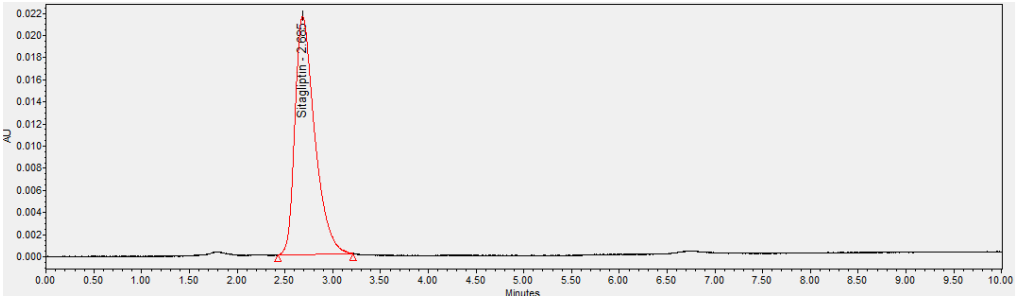

Fig 2.4.1 Chromatogram for Sitagliptin Standard

TABLE: 2.4.1 THE RESULT OF CHROMATOGRAPHIC STUDY FOR THE SITAGLIPTIN STANDARD

S.NO	Standard Area	RT
1	307920	2.685
2	310884	2.690
3	307636	2.684
Mean	308813	2.69

2.5 Preparation of Sample Solution

Weighed and powdered were twenty pills, equivalent to 50 mg of sitagliptin phosphate monohydrate and 100ml volumetric flask containing 50 mg of sitagliptin worth of tablet powder was filled with mobile phase and allowed to dissolve.

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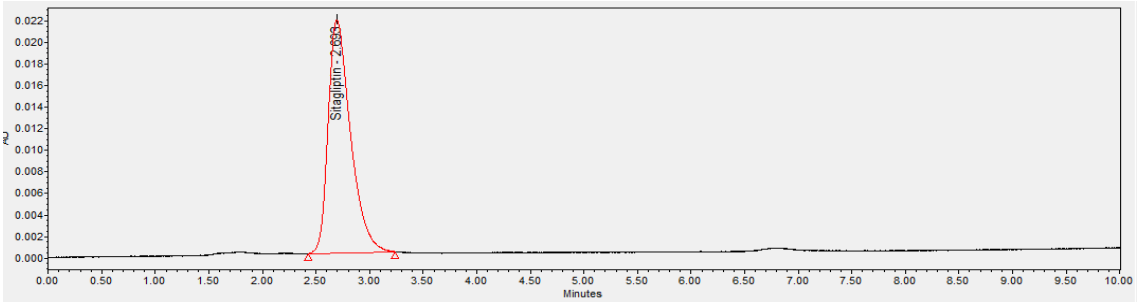

Fig 2.5.1 Chromatogram for Sitagliptin Sample

TABLE: 2.6.1 THE RESULT OF CHROMATOGRAPHIC STUDY FOR THE SITAGLIPTIN SAMPLE

S.NO	Sample Area	RT
1	311531	2.692
2	311906	2.693
3	308455	2.693
Mean	310631	2.7

2.7 System Suitability

System Suitability was performed by injecting 3 replicate injection of drug into the system to observe sharp peaks of Sitagliptin Phosphate Monohydrate at retention times of 2.70 min, respectively in reference to the standard solution and to determine the test concentration, number of Theoretical plates, and Tailing factor Retention time : 2.70 mins

Ststem Suitability Parameters	**SITAGLIPTIN**
RETENTION TIME (MIN)	2.70 mins
THEORETICAL PLATES	2562
TAILING FACTOR	1.5

3 RESULTS AND DISCUSSION

3.1 Fixed Chromatographic Conditions

Stationary phase : Qualisil C18 BDS Column(150×4.5mm,5µ)
Mobile phase : Potassium dihydrogen phosphate : Acetonitrile(pH :4.5)60:40 %v/v
Detection wavelength : 228nm
Temperature : Room temperature
Mode : Isocratic elution

3.2 Linearity

The calibration curve was analyzed using least squares linear regression to determine linearity. Between 20 and 100 µg/ml, the calibration curve (Figure 3.2.1, Table 3.2.1) was linear. Plotting peak regions against corresponding concentrations produced a curve that was subjected to linear regression analysis. It was discovered that the correlation coefficient was, respectively, 0.99961.

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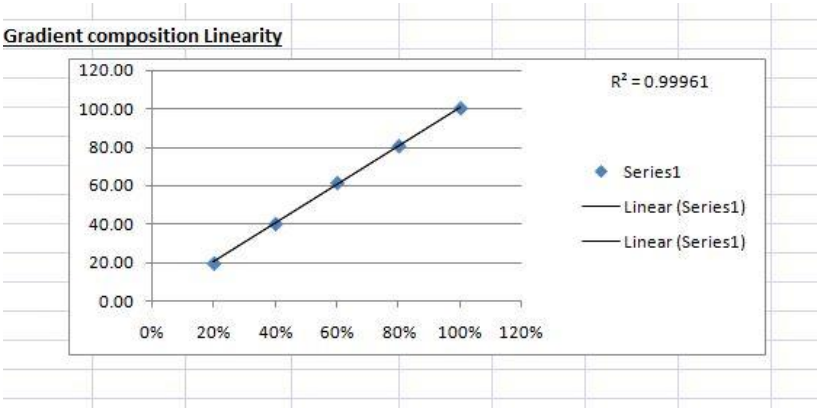

Fig 3.2.1 Calibration curve of Sitagliptin

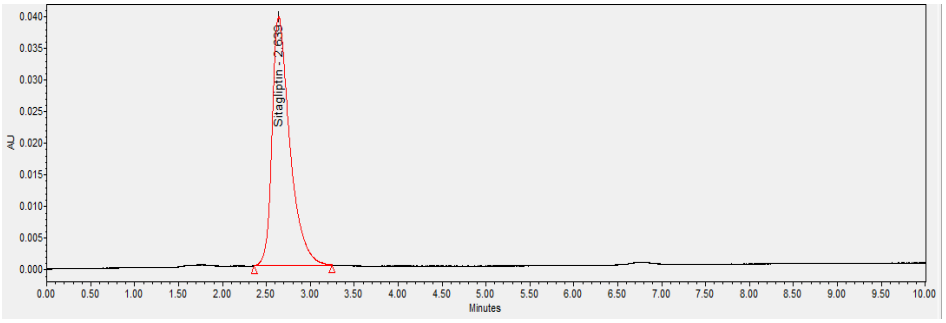

Fig 3.2.2 Chromatogram for Sitagliptin Linearity

TABLE: 3.2.1 THE RESULT OF CHROMATOGRAPHIC STUDY FOR THE SITAGLIPTIN LINEARITY

S.NO	Concentration (µg/ml)	Peak Area
1	20%	108290
2	40%	220518
3	60%	337195
4	80%	441964
5	100%	549338

3.3 Accuracy

Recovery studies were conducted by examining the samples by analyzing the added concentration of the drug as well as the measured concentration in order to assess the accuracy of the suggested approach.Every sample received three injections.(80,100,120%)

Recovery: 98.49%

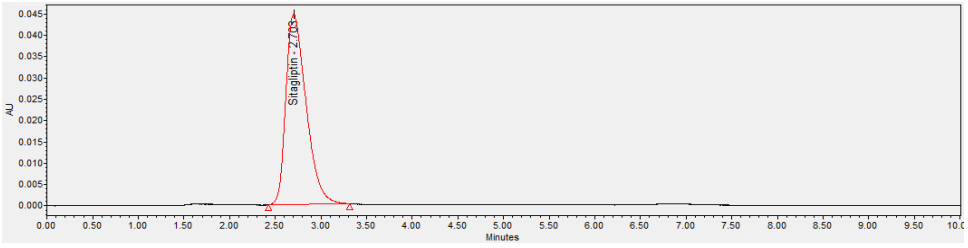

Fig 3.3.1 Chromatogram Recovery of Sitagliptin

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TABLE: 3.3.1 THE RESULT OF CHROMATOGRAPHIC STUDY FOR THE SITAGLIPTIN ACCURACY

Recovery	Amount Taken	Area	Average Area	Amount recovery mg/ml	% recovery	Average % recovery
80%	80(40mg)	461971	462691	39.19mg	98.34%	98.49%
	80(40mg)	462302		39.36mg	98.41%	
	80(40mg)	463801		39.49mg	98.73%	
100%	100(50mg)	570676	569906	49.31mg	98.63%	98.49%
	100(50mg)	570130		49.26mg	98.53%	
	100(50mg)	568914		49.32mg	98.32%	
120%	120(60mg)	682587	682806	59.08mg	98.46%	98.49%
	120(60mg)	683731		59.18mg	98.63%	
	120(60mg)	682102		59.03mg	98.39%	

3.4 Precision

Precision research examples include repeatability intraday and interday precision tests.The intraday (Repeatability) and interday precision experiments were finished by computing corresponding answers three times on the same day (Intraday) and three distinct days (Interday) for the three different concentrations of STG.The precision study's findings were presented as a RSD%.

RSD% - 0.2%

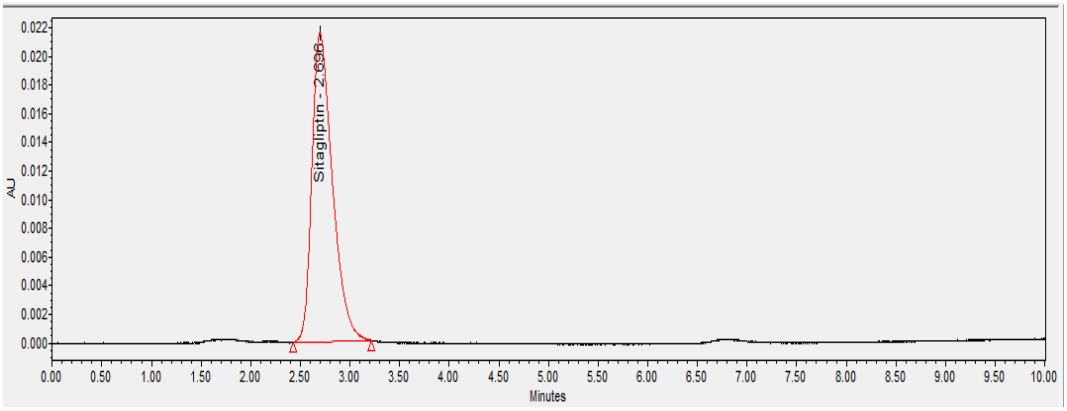

Fig 3.4.1 Chromatogram for Sitagliptin of Precision

TABLE: 3.4.1 THE RESULT OF CHROMATOGRAPHIC STUDY FOR THE SITAGLIPTIN PRECISION

INJECTION NO	Area of Sitagliptin	RT
1	308915	2.696
2	309756	2.700
3	312713	2.702
4	312808	2.705
5	310678	2.706
Mean = 310973.8 , %RSD = 0.2 , RT of 0.6 for Area , Std DV = 1746.4 for area		

1.Intra-day Precision:

The standard Sitagliptin solution is analyzed three times throughout the same day in order to assess the intra-day precision.

2.Inter-day Precision:

Three consecutive days of analysis the Sitagliptin standard solution is used to assess the inter-day precision .

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S,NO	Precision	Sitagliptin (%RSD)
1	Inter-Day	0.2%
2	Intra-Day	0.2%

3.5 Robustness

The examination of aliquots from homogenous batches was done to test the robustness of the suggested approach. Physical parameters such as injection volume and wavelength, pH, Flow rate were varied, but the responses remained within the assay's bounds.

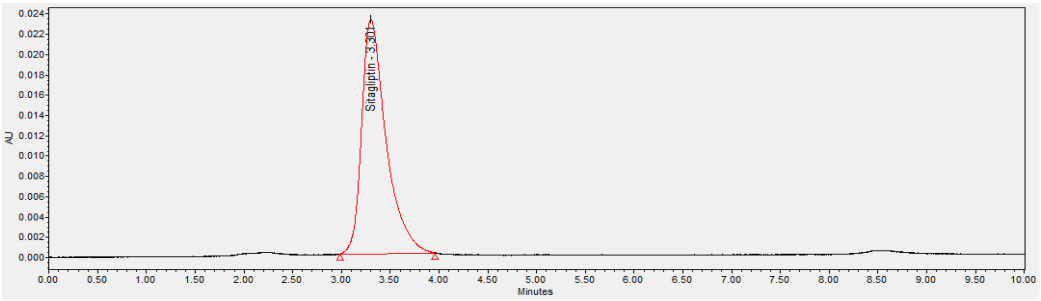

Fig 3.5.1 Chromatogram for flow rate for 0.8ml/min of Sitagliptin

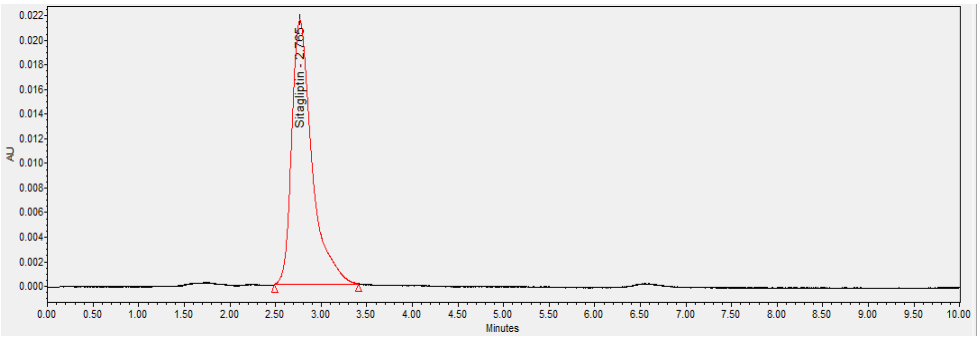

Figure 3.5.2 Chromatogram for pH for 5.5 of Sitagliptin

TABLE: 3.5.1 THE RESULT OF CHROMATOGRAPHIC STUDY FOR THE SITAGLIPTIN ROBUSNESS

S.NO	Condition	%RSD of Sitagliptin	RT
1	Flowrate 0.8ml	0.1 Area	0.0
2	Flowrate 1.2ml	0.1 Area	0.0
3	pH 3.5	1.1 Area	3.2
4	pH 5.5	0.0 Area	0.1

SUMMARY AND CONCLUSIONS

The current study aims to offer an updated RP-HPLC method that is sensitive, straightforward, accurate, and affordable. Without the interference of other formulation ingredients, it is effectively used to determine the amount of sitagliptin in pharmaceutical preparations. To provide a sufficient separation of eluted chemicals, this method's HPLC settings were optimised. To obtain the best possible outcomes, a variety of mobile phase compositions were initially tried. Peak parameters-height, tailing, theoretical plates, run time, etc.were used to determine the mobile phase and flow rate. This system, which uses an flow rate of 1.0 ml/min and a mixed phosphate buffer with acetonitrile (60:40) at pH 4.5 orthophosphoric acid, is very reliable. A better drug detector response was observed at 228 nm, which was the ideal wavelength for detection. It was shown that the average retention time for sitagliptin was 2.70 minutes.

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For Sitagliptin, the calibration was linear in the concentration range of 20–100 µg/ml. The method's precision and accuracy are indicated by the low percentage R.S.D. figures. For Sitagliptin, the mean recoveries were found to be 100.03%. By analysing aliquots from homogeneous slots under similar operational and environmental settings by several analyzers, the robustness of the suggested procedures was assessed; the reported percentage R.S.D was shown to be less than 2%. The proposed method was validated using ICH guidelines, and all of the approaches' outcomes were extremely similar to one another and to the label value of pharmaceutical formulations used in commerce. As a result, the outcomes produced by the suggested strategy do not differ much. It is therefore recommended that the indicated isocratic RP-HPLC procedures be used successfully for the regular analysis of sitagliptin in tablet formulation.

Summary of validation data for Sitagliptin

S.No	Parameter	Acceptance criteria	HPLC	
1	%Recovery	98-102%	100.03%	
2	Linearity Range	Correlation coefficient should be NLT 0.999	0.999	
3	No. of theoretical plates	NLT 2000	2562	
4	Accuracy	98%-102%	98.49%	
5	Method Precision	%RSD (NMT 2%)	0.2	
6	Intermediate Precision	%RSD (NMT 2%)	0.2	
7	Robustness	%RSD (NMT 5%)	pH	Flow rate
			3.2	0.0

Reference :

1. **Pradnya Lokhande**.., Development and Validation of an RP-HPLC metho for Analysis of Sitagliptin . International Journal of Trend in Scientific and Development Volume 3 Issue 6 , 2019.
2. **Sujani PV et.**al.., Validated RP-HPLC method the Estimation of Simvastatin and Sitagliptin. Scholars Academic Journal of Pharmacy.,2014 ;3(3):265-270
3. **Sai Lakshmi .E et.al**.., Development and Validation of RP-HPLC method for the Estimation of Sitagliptin phosphate in tablet dosage form.International Journal of Applied Pharmaceutical Sciences and Research 2017;2(3);41-45
4. **B.Prasanthi et.al**.., Analytical method Development and Validation for the Estimation of Metformin and Sitagliptin in Bulk and tablet dosage form by RP-HPLC. Indo American Journal of Pharmaceutical Sciences 2018, 05(2). 882-889
5. **Sudhir Adsul et.al**.., RP- HPLC method Development and Validation For Simultaneous Estimation for Metformin and Sitagliptin in Bulk and Tablet Formulation. Internationl Journal of ChemTech Research, 2018,11(11): 428-435
6. **Prasad P.B.N et.al**.., Development and Validation of a method for Simultaneous Determination of Metformin Hydrochloride and Sitagliptin phosphate in a formulation by RP-HPL. American Journal of Analytical Chemistry, 2014, 5, 737-742
7. **Mohamed Karam Qassas et.al**.., A Validated HPLC Stability Indicating Method for the Determination of Sitagliptin inBulk Drug Substance and Tablets. International Journal of Pharmaceutical Sciences Review and Research., 32(1), May – June 2015; Article No. 33, Pages: 194-198
8. **Shyam Rangari et.al**.., RP-HPLC method Development for simultaneous estimation of Sitagliptin phosphate and Metformin hydrochloride in pharmaceutical dosage form.International Journal of Research in Pharmacy and Chemistry 2021, 11(3), 64-70
9. **Vinit Dattatray Chavhan et.al**.., Development and Validation of RP-HPLC method for simultaneous estimation of Sitagliptin and Simvastatin in bulk and

tablet dosage form. Journal of Applied Pharmacy Vol.6; Issue 3 : 327-338; July, 2014

10. **Srinivasa Rao Atla et.al**.., Validated RP-HPLC method for the Simultaneous estimation of Sitagliptin and Simvastatin in dosage forms. International Journal of Chemical and Analytical Science, 2012, 3(11), 1611-1614
11. **Patel Tushar .R et.al**.., Stability indicating RP-HPLC method for Simultaneous estimation of Simvastatin and Sitagliptin in tablet dosage form. Indo American Journal Of Pharm Research.2014:4(04)
12. **Shaoo S.K et.**al.., Devlopement and Validation of RP –HPLC method for determination of Metformin and Sitagliptin in bulk and pharmaceutical dosage form. Journal of Applied Pharmaceutical Research 2017, 5 (2): 34 – 39
13. **Vasanth P M et.al**..,Method Development and Validation and Metformin using Reverse Phase HPLC method in bulk and tablet dosage form. Scholars Research Library Der Pharmacia Lettre, 2013, 5 (5):168-174 (http://scholarsresearchlibrary.com/archive.html)
14. **Swati D. Bhende et.al**.., RP-HPLC Method for the Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Combined Tablet Dosage Forms. Oriental Journal of Chemistry., Vol. 28(1), 463-469 (2012)
15. **Sankar A.S,K et.al**.., Development and Validation for simultaneous estimation of Sitagliptin and Metformin in pharmaceutical dosage form using RP-HPLC method. International Journal of PharmTech Research.2013, Vol.5, No.4, pp 1736-1744
16. **Lavanya.R et.al**.., Development and Validation of RP-HPLC for the estimation of Sitagliptin Phosphate in bulk and its tablet dosage form. Journal of Advanced Pharmacy Education & Research.2013 ,Vol 3 ,Issue 4
17. **Eyad S.M. Abu-Nameh et.al..,** Simultaneous Determination and stability assessment of Metformin and Sitagliptin in pharmaceutical form by High Performance Liquid Chromatography. Jordan Journal of Pharmaceutical Sciences, Volume 10, No. 2, 2017
18. **Narendra Nyola et.al..,** Simultaneous estimation of Sitagliptin phosphate monohydrate and metformin hydrochloridein bulk and pharmaceutical formulation by RP-HPLC. J Pharm Educ Res Vol. 3, Issue No. 2, December 2012
19. **Meher Vijay Dalawai et.al..,** Development and validation of stability indicating assay method by HPLC for the analysis of sitagliptin phospahte in bulk drug substances. Journal of Chemical and Pharmaceutical Research, 2015, 7(10):781-787
20. **Imran A Sheikh et.al..,** Application of validated HPLC method for degradation study of Sitaglptin and Metformin HCL. International Journal of Advances in Pharmaceutical Analysis 2017; 07(02): 21-24