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DEVELOPMENT OF A SIMPLE, RAPID AND SPECIFIC RP-HPLC METHOD FOR THE ESTIMATION OF METFORMIN AND SITAGLIPTIN IN BULK AND COMBINED PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

To develop and validate RP-HPLC method for the determination of Metformin and Sitagliptin dosage form and to develop and validate calibration curve and derivative method for the determination of Metformin and Sitagliptin in bulk and tablet dosage forms. To develop a simple, rapid and specific RP-HPLC method for the estimation of Metformin and Sitagliptin in bulk and combined pharmaceutical dosage forms. To develop a simple, rapid, accurate ecofriendly less cost methods for the determination of Metformin and Sitagliptin in bulk and tablet dosage forms. To validate the proposed methods in accordance with the analytical parameters mentioned in the ICH guidelines, such as system suitability, accuracy, precision, specificity, linearity, robustness.

KEY WORDS

Metformin HCl, Sitagliptin, RP-HPLC, ICH guidelines

INTRODUCTION:

The studies carried out on the stability indicating RP-HPLC method for the estimation of "Sitagliptin and Metformin HCl" in pharmaceutical dosage form determination of Sitagliptin and Metformin HCl in bulk and pharmaceutical dosage form. Metformin and sitagliptin are oral diabetes medicines that help control blood sugar levels. Metformin works by decreasing glucose (sugar) production in the liver and decreasing absorption of glucose by the intestines. Sitagliptin works by regulating the levels of insulin your body produces after eating. HPLC methods are useful in the determination of drugs in pharmaceutical formulations especially those containing more than one active components. Therefore, the aim of this work was to develop a relatively simple HPLC method for simultaneous quantification of Sitagliptin and Metformin HCL without the necessity of sample pre-treatment. This paper describes the development and validation of reliable, simple, stable and economic reverse phase HPLC assay, using UV detection for the simultaneous determination of Sitagliptin and Metformin HCL. The method appears to be suitable for quality control in pharmaceutical industry due to its sensitivity, simplicity, selectivity and lack of excipients interference.

MATERIALS AND METHODS:

Ortho phosphoric acid, Acetonitrile manufactured by merck using HPLC grade.

DETERMENATION OF WORKING WAVELENGTH (λ_{max}):

The wavelength of maximum absorption of the solution of the drug in acetonitrile were scanned using Photodiode spectrophotometer within the wavelength region of 200–400 nm against acetonitrile as blank. The spectra of drug show at 285 nm **Fig.1**, Thus 285 nm was selected as detector wavelength for the HPLC chromatographic method.

CHROMATOGRAPHIC CONDITIONS:

During the selection of chromatographic conditions number of trails was carried out and the best trail was selected for optimized method.

OPTIMIZED METHOD: Chromatographic conditions are, Buffer was prepare with1ml Ortho Phospharic acid is dissolved in 1 liter water, Column specifications was Luna C18 250mmx4.6mm, 5µ particle size, Elution mode was Isocratic, Mobile phase: Buffer: ACN (50: 50) (v/v), Flow rate: 1.0 mL /min, Detection wavelength 285 nm, Injection volume 20 µL, Run time: 10 min.

Observation: A sharp pin pointed peak 3.974 and 5.721 **Fig: 2** was observed, so this trail was considered as optimized method.

Conclusion: The metformin was observed at 3.974 min with peak area 5784524, theoretical plates 4566 and tailing factor 1.02 and sitagliptin was observed at 5.721 min with peak area 347851, theoretical plates 3542 and tailing factor 1.01. Because of the satisfactory results, less retention time, this trial was optimized.

%ASSAY OF FORMULATION:

% Assay of Metformin and Sitagliptin was carried out in tablet formulation with 100.1 µg/mL and 10.04 µg/mL results were calculated by using the formula given below and reported in

Test area x STD weight x Test dilution x Avg. Weight x Potency x 100

= ----------

STD area x test weight x STD dilution x label claim x 100

GENERAL PREPARATIONS:

Preparation of Buffer:1ml Orthophospharic acid is dissolved in 1 liter of water. Filtered through 0.45µ membrane filter.

Preparation of Standard Solution and sample solution: Weigh accurately about 100.1 mg of Metformin working standard and 10.2 mg of Sitagliptin working standard into a 100-mL volumetric flask. Add 80 mL of diluent, sonicate to dissolve and dilute to volume with diluent. Further dilute 5mL of the above solution to 50 mL with the diluent.

Preparation of Sample solution: Weigh 10 tablets and take average weight, then crush the tablets to powder form after taking one tablet equivalent weight to weigh accurately about 136.06 mg of sample taken into a 100-mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume diluent. Further dilute 5 mL to 50 mL with the diluent. Filter through 0.45µ Nylon syringe filter.

METHOD VALIDATION: The validation of HPLC method for the determination of Metformin and Sitagliptin as per the protocol and to demonstrate that the method is appropriate for its intended use was studied for the following parameters. All the validation parameters were carried out according to ICH.

System suitability: The system suitability of developed method was conducted through the validation studies by using 100.1+10.2 µg/mL Metformin and Sitagliptin. System suitability prior to analysis was investigated by checking parameters like tailing factor, retention times and number of theoretical plates. The results were found to be within the limits.

Acceptance Criteria

1. The no. of Theoretical plates should not be less than 3000.
2. The Tailing factor should not be more than 2.0
3. The %RSD should not more than 2.0

Linearity and Range: Linearity of an analytical method is its ability to elicit the test results that are directly, or by well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. Linear correlation was obtained between peak area Vs concentration of Metformin and Sitagliptin were in the range of 10.01-150.15µg/mL and 1.06-15.02 µg/mL. The linearity of the calibration curve was validated by the high value of correlation co-efficient of regression equation.

The Range of an analytical method is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated with precision, accuracy and linearity. Correlation coefficient should be not less than 0.999.

Accuracy: The accuracy experiments were carried out by the standard addition method at 50%,100% and 150% levels of linearity and the recoveries obtained were 99.95 to 100.55% for both Metformin and Sitagliptin. The mean % recovery at each level should not be less than 98%-102%.

Precision: The precision of the instruments was checked by repeatedly injecting (n=6) solutions of 100.01µg/mL Metformin and 10.2 µg/mL Sitagliptin.

Intermediate Precision (Reproducibility): The intra-day and inter-day precision of the proposed methods were determined by the corresponding responses three times on the same day and on three different days over a period of one week for three different concentration of 100.01 µg/mL Metformin and 10.2µg/mL Sitagliptin.

The low % RSD values of for Metformin and Sitagliptin were reveal that the proposed method was precise. The % RSD for the absorbance of six replicate injections results should not be more than 2%.

Robustness and Ruggedness: Robustness of the method was determined by carrying out the analysis at two different mobile phases (i.e. 40±5) and two different flow rates (i.e. 1±0.2 mL/min). The high % RSD values of robustness and for Metformin and Sitagliptin with change in flow rate indicates that the method is not robust for change in flow rate. The low % RSD values of robustness and for Metformin and Sitagliptin with change in Organic phase that the proposed method is robust. Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective peak areas were noted. The result was indicated by % RSD.

STABILITY STUDY: 100.01 µg/mL Metformin and 10.2 µg/mL Sitagliptin was prepared and stability study was carried out at different time intervals and the results were recorded.

DEGRADATION STUDIES

Acid Degradation (5N HCl): Diluted sample in a 100ml volumetric flask, add 50ml of diluent. Add 3ml of 5N HCl and heated at 70°C for 30 mins on a water bath. Remove the flask from the water bath and allow the flask to cool at room temperature. Add 3 ml of 5N NaOH to neutralize the solution. Cooled to room temperature and diluted to volume with diluent and mixed.

Base Degradation (5N NaOH): Diluted sample in a 100ml volumetric flask, add 50ml of diluent add 3ml of 5N NaOH and heated at 70°C for 30 mins on a water bath. Removed the flask from the water bath and allow the flask to cool at room temperature. Add 3ml of 5N HCl to neutralize the solution. Cooled to room temperature and diluted to volume with diluent and mixed.

Peroxide Degradation (30% H_2O_2): Diluted sample in a 100ml volumetric flask, add 50ml of diluents and add 3ml of 30% v/v H_2O_2 and heated at 70°C for 30 mins on a water bath. Remove the flask from the water bath and allow the flask to cool at room temperature. Diluted to volume with diluent and mixed.

Reduction Degradation (10% Sodium Bisulphate): Diluted sample in a 100ml volumetric flask, add 50ml of diluent Add 5ml of 10% w/v sodium Bisulphate and heated at 70°C for 1 hours on a water bath. Remove the flask from the water bath and allow the flask to cool at room temperature. Diluted to volume with diluent and mixed.

Hydrolysis Degradation: Diluted sample in a 100ml volumetric flask, add 20ml of diluent added 50ml of water to disperse and dissolve and heated at 70°C for 1 hour on a water bath. Remove the flask from the water bath and allow the flask to cool at room temperature and diluted to volume with diluent and mixed.

Thermal Degradation (105°C / 2 hr): Sample was exposed at 80°C for 3 hrs and analyzed the exposed sample are injected.

Humidity Degradation (25°C / 92% RH for 2 hr): Sample was exposed at 25°C / 92% RH for 3 hrs and analyzed the exposed sample are injected.

Photolytic Degradation: Sample was exposed to sun light for 5Hrs and analyzed the exposed samples are injected.

RESULTS AND DISCUSSION:

HPLC METHOD: Determination of Working Wavelength (λ_{max})

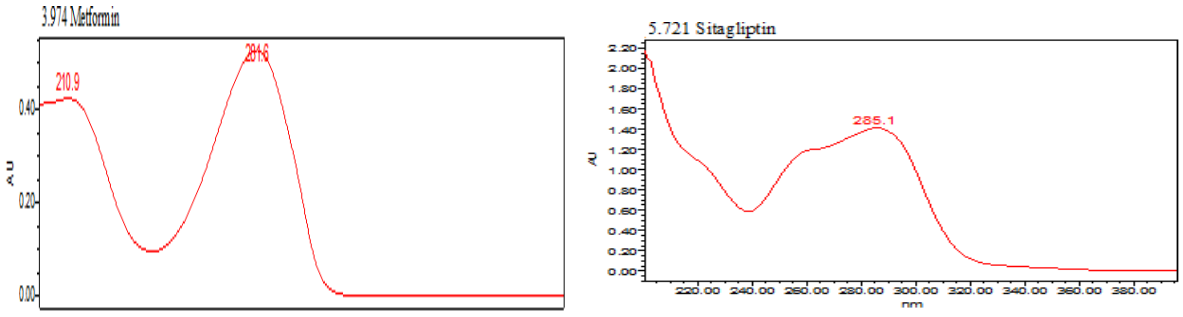


Fig 1: PDA-Spectrum of Metformin and Sitagliptin

Optimized method:

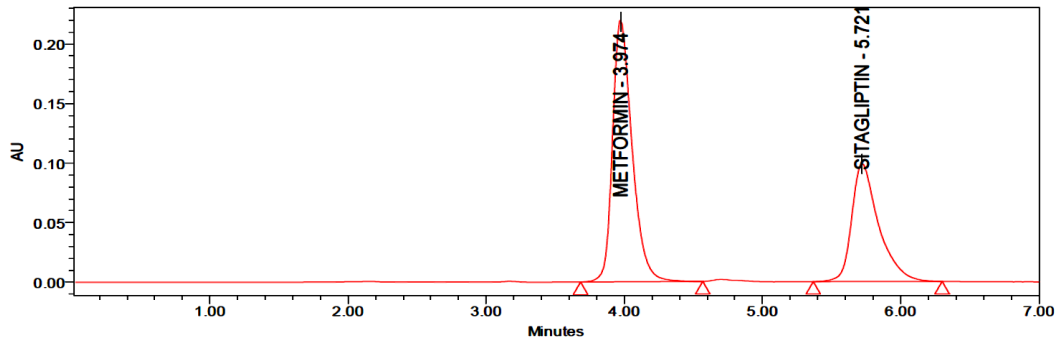


Fig 2: Chromatogram of Optimized method

Table 1: Optimized chromatography

SNo	Name	Retention Time	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Metformin	3.974	5784524	82.16		1.05	3868
2	Sitagliptin	5.721	347851	18.84	1.85	1.12	4564

Assay:

Table 2: % Assay of Metformin and Sitagliptin

Drug	Avg std area (n=6)	Avg sample area(n=6)	Avg wt of tab. (mg)	Stdwt (mg)	Sample wt(mg)	Lable amount (mg)	Std purity	Amount found (mg)	% assay
Metformin	5784663	5784663	680.3	100.1	136.06	500	100.8	100.5	100.4
Sitagliptin	348246	348246	680.3	10.2	136.06	50	99.9	100.1	100.3

ANALYTICAL METHOD VALIDATION (HPLC):

The method was validated for its linearity range, accuracy, precision.

Method validation is carried out as per ICH guidelines

System Suitability Studies:

Table 3: System suitability parameters

S.No	Drug	Retention time(min)	Plate count	Tailing factor
1	**Metformin**	3.969	3842	1.01
2	**Sitagliptin**	5.724	4578	1.08

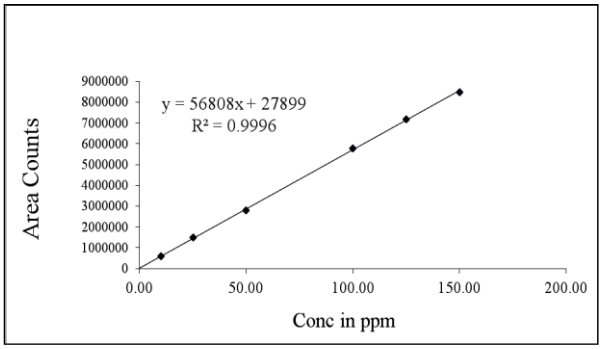
Linearity of Metformin and sitagliptin

Table 4: Results of linearity for Metformin

S.No	Metformin	
	Conc.(µg/mL)	Peak area
1	10.00	584563
2	25.00	1487463
3	50.00	2784632
4	100.00	5784663
5	125.00	7178963
6	150.00	8478932
Regression equation	y = 56808x+27899	
Slope	56895.11	
Intercept	0.99984	
R^2	0.9996	

Table 5: Results of linearity for Sitagliptin

S.No	Sitagliptin	
	Conc.(µg/mL)	Peak area
1	1.00	34245
2	2.50	78962
3	5.00	167896
4	10.00	348246
5	12.50	437892
6	15.00	524156
Regression equation	y = 35371x+5946.4	
Slope	35186.20	
Intercept	3881.18	
R^2	0.99986	



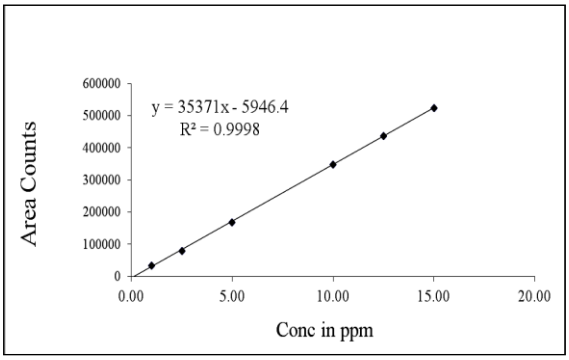


Figure 3 & 4: calibration curve for Metformin at 285 nm & calibration curves for Sitagliptin at 285 nm

Acceptance criteria: For linearity of test method, the squared co relation coefficient derived from least square fit of the data should not be less than 0.999.

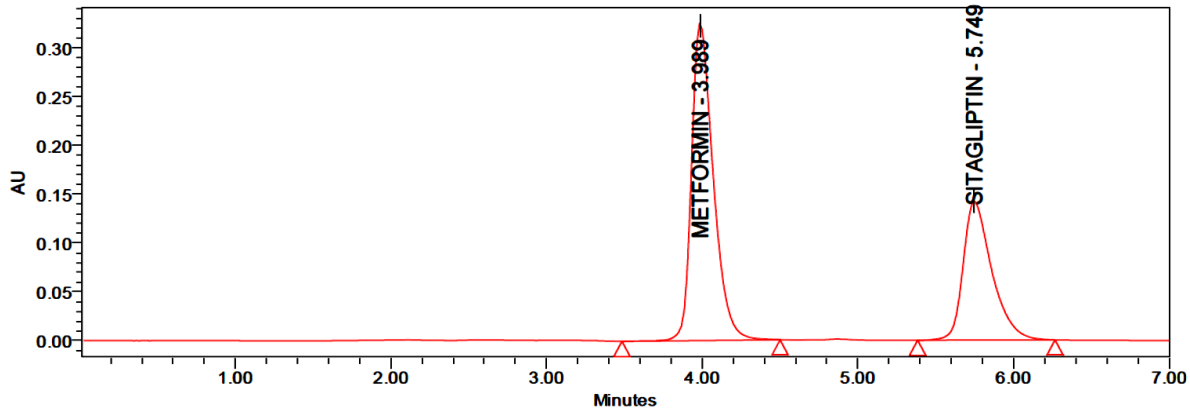


Figure 5: Chromatogram of Accuracy 150%-3

Acceptance criteria:

1. Individual % recovery and mean % recovery of both drugs should be between 98.0 and 102.0.
2. For replicate preparations the %RSD should not more than 2.0.

Table 6: Accuracy results of Metformin by RP-HPLC method

S.NO	% Level of Std	Conc. Of working std. Added (µg/mL)	Peak area	Amount recovered	% recovery	Mean recovery	% R.S. D
1	50	50+100	2378567				
			2378632	100.56	100.5		
			2377465				
2	100	100+100	5786515				
			5741126	100.05	100.0	100.3	0.0475
			5747563				
3	150	150+100	7587463				
			7588632	100.13	100.1		
			7587632				

Table 7: Accuracy results of Sitagliptin by RP-HPLC method

S.NO	% Level of Std	Conc. Of working std. Added (µg/mL)	Peak area	Amount recovered	% recovery	Mean recovery	% R.S.D
1	5	5+100	178624				
			174526	100.78	100.7		
			178532				
2	10	10+100	344248				
			348752	99.98	99.9	100.3	0.0462
			348564				
			518753				
3	15	15+100	518871	100.16	100.1		
			518742				

Precision:

Table 8: Precision studies by RP-HPLC method

S.NO	TYPE	Metformin		
		Mean area(n=6)	Std. deviation	% RSD
1	System precision	5787456	7485.58	1.11
2	Method precision	5688746	4578.16	1.06
3	Intermediate precision	5874632	4785.21	1.14

Table 9: Precision studies by RP-HPLC method

S.NO	TYPE	Sitagliptin		
		Mean area(n=6)	Std. deviation	% RSD
1	System precision	347896	2682.45	1.06
2	Method precision	338956	7852.16	0.82
3	Intermediate precision	347862	4827.32	1.01

Acceptance criteria:

The % assay for each individual preparation should be 95.02 to 105.0 of labeled amount of drug.

The % RSD for assay of six replicate preparations should not more than 2.0 for Metformin and Sitagliptin

Ruggedness and Robustness:

Table 10: Results of Ruggedness study by RP-HPLC

S.No	Parameter	Metformin	Limit	S.No	Parameter	Sitagliptin	Limit
1	% RSD	1.048	NMR 2.0%	1	% RSD	1.104	NMR 2.0%

Table 11: Results of Robustness study by RP-HPLC

Variations	Metformin			
	Retention time	Peak area	Plate count	% RSD
Org Plus	3.309	6287426	4058	1.17
Org Minus	4.962	5682765	4890	0.18
Flow rate 1.2ml/min	3.670	6180783	4863	1.52
Flow rate 0.8ml/min	4.392	5647415	5890	0.85

Table 12: Results of Robustness study by RP-HPLC

Variations	Sitagliptin Retention time	Peak area	Plate count	% RSD
Org Plus	4.772	368746	4852	1.03
Org Minus	7.151	342651	4158	1.18
Flow rate 1.2ml/min	4.814	358581	3986	1.02
Flow rate 0.8ml/min	7.209	349874	3862	1.00

Acceptance criteria: The % RSD of Metformin and Sitagliptin was should be within limits. I.e. < 2. Tailing factor was less than 2.

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Table 13: Results of stability study for Metformin and Sitagliptin

Time period (hours)	Metformin			
	Retention time	Peak area	Tailing factor	Plate count
Initial	3.974	5789614	1.21	4782
6 Hrs	3.969	5784128	1.13	4582
12 Hrs	3.968	5798510	1.22	4511
18Hrs	3.972	5789632	1.01	4289
24 Hrs	3.970	5743155	1.48	4813

Time period (hours)	Sitagliptin			
	Retention time	Peak area	Tailing factor	Plate count
Initial	5.271	348746	1.01	4785
6 Hrs	5.724	347896	1.02	4528
12 Hrs	5.721	345213	1.03	4685
18Hrs	5.726	347896	1.16	4782
24 Hrs	5.725	348852	1.14	4888

Acceptance criteria: All samples were found to be stable up to 24 hrs.

LOD AND LOQ: LOD and LOQ were calculated by the method based on the standard deviation (Σ) and solpe of the calibration curve, using the formula

LOD = 3.3 Σ / S

LOQ = 10 Σ / S

Where,

Σ = the standard deviation of the response

S = the slope of the calibration curve

The LOD and LOQ were calculated as per formula and were shown in the below table.

Table 14: Limit of Detection and Limit of Quantification

Sample	LOD	LOQ
Metformin	0.1485	1.0876
Sitagliptin	0.00171	0.0147

Table 15 & 16 Results of degradation study for Metformin and Sitagliptin

	Sitagliptin				Peak Purity			
	Sample Weight in mg	Area Counts Injections	Mean Area Count	% Label Claim	%Degradation	Purity Angle	Purity Thresold	Pass/Fail
Control	136.06	346954	346954	100.8	-0.5	21.86	1.048	Pass
Acid	135.85	278961	278961	75.5	24.5	26.08	1.046	Pass
Alkali	136.24	287416	287416	76.2	26.2	20.14	1.145	Pass
Peroxide	136.58	276321	276321	77.5	28.5	21.16	1.148	Pass
Reduction	135.92	281245	281245	70.8	27.2	21.25	1.446	Pass
Thermal	137.52	278561	278561	79.2	26.5	22.68	1.146	Pass
Photo	136.28	286412	286412	77.1	22.8	24.18	1.149	Pass
Hydrolysis	135.99	282456	282456	76.3	26.3	22.70	1.414	Pass

	Metformin				Peak Purity			
	Sample Weight in mg	Area Counts Injections	Mean Area Count	% Label Claim	% Degradation	Purity Angle	Purity Thresold	Pass/Fail
Control	136.06	5787456	5787456	100.5	-0.2	28.562	0.782	Pass
Acid	135.85	4687413	4687413	75.36	28.52	26.441	0.985	Pass
Alkali	136.24	4589321	4589321	70.58	26.41	28.641	0.952	Pass
Peroxide	136.58	4638415	4638415	74.35	28.36	27.582	0.992	Pass
Reduction	135.92	4689332	4689332	75.58	20.52	28.368	0.982	Pass
Thermal	137.52	4589632	4589632	73.38	21.47	29.328	0.986	Pass
Photo	136.28	4692714	4692714	72.88	20.55	26.258	0.987	Pass
Hydrolysis	135.99	4587131	4587131	77.66	26.24	25.663	0.989	Pass

SUMMARY AND CONCLUSIONS:

An attempt has been made to develop a new stability indicating validated RP-HPLC method for the estimation of Metformin and Sitagliptin in bulk and in dosage form. As the literature survey revealed that only one method are available for estimation of Metformin and Sitagliptin in bulk and in dosage forms so there is a need for a simple, economical and proper method of estimation of Metformin and Sitagliptin bulk and in dosage form.

Luna C18, 250mm x 4.6mm, 5µm column with UV detector with an injection volume of 10 µL was injected and eluted with the mobile phase containing buffer: acetonitrile (50: 50v/v). This is pumped at a flow rate of 1mL/min and detected by UV (285 nm) detector. The peaks of Metformin and Sitagliptin were eluted at retention times of 3.98 and 5.75 min respectively.

After method was developed, it was validated according to ICH guidelines for system suitability, specificity and linearity, sensitivity parameters, precision, accuracy and robustness

studies. the validation results were found well within the limits(%RSD of areas were<2 for assay and recoveries in the range of 98%-102% for assay,r^2>0.999) indicating that the developed method is simple, rapid, accurate, precise, specific, robust and economical and less time consuming.

The proposed RP-HPLC, method were suitable methods for the determination of Metformin and Sitagliptin in dosage forms. All the parameters of developed methods met the criteria of ICH guidelines for method validation. No tedious extraction procedures were involved. These methods are also having an advantage than reported method of good resolution and with retention time. The developed method has good recovery and sensitivity. The run time required for recording chromatogram was below 10 min. Suitable for the analysis of raw materials and formulations. Hence, the developed chromatographic method for Metformin HCL and Sitagliptin is said to be rapid, simple, precise, accurate, specific and cost effective that can be effectively applied for the routine analysis.

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