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RESEARCH ARTICLE

VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF JANUMET XR (SITAGLIPTIN/METFORMIN HCL EXTENDED-RELEASE FIXED DOSE COMBINATION (FDC) CONTENT IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract

A simple, accurate, precise and rapid stability indicating reverse phase High performance chromatography method was used for estimation of Sitagliptin/metformin HCl ER tablets in bulk and fixed-dose combination solid oral dosage form. The proposed analytical method has been validated for specificity, Linearity, Accuracy, Precision and Robustness. The chromatography was achieved in a Avantor, ACE C18 (Length 150 x Diameter 4.6mm Particle size 5µm) column with gradient flow. The optimal chromatographic condition consisted of mobile phase pH 3.5 at a flow rate of 1.2 mL/min, with a column temperature of 35°C, run time 17 minutes and detector wavelength of 210 nm (Sitagliptin& Propyl gallate and Metformin HCl).

Introduction:-

Sitagliptin/metformin HCl, a fixed-dose combination is used to treat oral diabetes medicines that help control blood sugar levels [1]. Metformin works by decreasing glucose (sugar) production in the liver and decreasing absorption of glucose by the intestines. Metformin HCl is chemically N, N-dimethylimidodicarbonimidic diamide hydrochloride. Its empirical formula is $C4H_{11}N_5$.HCl, its molecular weight is 165.63g/mol, and its structural formula is presented in Figure 1. Metformin is prescribed as a first-line therapy in type-2 diabetes.3 Metformin exerts a glucose-lowering effect (i) via inhibition of gluconeogenesis in the liver, (ii) by delaying the action of glucagon, (iii) by facilitating the action of insulin, and(iv) by delaying glucose absorption from the intestine.

Sitagliptin works by regulating the levels of insulin your body procedure after eating. Sitagliptin is chemically 7-[(3*R*)-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. Its empirical formula is $C_{16}H_{15}F_6N_5O.H_3PO_4.H_2O$, and its structural formula is presented in Figure 2. Sitagliptin is used for the treatment of type 2 diabetis. It is effective in lowering of HbA1c, fasting as well as postprandial glucose in monotherapy and in combination with other oral anti diabetic agents.

Various HPLC estimations have been reported in the literature for the determination of sitagliptin/metformin HCl present in pharmaceutical dosage forms.[2,3] Only few methods were reported for the simultaneous estimation of Sitagliptin/metformin HCl by spectrophotometry[4,5,6], tandem mass spectrometry[7,8,9], UPLC[10], HPTLC[11,12] and HPLC[13,14]. Hence, in the current study we made an attempt to develop a simple, selective and precise RP-HPLC method for the simultaneous estimation of sitagliptin/metformin HCl in bulk and finished dosage forms.

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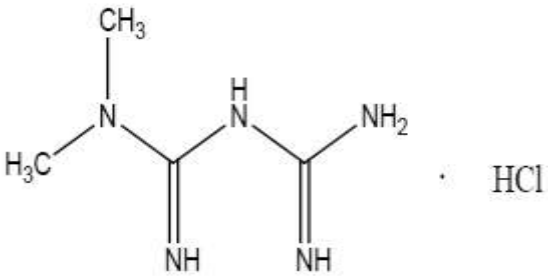


Figure. 1:- Structure of Metformin HCl.

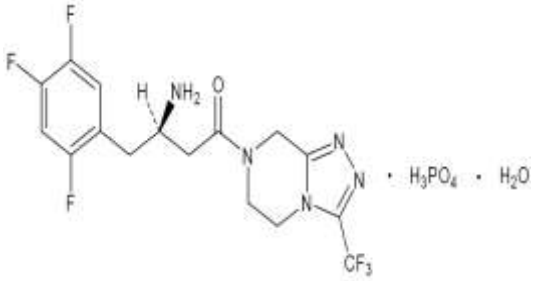


Figure. 2:- Structure of Sitagliptin Phosphate Monohydrate.

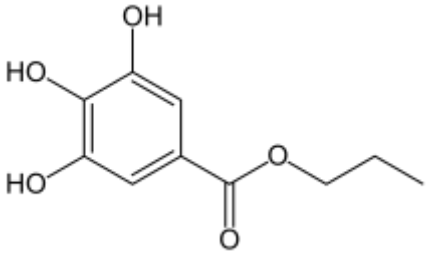


Figure. 3:- Structure of Propyl Gallate.

Table 1:- Degradation impurities in Metformin HCl and Sitagliptin Phosphate.

S. No:	IUPAC Name	Imp code
1	(1-Cyanoguanidine)	RC-A
2	R)-3-Amino-4-(2,4,5-trifluorophenyl) butanoic acid.	XG-0
3	2-[[(R)-4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro- [1,2,4] triazolo[4,3-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-yl] amino] succinic acid.	XG-H
4	This degradation product may be present only for Tablets containing sodium stearyl fumarate as an excipient in the formulation.	XGP-A-1
		XGP-A-2
5	10-(2,4,5-Trifluorobenzyl)-3-(trifluoromethyl)-6,7,10,11-tetrahydro- [1,2,4] triazolo[3,4-c][1,4,7]triazecine-8,12(5H,9H)-dione.	XG-Q
6	E)-1-{3-(Trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl}-4-(2,4,5-trifluorophenyl) but-3-en-1-one.	XG-F
7	E)-1-{3-(Trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl}-4-(2,4,5-trifluorophenyl) but-2-en-1-one.	XG-G
8	(3*S*)-3-amino-1-[3-(trifluoromethyl)-5,6-dihydro [1,2,4] triazolo[4,3-*a*] pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl) butan-1-one,	XG-E

Materials and Methods:-

Sitagliptin phosphate monohydrate, propyl gallate and Metformin HCl drug substance, working standard, and finished dosage forms were manufactured by Changzhou Pharmaceutical Factory, China. Other chemicals such as Trisodium phosphate, 1-Heptane sulfonic acid sodium salt, Orthophosphoric acid, Triethylamine all reagents used

were of analytical grade. Methanol (Tedia), Acetonitrile (Sigma) and Milli-Q water was used for the mobile phase and diluent preparation.

Instrument Details:
HPLC Shimadzhu LC20, with PDA detector and Empower 3 software was used for the purpose of method validation. This HPLC is comprised of quaternary pump. Analytical balance (Mettler Toledo) and pH Meter (Thermo).

Method development and cinematographic conditions:
Various mobile phase types were investigated in the development of a stability-indicating LC method for the analysis of Sitagliptin/metformin HCl ER Tablets. The suitability of mobile phase was decided on the basis of selectivity and sensitivity of the assay, stability studies and separation among impurities formed during forced degradation studies.

Finally good separations were achieved in Avantor, ACE C18(150x4.6mm,5µm) analytical column. The mobile phase with a flow rate of 1.2 mL/min consisted of Mobile phase A: Buffer: Methanol (90:10) and Mobile phase B: Acetonitrile: Methanol (30:70). Buffer Preparation: 2.8g/L trisodium phosphate and 2g/L of 1-heptane sodium salt anhydrous (Adjust the pH 3.5 ± 0.2 with diluted phosphoric acid). The gradient as mentioned in **Table 1.** The mobile phase was degassed and filtered by using 0.45µm membrane filter. The flow rate is 1.2 mL/min with an injection volume of 10µL. The analysis was performed at a column temperature of 35°C with the detection at wavelength of 210nm (Sitagliptin/Metformin HCl). For complete extraction of actives from formulations, trials were taken and 50% methanol was finalized as a diluent.

Table 2:- Mobile Phase Gradient Program for Chromatographic Method.

Time (min)	Mobile Phase A	Mobile Phase B
0.00	80	20
7.50	60	40
11.00	60	40
11.10	80	20
17.00	80	20

Solution preparations:
Preparation of Standard Solution:
Propyl gallate stock:
Accurately weigh about 11mg of propyl gallate standards were weighed and taken into 200 mL volumetric flask to this add 10mL of methanol, sonicate to dissolve completely, then dilute to volume with diluent and mix well

Accurately weigh about 16mg of Sitagliptin phosphate (Equivalent to 12.5mg of sitagliptin) and 125 mg of metformin Hydrochloride standards were weighed and taken into 50 mL volumetric flask to this add 10mL of methanol, sonicate to dissolve completely, then dilute to volume with diluent and mix well. Further take 5mL of this solution and 3mL of **Propyl gallate stock solution**into 50mL volumetric flask, then dilute to volume with diluent and mix well (Concentration of Sitagliptin, propyl gallate and Metformin HCl is25µg/mL, 3µg/mL and 250 µg/mL respectively).

Preparation of sample solution:
Select the tablets randomly and weigh 5 tablets, drop5 tablets into 1000mL of volumetric flask containing 500mL of methanol and **magnetic laboratory stirrer bead(Teflon coat**) then stir for 45minutes with suitable stirring speed (**adjust the stirring speed accordingly uniform stir**) at room temperature, take the sample flask from the stirrer and to this add water up to 80% of total flask volume, then sonicate for 15 minutes with intermediate shaking, then dilute to volume with water and mix well. Centrifuge the solution at 10000rpm for 10minutes, then take the clear supernatant solution 5mL to 50mL, and dilute to volume with diluent and mix well and filter the solution through 0.45µm.

Analytical Method Validation:
The optimized chromatographic conditions were validated for assay of Sitagliptin/Metformin in Sitagliptin/Metformin HCl ER Tablets by evaluating specificity, linearity, precision, accuracy, robustness and system suitability parameters in accordance with the ICH guideline Q2 (R1). [15,16]

Specificity:
Specificity-Blank and Placebo interference:
To establish the interference of blank, placebo, degradation impurities, study was conducted. Assay was performed on placebo in duplicate equivalent to concentration of test preparation as per proposed method. Established the degradation studies on different conditions and reported mass balance.

Linearity:
Established the Linearity by plotting a graph of concentration versus peak response and determining the correlation coefficient, slope and Y-intercept. A series of solutions of Sitagliptin, propyl gallate and Metformin HCl, the standard solutions were prepared in the concentration range from 6.25µg/mL to 50 µg/mL for Sitagliptin, 1.3µg/mL to 10.9µg/mL for propyl gallate and 63µg/mLto 500 µg/mL for Metformin HCL.

Method Precision and Intermediate Precision:
The precision study was confirmed by preparing six preparations, %RSD of six assay values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different day with different analyst and column.

Accuracy:
The (%) recovery level was confirmed of Sitagliptin, propyl gallate and Metformin HCl from spiked placebo was conducted at three different spike levels i.e., 50%, 100% and 150 %. Samples were prepared by mixing placebo with Sitagliptin/Metformin HCl drug substances equivalent to test concentration. Sample solutions were prepared in triplicate for each spike level and (%) recovery, (%) RSD were calculated.

Solution Stability:
Conducted the solution stability of Standard and Sample Solutions at room temperature, and refrigerator conditions, as per proposed method. % Difference between the areas obtained for Sitagliptin, propyl gallate and Metformin HCl at initial and different time interval should not be more than 2.0. So sample and standard solution was stable up to 48hrs on room temperature and refrigeration conditions.

Robustness:
The robustness studies were evaluated by deliberate changes in Chromatographic conditions. The conditions studied were flow rate (altered by ±0.10 mL/min), wavelength (Altered by ±2 nm), variation in mobile phase buffer pH (3.5±0.2 absolute), and Column Oven temperature (±5°C), standard solution was prepared and injected into HPLC system. The system suitability parameters were evaluated for each deliberate variation.

System suitability:
System Suitability testing is an integral part of liquid chromatographic method validation performed to check and ensure on-going performance of a chromatographic system. The System Suitability was estimated by five replicate injections standard solution at 100% of test concentration and also 2 injections of check standard solutions. The column efficiency as determined from Sitagliptin/Metformin HCl peaks is not less than 2000 USP plate count, the USP Tailing for the same peaks are not more than 2.0. %RSD for corresponding peak areas of five replicate injections of the standard solution should not be more than 2.0% and similarity factor between Standard solution and check standard solution should be (0.98 to 1.02).

Results And Discussion:-

Analytical Method Validation:
The content test method is validated for Specificity, Linearity, Precision, Accuracy (Recovery), solution stability, Robustness and System Suitability and was found to be meeting the predetermined acceptance criteria.

Specificity:
Specificity-blank and placebo interference:
Interference study:
From the chromatograms of blank, placebo, and degradation impurity solutions showed there is no inference at the retention time of Sitagliptin/Metformin HCl peaks. The chromatogram of blank, placebo, standard and sample using the proposed method is shown in Figure 4, Figure 5, Figure 6,Figure 6 and Figure 7.

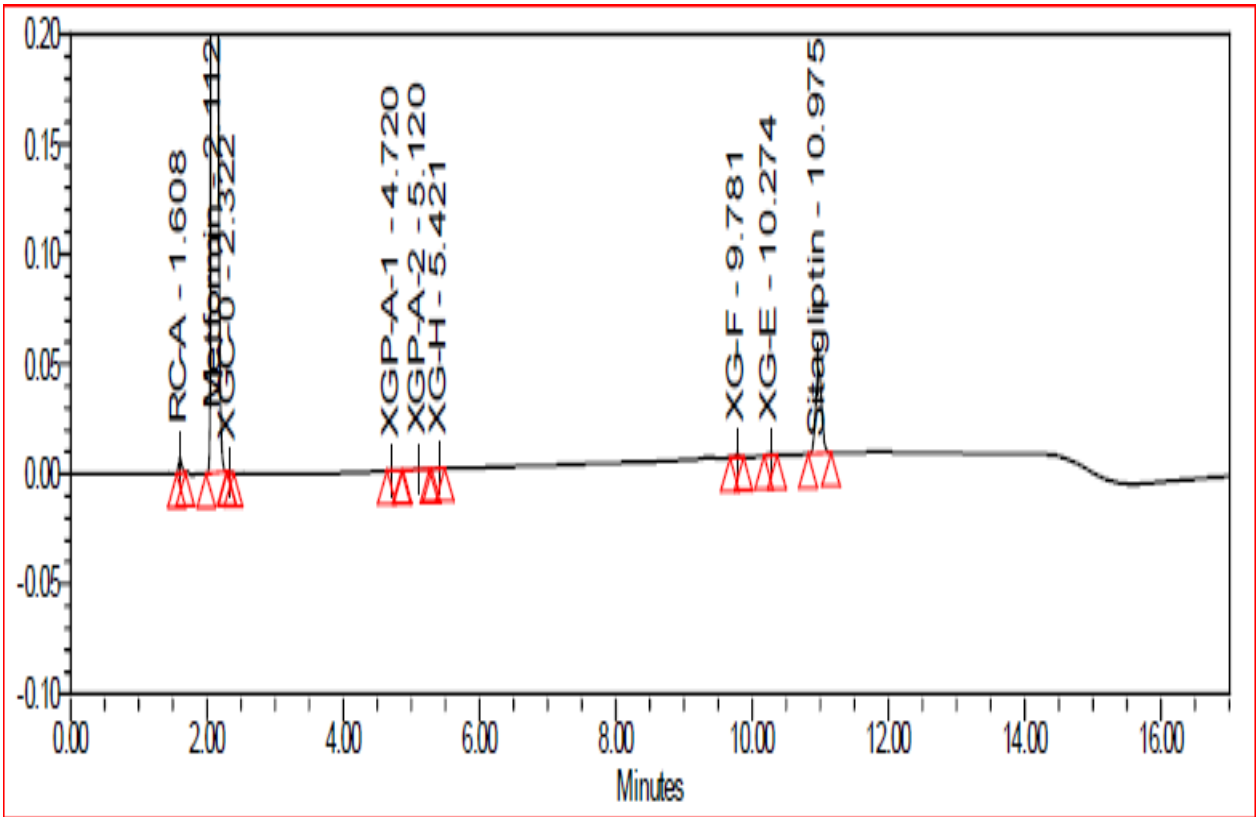


Figure 4:- (System suitability chromatogram).

Table 3:- Summery of system suitability results.

Name of Imp	Spiked solution with 1% of test 0.025mg/mL test Concentration			
	RT (min)	Resolution	Plate Count	Tailing Factor
RC-A	1.608	NA	5637	1.0
Metformin	2.112	4.9	5362	1.0
XGC-0	2.322	2.2	14221	1.0
XGP-A-1	4.720	20.3	13052	1.0
XGP-A-2	5.120	1.7	11029	1.0
XG-H	5.421	28.4	19639	1.0
XG-Q	9.306	3.2	67481	1.0
XG-F	9.781	4.4	73154	1.0
XG-E	10.274	1.9	74141	1.0
Sitagliptin	10.975	NA	74524	1.0

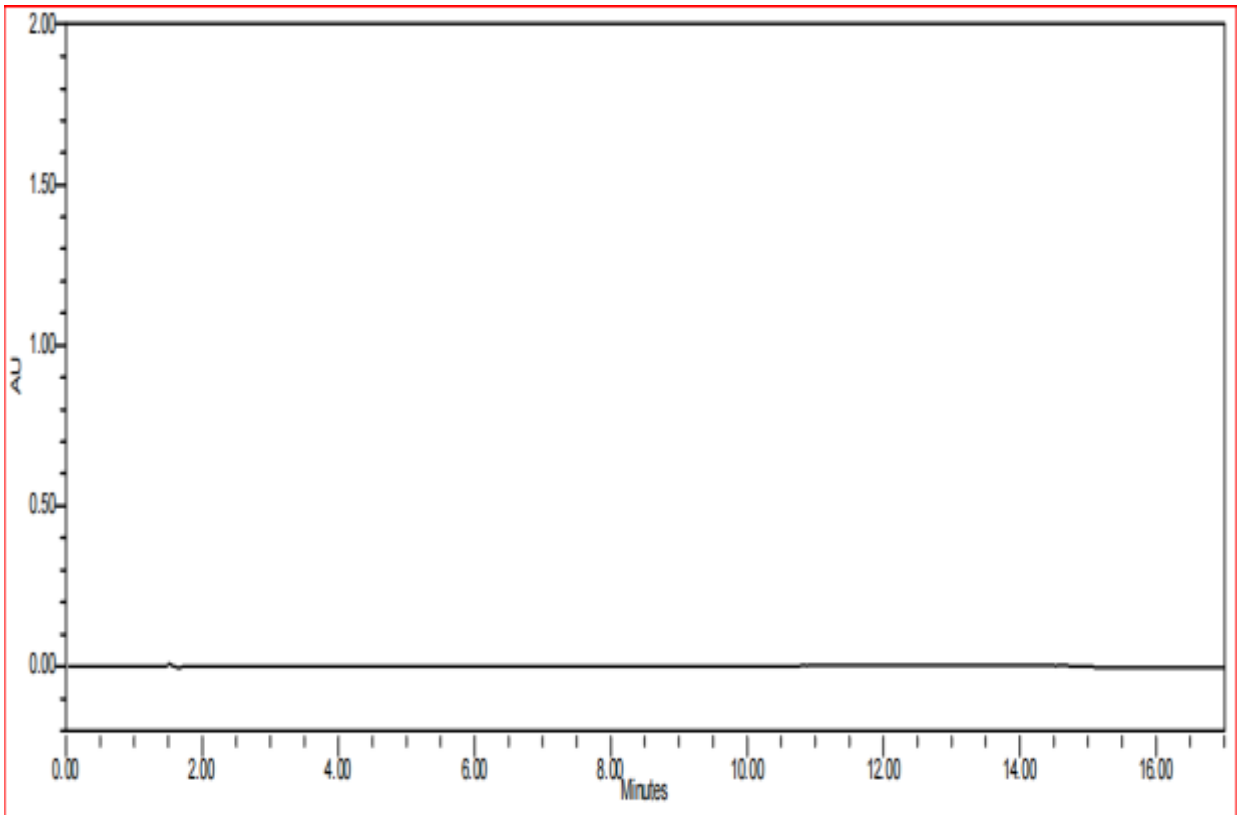


Figure 4:- (Blank solution Chromatogram).

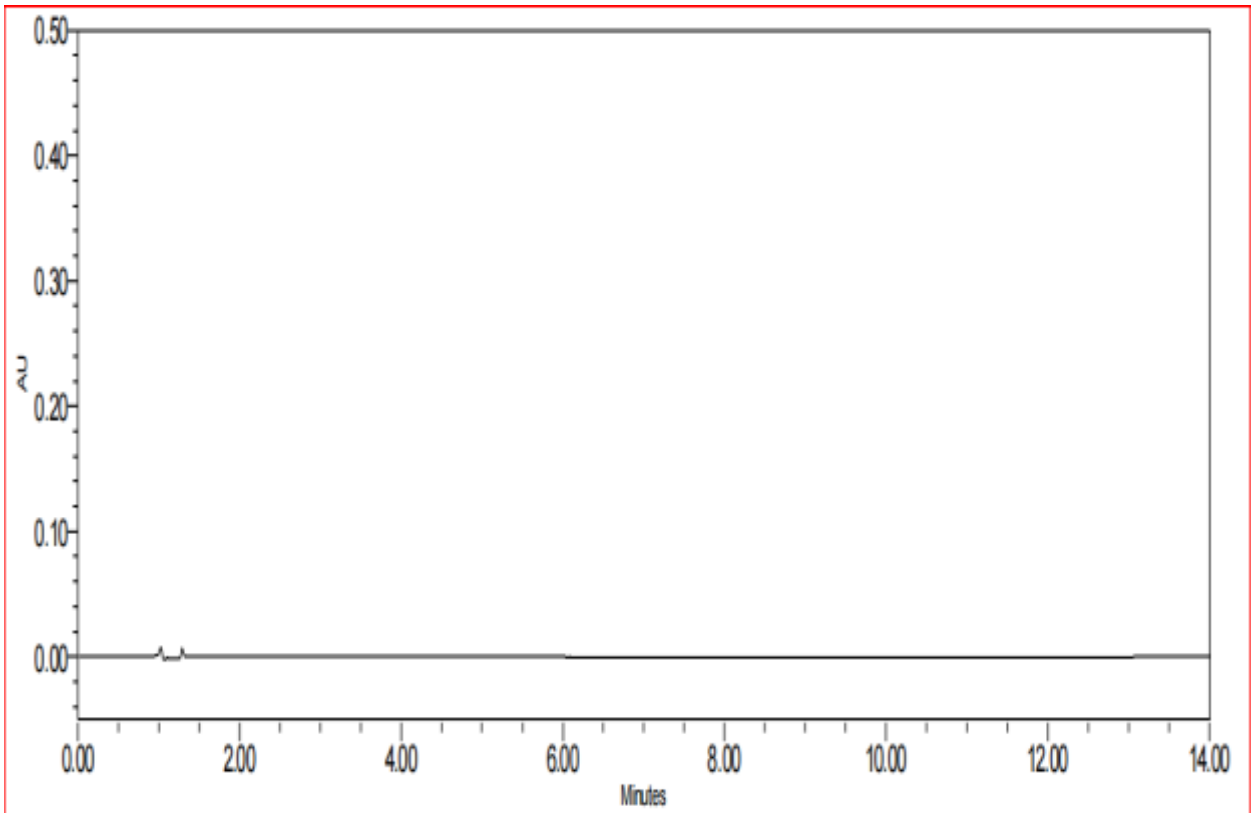


Figure 5:- (Placebo solution Chromatogram).

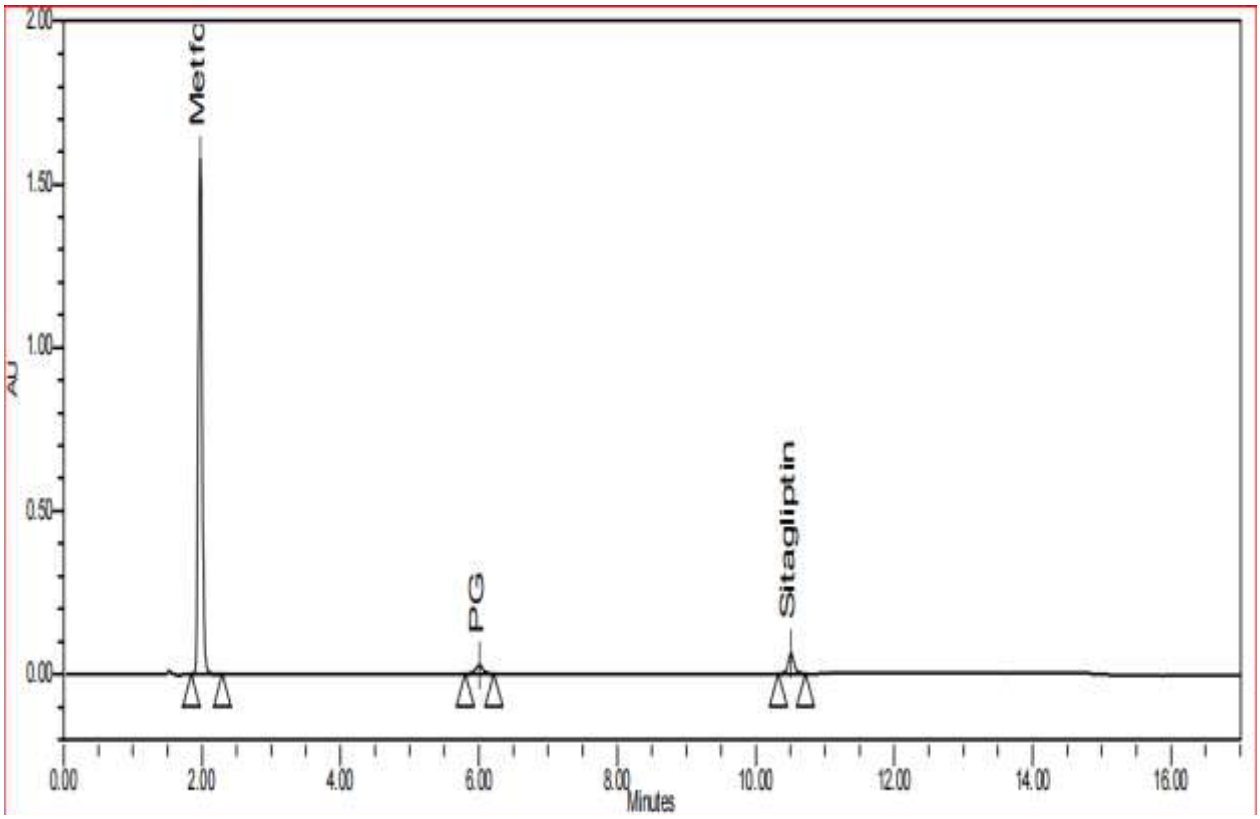


Figure 6:- (Standard solution Chromatogram).

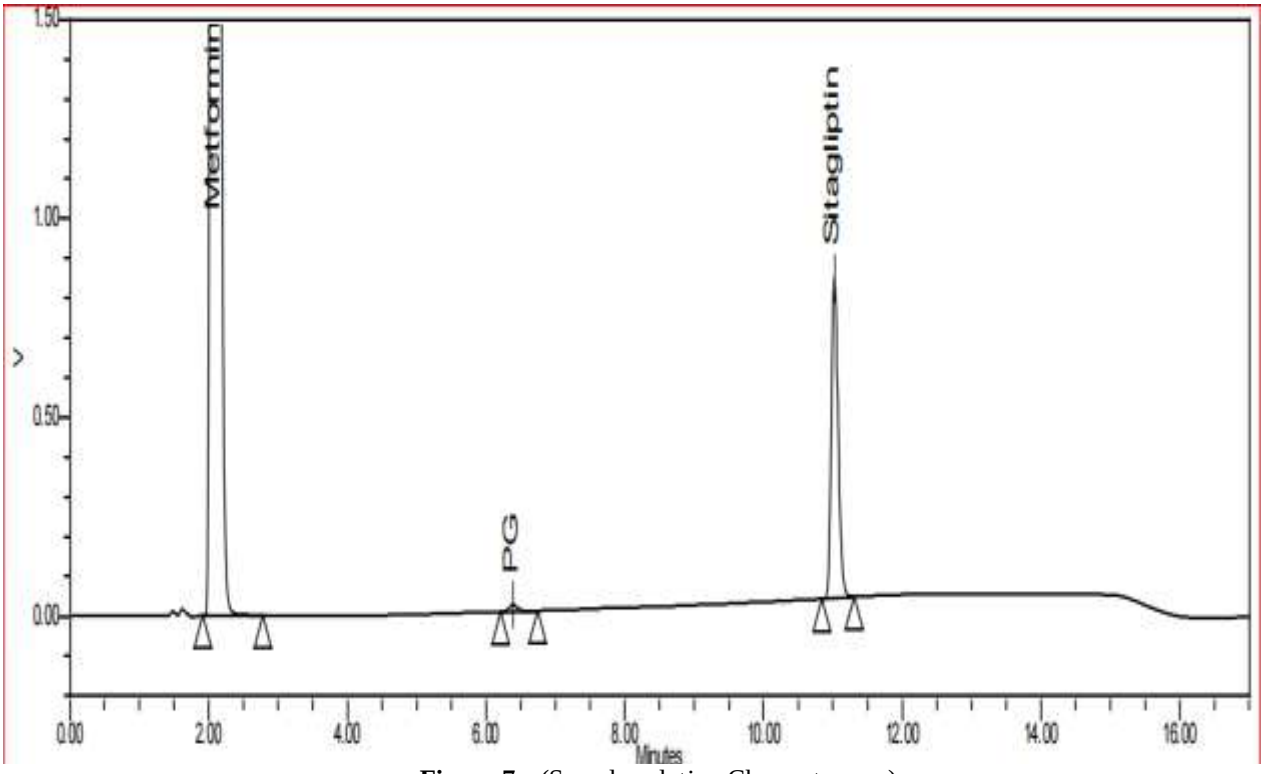


Figure 7:- (Sample solution Chromatogram).

Force degradation study summary results:

Table 2:- Degradation summary results.

Sitagliptin						
Degradation Conditions		**Degradation Content (%)**	**Total Degradation**	**(%) Mass Balance**	**Purity Angle**	**Purity Threshold**
Controlled Sample		98.5	0.06	98.56	0.025	0.152
Acid Degradation	1mol/L HCl-2mL-48h	95.5	0.09	95.59	0.031	0152
Base Degradation	1mol/LNaOH-2mL-48h	98.2	0.03	98.23	0.035	0.145
Oxidation Degradation	10%H_2O_2-2mL-48h	95.1	0.07	95.17	0.038	0.182
Temperature (Solid)	60℃-148h	100.2	0.01	100.21	0.031	0.196
Temperature (Liquid)	60℃-24h	99.5	0.03	99.53	0.039	0.156
Light (Solid)	4500LUX -48h	98.6	0.03	98.63	0.042	0.125
Light (Liquid)	4500LUX -48h	98.9	0.02	98.92	0.041	0.165
Humidity	92.5%RH -48h	95.7	0.08	95.78	0.040	0.155
Metformin HCl						
Degradation Conditions		**Degradation Content (%)**	**Total Degradation**	**(%) Mass Balance**	**Purity Angle**	**Purity Threshold**
Controlled Sample		100.5	0.01	100.51	0.251	0.456
Acid Degradation	1mol/L HCl-2ml-48h	99.5	0.02	99.52	0.244	0.425
Base Degradation	1mol/LNaOH-2ml-48h	99.6	0.02	99.62	0.236	0.435
Oxidation Degradation	10%H_2O_2-2ml-48h	98.1	0.01	98.2	0.252	0.482
Temperature (Solid)	60℃-148h	98.9	0.06	98.96	0.325	0.442
Temperature (Liquid)	60℃-24h	101.2	0.01	101.21	0.260	0.435
Light (Solid)	4500LUX -48h	100.5	0.01	100.51	0.234	0.463
Light (Liquid)	4500LUX -48h	102.1	0.02	102.12	0.228	0.432
Humidity	92.5%RH -48h	100.5	0.01	100.51	0.214	0.425
Propyl Gallate						
Degradation Conditions		**Degradation Content (%)**	**Total Degradation**	**(%) Mass Balance**	**Purity Angle**	**Purity Threshold**
Controlled Sample		103.5	0.00	103.5	1.256	3.263
Acid Degradation	1mol/L HCl-2ml-48h	102.3	0.01	102.31	1.236	3.254
Base Degradation	1mol/LNaOH-2ml-48h	98.5	0.03	98.53	1.245	3.652
Oxidation Degradation	10%H_2O_2-2ml-48h	97.2	0.05	97.25	1.352	3.255
Temperature (Solid)	60℃-148h	96.6	0.09	96.69	1.298	3.522
Temperature (Liquid)	60℃-24h	100.2	0.00	100.2	1.242	3.566
Light (Solid)	4500LUX -48h	103.2	0.00	103.2	1.235	3.255
Light (Liquid)	4500LUX -48h	101.5	0.00	101.5	1.277	3.855
Humidity	92.5%RH -48h	100.9	0.00	100.9	1.236	3.566

Linearity:
Calibration curve obtained by the least square regression analysis between peak area and concentration showed linear relationship with a correlation coefficient of greater than 0.999 over the calibration ranges tested for both the actives. Correlation obtained between peak area and concentration of Sitagliptin, propyl gallate and Metformin HCl. Linearity graph of Sitagliptin, propyl gallate and Metformin HCl and are shown in table 3,4and table 5.

Table 3:- Linearity Results for Metformin HCl.

(%) Level	Concentration (µg/mL)	Peak Area
50	62.367	1597251
80	124.733	3195655
100	249.466	6391425
120	374.200	9587365
200	498.933	12783125
y-Intercept	-482.500000	
Slope of regression line	25622.1038	
Correlation Coefficient	1.0000	

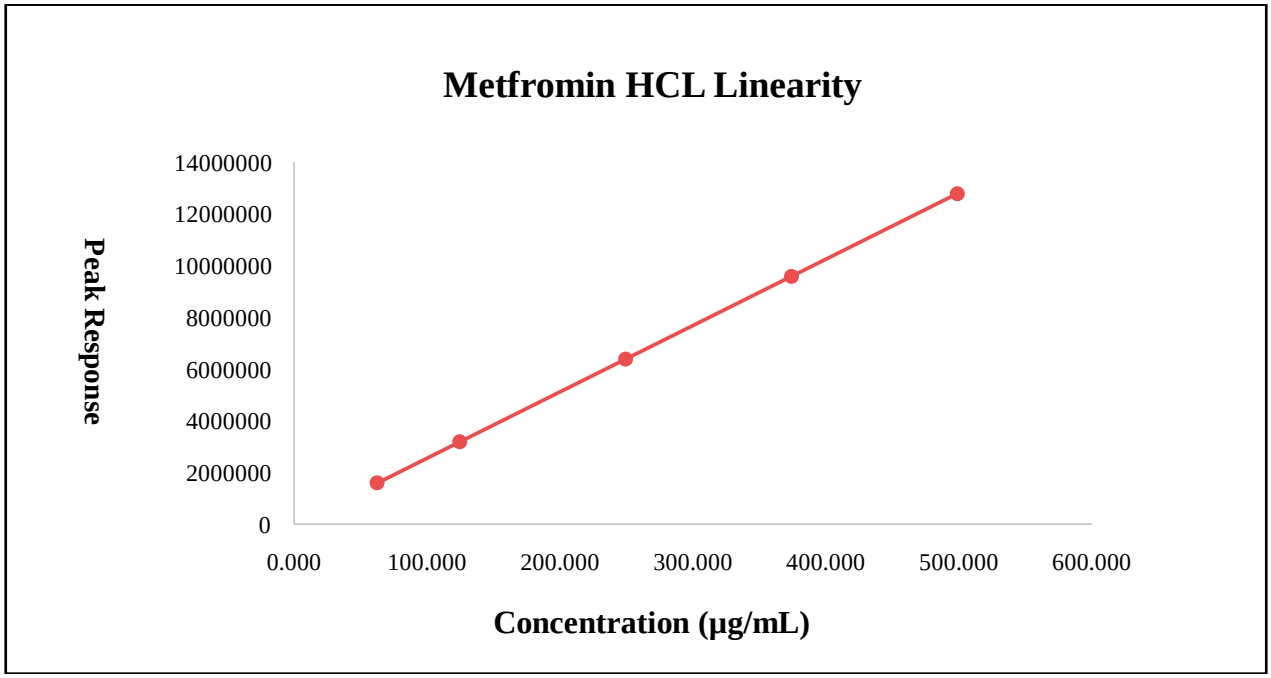


Table 4:- Linearity Results for Sitagliptin.

(%) Level	Concentration (µg/mL)	Peak Area
50	6.658	92115
80	13.315	184235
100	26.630	368425
120	39.945	552723
200	53.260	736985
y-Intercept	-25.12195122	
Slope of regression line	13837.535	
Correlation Coefficient	1.0000	

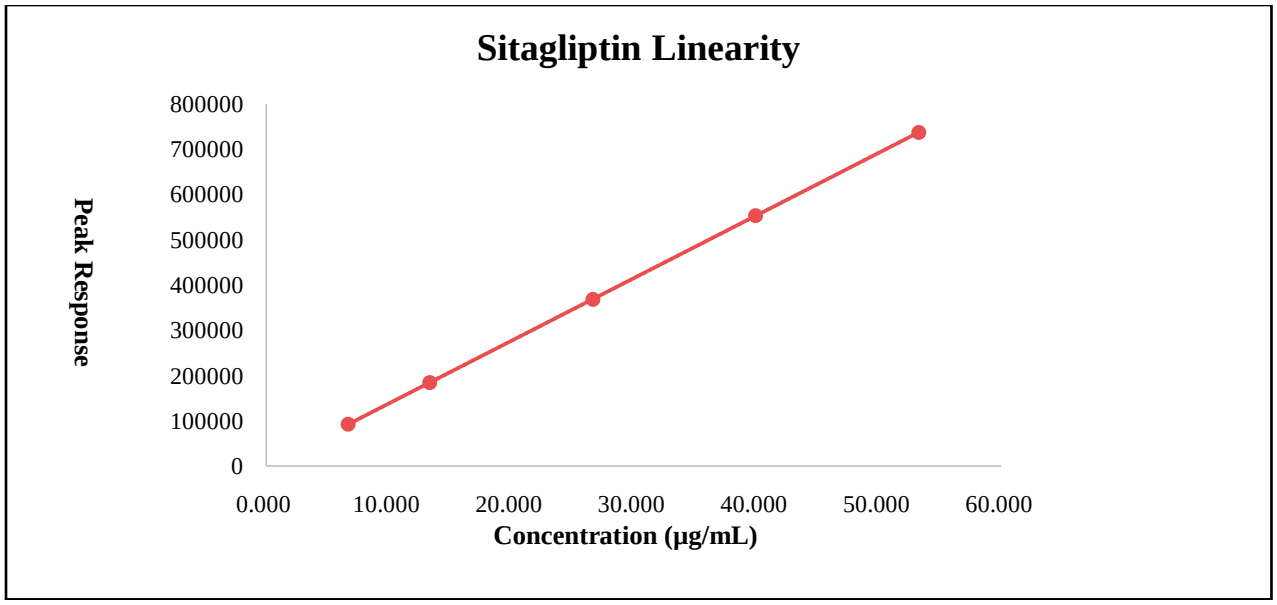
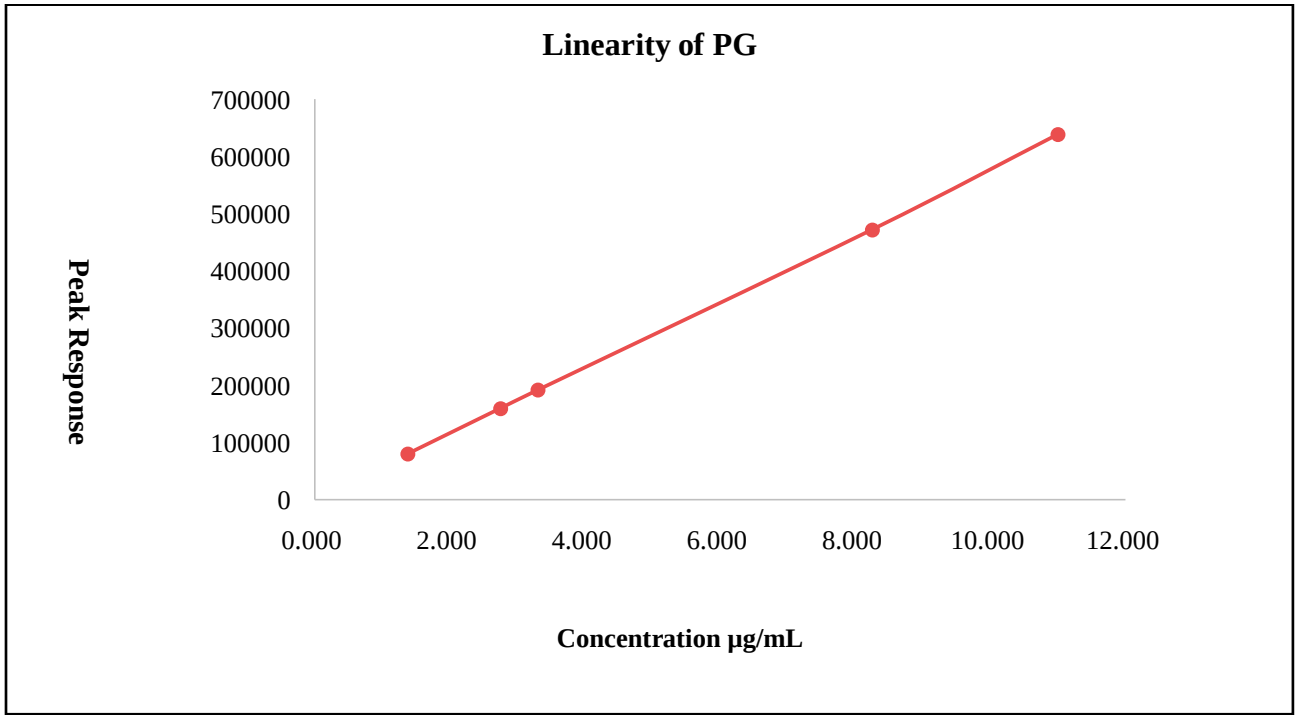


Table 5:- Linearity Results for Propyl Gallate.

(%) Level	Concentration (µg/mL)	Peak Area
50	1.375	79358
80	2.750	159012
100	3.300	190855
120	8.249	471252
200	10.998	637851
y-Intercept	-329.22858	
Slope of regression line	57739.38788	
Correlation Coefficient	0.9999	



Method Precision and Intermediate Precision:
The average % contents of Sitagliptin/Metformin HCl, PG in tablets were found to be 100.7,99.6 and 100.4. The %RSD found to be 0.9,0.6 and 0.7. The average results between method precision and intermediate precision have also shown less than 1.0% RSD. The results were given in Table 6.

Table 6:- Method Precision and Intermediate Precision results.

S.No.:	% Content (Sitagliptin)		% Content (Metformin HCl)		% Content (PG)	
	Method Precision	Intermediate precision	Method Precision	Intermediate precision	Method Precision	Intermediate precision
1	102.1	100.2	99.1	100.1	100.5	100.2
2	100.1	100.5	99.5	100.5	98.5	100.9
3	99.5	101.3	99.8	99.5	100.2	101.2
4	100.2	102.5	100.1	98.6	100.3	100.8
5	100.6	100.2	100.0	99.2	100.5	99.8
6	101.0	99.8	99.0	99.3	99.5	99.6
Mean	100.6	100.8	99.6	99.5	99.9	100.4
SD	0.8976	0.9935	0.4622	0.6772	0.7859	0.6463
%RSD	0.9	1.0	0.5	0.7	0.8	0.6
Overall mean	100.7		99.6		100.4	
%RSD	0.9		0.6		0.7	

Accuracy:
Accuracy was conducted at three different levels including 50%, 100% and 150% of the test concentration level for three components. The observed recovery results were found in the range between 98 to 102%. The recovery results indicated that the test method has an acceptable level of accuracy for the assay of Sitagliptin/Metformin HCl, PG in Sitagliptin/Metformin HCl Tablets from 50% to 150% test concentration. The results were given in Table 7.

Table 7:- Recovery on synthetic mixture of both drug substances as excipients.

Level (%)	Sitagliptin (AVG)		%RSD	Metformin HCl (AVG)		%RSD	PG (AVG)		%RSD
50%-1	100.2	100.1	0.5	100.2	100.6	0.3	98.5	98.9	0.3
50%-2	99.5			100.6			99.0		
50%-3	100.5			100.9			99.1		
100%-1	100.5	100.2	0.6	100.2	100.2	0.3	99.0	98.7	0.3
100%-2	100.6			100.5			98.5		
100%-3	99.5			99.9			98.6		
150%-1	99.2	100.0	0.7	100.2	100.2	0.4	98.3	98.8	0.6
150%-2	100.5			99.8			99.5		
150%-3	100.3			100.6			98.6		

Solution Stability:
The reference solution and the test sample solution considered, were respectively placed at room temperature and refrigerator for a period of 48 hours. The results were given in Table 8 and Table 9.

Table 8:- Solution stability in reference solution.

Stability of reference solution (Metformin)				
Time	(~25°C) Room Temperature		(~5℃) Refrigerator	
	Peak Area	% Difference	Peak Area	% Difference
0h	6391425	NA	6391425	NA
24h	6391321	0.0	6391325	0.0
48h	6391523	0.0	6391401	0.0
Stability of reference solution (Sitagliptin)				

Time	(~25°C) Room Temperature		（~5℃） Refrigerator	
	Peak Area	% Difference	Peak Area	% Difference
0h	368425	NA	368521	NA
24h	368385	0.0	368424	0.0
48h	368352	0.0	368503	0.0
Stability of reference solution (PG)				
Time	**(~25°C) Room Temperature**		**（~5℃） Refrigerator**	
	Peak Area	**% Difference**	**Peak Area**	**% Difference**
0h	190855	NA	190855	NA
24h	190922	0.0	190799	0.0
48h	190900	0.0	190785	0.0

Table 9:- Solution stability in sample solution.

Test Solution Stability (Metformin)				
Time	**(~25) Room Temperature**		**(~5℃） Refrigerator**	
	Peak Area	**% Difference**	**Peak Area**	**% Difference**
0h	6593258	NA	6593258	NA
24h	6562321	0.5	6649852	0.9
48h	6568921	0.4	6591253	0.0
Test Solution Stability (Sitagliptin)				
Time	**(~25°C) Room Temperature**		**（~5℃） Refrigerator**	
	Peak Area	**% Difference**	**Peak Area**	**% Difference**
0h	359824	NA	359824	NA
24h	356891	0.8	356852	0.8
48h	358921	0.3	356621	0.9
Test Solution Stability (PG)				
Time	**(~25°C) Room Temperature**		**（~5℃） Refrigerator**	
	Peak Area	**% Difference**	**Peak Area**	**% Difference**
0h	189521	NA	189521	NA
24h	190122	0.3	188521	0.5
48h	190012	0.3	187542	1.0

Robustness:

The reference solution was injected in different conditions and there are no abnormal results, in all the conditions system suitability is good. The results were given in Table 9, Table10 and Table 11.

Table 9:- Robustness results of reference solution (Metformin HCl).

Metformin HCl					
Condition		**Retention time**	**RSD% Peak Area**	**Theoretical Plates**	**Tailing Factor**
Normal Condition		1.921	0.2	47524	1.1
Flow	1.1ml/min	2.012	0.2	47124	1.0
	1.3 ml/min	1.821	0.2	47241	1.0
Wavelength	268 nm	1.935	0.2	47414	1.0
	272nm	1.931	0.3	47454	1.0
Column Temperature	35 ℃	1.991	0.2	47332	1.0
	45℃	1.921	0.1	47325	1.0
Sample Temperature	15 ℃	1.925	0.2	47410	1.0
	25 ℃	1.923	0.3	47394	1.0
Organic phase % in mobile phase A	86:14	2.001	0.2	47525	1.1
	84:16	2.012	0.1	47542	1.1

Buffer preparation (pH)	2.8	1.981	0.2	47652	1.1
	3.2	1.985	0.2	47652	1.0

Table 10:- Robustness results of reference solution (Sitagliptin).

Hydrochlorothiazide					
Condition		**Retention time**	**%RSD Peak Area**	**Theoretical Plates**	**Tailing Factor**
Normal Condition		11.521	0.3	25111	1.1
Flow	1.1ml/min	11.852	0.2	25134	1.0
	1.3 ml/min	11.921	0.2	25155	1.0
Wavelength	268 nm	11.452	0.2	24965	1.0
	272nm	11.452	0.3	24934	1.2
Column Temperature	35℃	11.852	0.2	25335	1.1
	45℃	11.932	0.1	25362	1.1
Sample Temperature	15℃	11.521	0.1	25632	1.0
	25℃	11.536	0.0	25633	1.0
Organic phase % in mobile phase A	86:14	11.325	0.2	25142	1.1
	84:16	11.865	0.3	25632	1.0
Buffer preparation (pH)	3.2	11.555	0.1	25335	1.0
	3.7	11.632	0.2	25235	1.0

Table 11:- Robustness results of reference solution (Propyl Gallate).

Propyl Gallate					
Condition		**Retention time**	**%RSD Peak Area**	**Theoretical Plates**	**Tailing Factor**
Normal Condition		6.521	0.1	33325	1.0
Flow	1.1ml/min	6.921	0.2	33253	1.0
	1.3 ml/min	6.025	0.1	33254	1.0
Wavelength	268 nm	6.452	0.1	33255	1.0
	272nm	6.423	0.1	33352	1.0
Column Temperature	35℃	6.852	0.1	32554	1.0
	45℃	6.324	0.1	33652	1.2
Sample Temperature	15℃	6.525	0.2	36582	1.0
	25℃	6.235	0.2	36524	1.0
Organic phase % in mobile phase A	86:14	6.772	0.2	33524	1.0
	84:16	6.335	0.2	32545	1.0
Buffer preparation (pH)	2.8	6.253	0.0	33652	1.0
	3.2	6.325	0.0	36521	1.0

Conclusion:-

The Validated HPLC results shows that the of Sitagliptin/Metformin HCl in bulk and tablets dosage forms. The method, specific, precise, robust, stable, and can be applied for the routine and stability analysis for commercially available formulation.

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