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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HCL AND SITAGLIPTIN IN PHARMACEUTICAL DOSAGE FORMS

K. Vamsee Krishna Reddy[1]* and R. Madhusudhan Raju[2]

[1]Department of Chemistry, Osmania university, Hyderabad, India.

[2]Department College of Technology, Osmania university,Hyderabad, India.

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***Correspondence for Author**
K. Vamsee Krishna Reddy
Department of Chemistry, Osmania university,Hyderabad, India.

ABSTRACT

A simple, rapid, precise and accurate reverse-phase HPLC method was developed and validated for the simultaneous determination of Metformin HCL and Sitagliptin in commercial tablets. The method has shown adequate separation for Metformin HCL and Sitagliptin. Separation was achieved on Kromasil C8 (250 mm × 4.6mm; 5 µm) column using isocratic method with 0.1NaH2PO4:Methanol(60:40) system at room temperature and the detection was carried out at 235nm using photodiode array (PDA) detector. The linearity of the proposed method was investigated in the range of 50-150µg/ml (r^2=0.9998), 5-15µg/ml (r^2=0.999) for Metformin HCL and Sitagliptin Respectively. The limit of detection (LOD) was 0.341and 0.0495 for Metformin HCL and Sitagliptin respectively. The limit of quantification (LOQ) was 1.136 and 0.1650 for Metformin HCL and Sitagliptin respectively. The relative standard deviation (RSD) of six replicates is less than 2%. This HPLC method is applied successfully to the simultaneous quantitative analysis of Metformin HCL and Sitagliptin in commercial tablets.

KEYWORDS: RP-HPLC, Metformin HCL ,Sitagliptin,Simultaneous Estimation.

INTRODUCTION

Metformin,[1,2] is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). Chemically,Metformin HCL is described as 1-carbamimida mido-N,N-dimethylmethanimidamide. Metformin decreases blood glucose

levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by metformin of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats. Activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors.

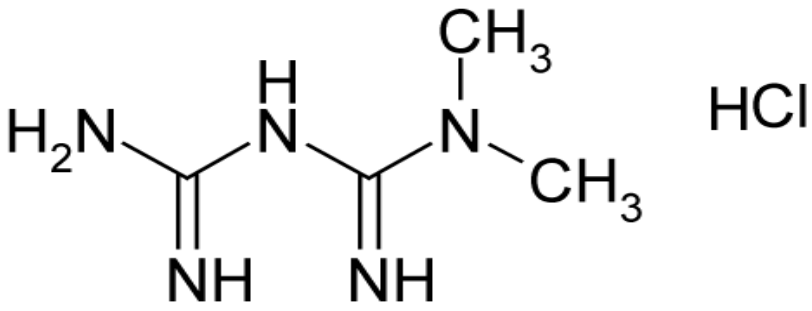

Figure 1: Chemical structure of Metformin HCL

Sitagliptin,[3,4] is a new oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs.The chemical name of stavudine is (3R)-3-amino-1-[3-(trifluoromethyl)-5H,6H,7H,8H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan-1-one. The empirical formula is $C_{16}H_{15}F_6N_5O$. Its molecular weight is 407.3 and its structural formula is:

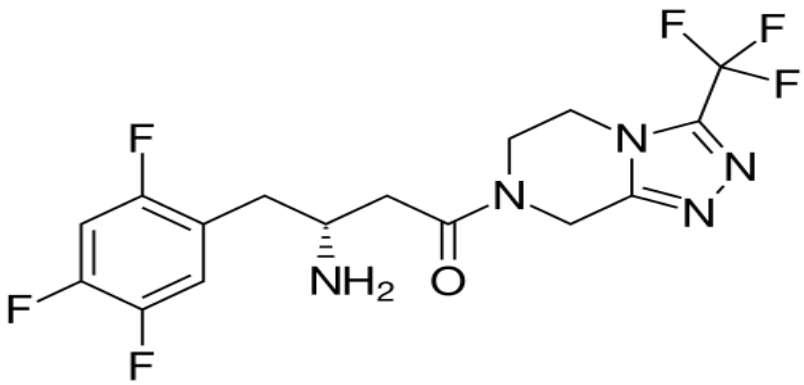

Figure 2: Chemical structure of Sitagliptin

Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1and GIP, gastrointestinal hormones released in

response to a meal. By preventing GLP-1 and GIP inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas.

The literature reports, many methods for simultaneous quantitative determination of Metformin HCL and Sitagliptin in bulk, tablet dosage form, capsule dosage form and human plasma. These methods include simultaneous estimation of Metformin HCL and Sitagliptin by UV spectrophotometry,[5,6] Flourometry,[7] UPLC,[8] MS/MS,[9] HPTLC,[10] and HPLC.[11,22]

The aim of the present investigation is to develop and validate a sensitive, precise and accurate RP-HPLC method for the simultaneous quantification of Metformin HCL and Sitagliptin in bulk and in its combined pharmaceutical formulation. The proposed method is validated as per ICH guidelines.[23,24]

MATERIALS AND METHOD

Pure standards and Chemicals

Metformin HCL and Sitagliptin was a gift sample by Lara drugs Pvt Ltd., Hyderabad. Sodium dihydrogen phosphate,methanol of HPLC grade was purchased from Merck (India) Ltd., Mumbai and HPLC grade water from milli Q water.

Instrumentation

Analysis was carried out using Waters 2695 alliance HPLC system with binary HPLC pump and Waters 2998 PDA detector. Waters Empower2 version software was used for the acquisition of the chromatographic data.

Chromatographic conditions

The HPLC separation and quantification of the Metformin HCL and Sitagliptin were made on the Kromasil C8 Analytical column (250 mm × 4.6mm; 5 µm). An isocratic mobile phase consisting of 0.1M Sodium dihydrogen phosphate:Methanol in the proportion of 60:40 v/v at a temperature of 30 °C was the optimized mobile composition and column temperature. The eluate was monitored at 235 nm. The mobile was pumped into the column at a flow rate of 1.0 mL/min and the run time was 8 min. The volume of injection loop was 10µL. Prior to injection of the drug solution the column was equilibrated for at least 10 min with the mobile phase flowing through the system.

Preparation of standard solutions

Accurately weigh and transfer 500 mg Metformin HCL and 50 mg Sitagliptin into 100 ml of volumetric flask and add 10 ml of mobile phase and sonicate for 10 min and makeup the volume upto the mark with mobile phase. This solution is used as stock standard solution. Pipette out 2 ml of stock standard stock into 100 ml volumetric flask and dilute to volume with mobile phase.

Preparation of Sample Solutions

Accurately weigh 708.0mg of sample. Transfer the sample powder into 100 ml of volumetric flask add 10 ml mobile phase and sonicate for 20 min to completely dissolve the drugs. The volume was made up to the mark with mobile phase and filter through the 0.45 µm filter paper. Transfer 2 ml of above prepared solution into 100 ml volumetric flask and make up the volume to mark with mobile phase.

System Suitability Studies

System suitability for chromatographic separation was checked on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system meet the standards required by the method. Mixed standard solution of Metformin HCL and Sitagliptin solution was injected in six replicates and system suitability parameters were determined System suitability parameters established for the developed method include number of theoretical plates, resolution and tailing factor.

Linearity and Range

The linearity was established by least squares linear regression analysis of the calibration curve for Metformin HCL and Sitagliptin standard solutions by plotting the concentrations of the compound versus peak area response.

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out 3 times. The percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate. The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample

solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions.It was observed that there were no marked changes in the chromatograms which demonstrated that the RP-HPLC method developed is robust.

Limit of quantification (LOQ) and detection (LOD)

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Metformin HCL and Sitagliptin. RSD (σ) method predicted using following formulas (a) and (b). Precision was established at this predicted level was applied; the LOQ and LOD values were,

(a) LOQ = 10 σ / S

(b) LOD = 3.3 σ / S

Where σ = residual standard deviation of response

S = slope of the calibration curve.

RESULTS AND DISCUSSION

System Suitability Studies

The column efficiency, resolution and tailing factor were calculated for the standard solutions (Table 1).The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 2 %Relative standard deviation range during routine performance of the method.

Table 1: System suitability parameters

Parameter	Metformin HCL	Sitagliptin
Retention time	2.744	5.203
Theoretical plates	7071	6260
Tailing factor	1.33	1.19
% RSD	0.1	0.5

Linearity and range

The range of linearity of the method was 50-150 µg/ml for Metformin HCL and 5-15 µg/ml for Sitagliptin. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was Y = 15946 (R^2=0.9998) for Metformin HCL and Y = 21762 (R^2=0.9999) for Sitagliptin. The results

shows an excellent correlation exists between peak area and concentration of Metformin HCL and Sitagliptin within concentration range indicated above. The results for calibration data are shown in Table 2 and calibration curves are given in Figure 3& 4.

Table 2: Linearity studies for Metformin HCL and Sitagliptin by proposed method.

Metformin HCL		Sitagliptin	
Area	**Amount of drug (µg/mL)**	**Area**	**Amount of drug (µg/mL)**
797097	50	1088471	5
1195796	75.00	1632923	7.5
1594713	100.00	2156898	10
1992713	125	2720542	12.5
2391941	150	3264875	15.00

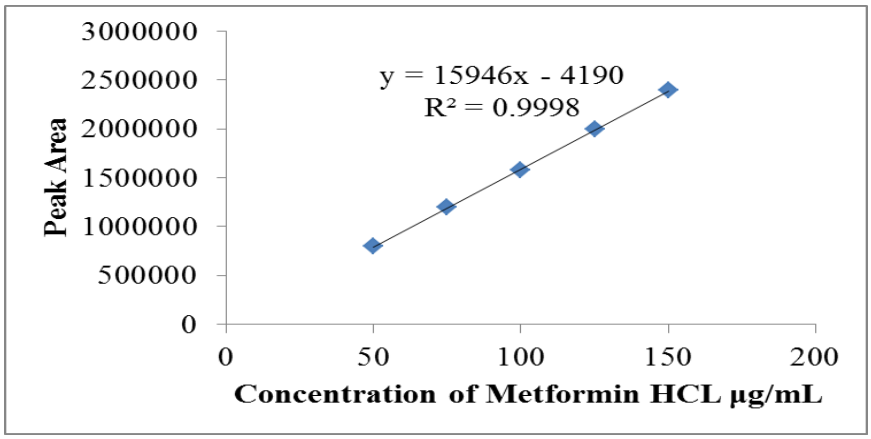

Figure 3: Linearity curve for Metformin HCL

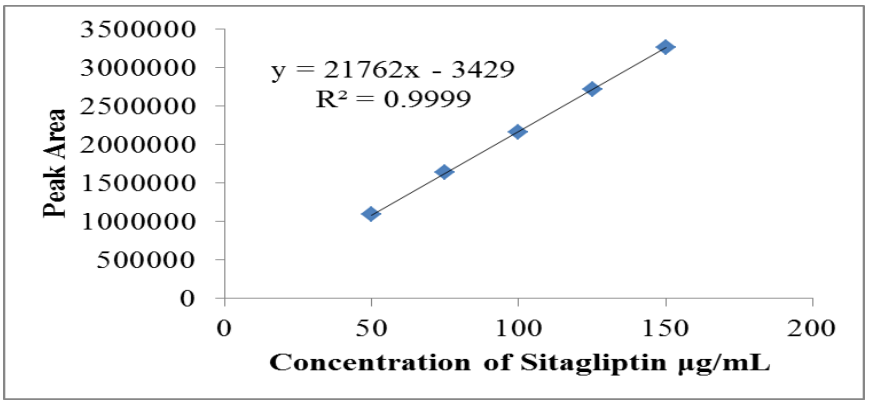

Figure 4: Linearity curve for Sitagliptin

Accuracy and Precision

The results of accuracy of the method were determined by recovery experiments. The percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate (Tables 3& 4).

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug and percentage RSD were calculated. The chromatograms of three different levels shown in Figure 5,6 & 7. From the results, the developed RP-HPLC method was considered to be precise (Table 5).

Table 3: Accuracy for Metformin HCL

Spiked Level	Sample Weight	µg/ml added	µg/ml found	% Recovery	% Mean
50%	354.00	49.500	49.70	100	100
50%	354.00	49.500	49.69	100	
50%	354.00	49.500	49.70	100	
100%	708.00	99.000	99.44	100	100
100%	708.00	99.000	99.19	100	
100%	708.00	99.000	99.40	100	
150%	1062.00	148.500	149.08	100	100
150%	1062.00	148.500	148.99	100	
150%	1062.00	148.500	149.04	100	

Table 4: Accuracy for Sitagliptin

Spiked Level	Sample Weight	µg/ml added	µg/ml found	% Recovery	% Mean
50%	354.00	5.000	4.96	99	99
50%	354.00	5.000	4.95	99	
50%	354.00	5.000	4.98	100	
100%	708.00	10.000	9.98	100	100
100%	708.00	10.000	9.95	99	
100%	708.00	10.000	9.98	100	
150%	1062.00	15.000	14.98	100	100
150%	1062.00	15.000	14.94	100	
150%	1062.00	15.000	14.95	100	

Table 5: Precision studies

Sample Wt(mg)	Metformin HCL		Sitagliptin	
	Peak area	% Assay	Peak area	% Assay
708.00	1590337	99	2171635	99
708.00	1598175	100	2176631	100
708.00	1593175	99	2175775	100
708.00	1595908	99	2175928	100
708.00	1597945	100	2179526	100
708.00	1590765	99	2172443	100

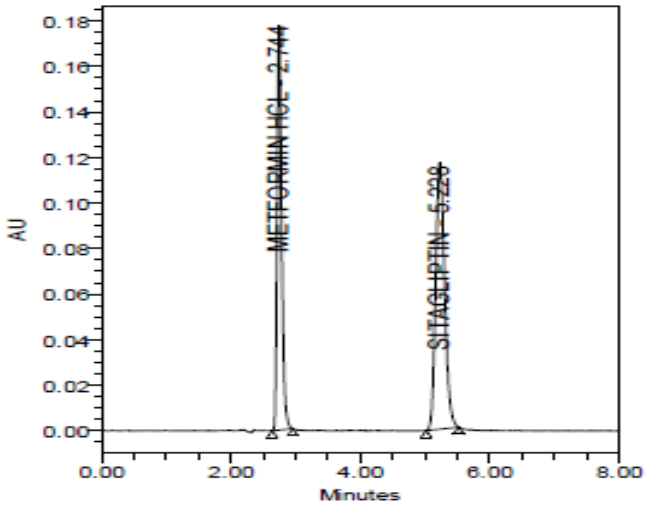

Figure 5: Chromatogram of Metformin HCL and Sitagliptin at 50 % accuracy level.

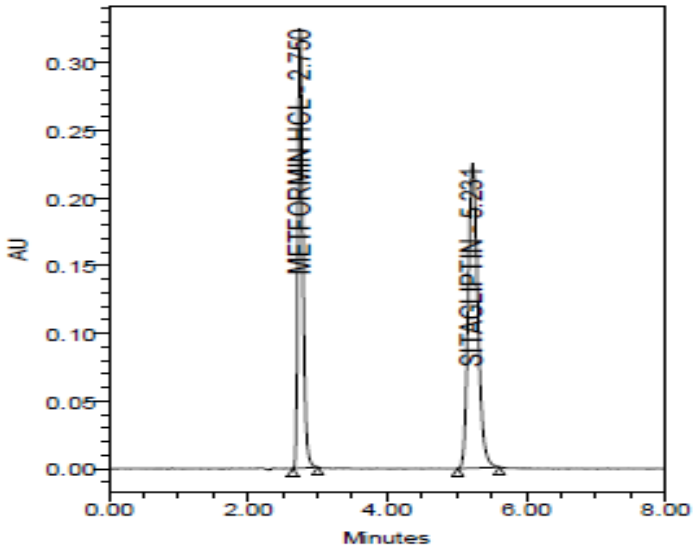

Figure 6: Chromatogram of Metformin HCL and Sitagliptin at 100 % accuracy level

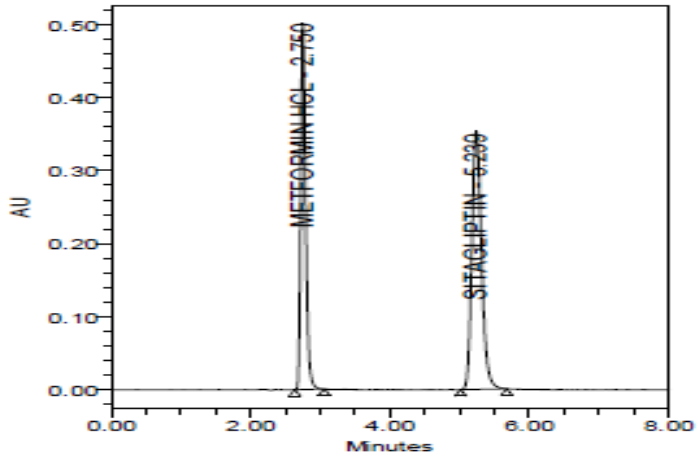

Figure 7: Chromatogram of Metformin HCL and Sitagliptin at 150 % accuracy level

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions.It was observed that there were no marked changes in the chromatograms which demonstrated that the RP-HPLC method developed is are robust (Table 6,7).

Table 6: Robustness for Metformin HCL

Sample. No	Sample Name	RT	Area	Theoretical plates	USP Tailing
1	Temp-1	3.009	1740015	8413	1.31
2	Temp-2	2.562	1483017	7388	1.36
3	Flow-1	3.017	1755810	8207	1.31
4	Flow-2	2.551	1485269	7261	1.36

Table 7: Robustness for Sitagliptin

Sample. No	Sample Name	RT	Area	Theoretical plates	USP Tailing
1	Temp-1	5.747	2381162	7742	1.21
2	Temp-2	4.931	2028832	6725	1.22
3	Flow-1	5.759	2377626	7657	1.19
4	Flow-2	4.919	2037843	6535	1.23

LOD & LOQ

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Metformin HCL and Sitagliptin. Relative standard deviation (σ) method was applied, the LOQ and LOD values were predicted using following formulas (a) and (b). Precision was established at these predicted levels.

(a) LOQ = 10 σ / S

(b) LOD = 3.3 σ / S

Where σ = residual standard deviation of response; s = slope of the calibration curve.

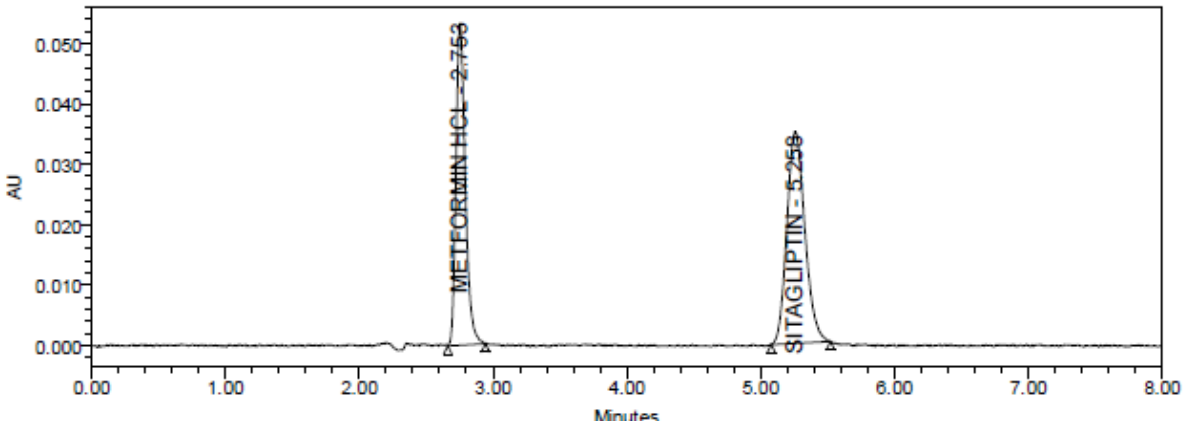

Figure 10: Chromatogram for LOD

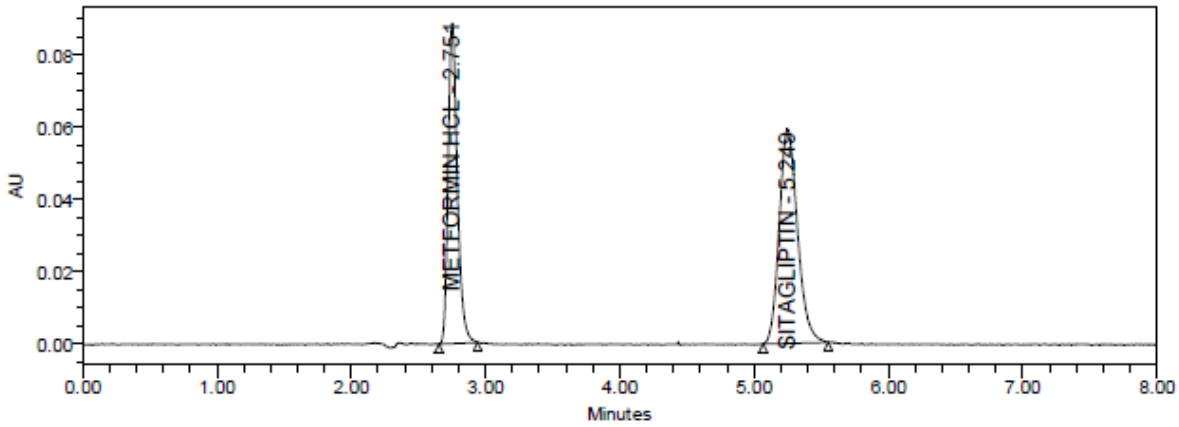

Figure 11: Chromatogram for LOQ

Table 8: LOD and LOQ for Metformin HCL and Sitagliptin

Sample. No	Sample Type	Sample Name	RT	Area	Value (µg/ml)
1	LOD	Metformin HCL	2.753	255878	0.341
2	LOQ	Metformin HCL	2.751	424156	1.136
1	LOD	Sitagliptin	5.258	327565	0.0495
2	LOQ	Sitagliptin	5.249	558421	0.1650

OVER ALL SUMMARY OF THE METHOD

System suitability results were given in by Table 1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1. Hence the system is suitable for analysis of the selected drugs. The result given in Table 6 concluded that the method precision passed for Metformin HCL and Sitagliptin studies. The method accuracy was evaluated by recovery studies. The percent recovery of Metformin HCL and Sitagliptin was found to be 100% & 100%, respectively. The percent recovery values are as per the acceptance criteria of ICH (97% - 103%). The low percentage indicated that the method is accurate. The results are shown in Tables 3 and 4. Linearity calibration curve was given below Figures 4, 5 and 6. The calibration graph was plotted by taking concentration versus area to construct the linear regression equation and to calculate the value of correlation co-efficient. Linear correlation was found to be Y = 15946 (R^2=0.9998) for Metformin HCL and Y = 21762 (R^2=0.9999) for Sitagliptin. Method robustness results were given by Tables 6&7. LOQ and LOD results are given by Table 8.

CONCLUSION

The proposed HPLC method can easily and conveniently adopted for routine quality control analysis of Metformin HCL and Sitagliptin in pure and its pharmaceutical dosage forms.

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