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HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF METFORMIN AND SITAGLIPTIN IN PHARMACEUTICAL DOSAGE FORMS AND ITS APPLICATIONS TO DISSOLUTION STUDY

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Conflict of interest: Nil

Abstract

A novel approach was used to develop and validate a rapid, specific, accurate and precise Reverse phase High performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of Metformin and Sitagliptin in pharmaceutical dosage forms and its applications to dissolution study. The chromatographic separation was carried out on a C_8 (250mm X 4.6 mm i.d., 5µm) column with a mobile phase of 40 Acetonitrile: 60 Phosphate Buffer (pH 6.8), using UV detector at 257 nm at 1ml min^{-1} flow rate. The retention time for Metformin was 2.11 minutes and 5.30 minutes for Sitagliptin. The Linearity for Metformin was found to be 10-80 µg ml^{-1} with R^2 value of 0.9998 and for Sitagliptin 1-8 µg ml^{-1} with R^2 value of 0.9976. Dissolution study of both for Metformin and Sitagliptin was carried, Percentage of drug release was established which was found to be 96.23% and 102.64% respectively, in the period of 50 minutes.

Keywords: RP-HPLC, Metformin, Sitagliptin, Dissolution study

INTRODUCTION

Metformin(MET3-(diaminomethylidene)-1,1-dimethylguanidine hydrochloride (fig. 1) and Sitagliptin (STG), [(2R)-1-(2,4,5-trifluorophenyl)-4-oxo-4-[3-(trifluoromethyl)-5,6 dihydro [1,2,4] triazolo [4,3-α]pyrazin-7(8H)-yl] butan-2-amine] (fig. 2) are two well-known anti diabetic drugs. Metformin is the drug of choice for the treatment of type II diabetes, particularly in overweight and obese people and individuals with normal kidney function. It works by lowering blood sugar and helping the body use insulin more efficiently. It is available in 500 mg, 850 mg and 1000 mg tablets (immediate release) and in 500 mg and 750 mg (slow release) for oral administration[1-2]. Sitagliptin is an oral anti-diabetic, which is available in 25 mg, 50 mg and 100 mg tablets for oral administration. It is used for the improvement of glycemic control in patients with type II diabetes mellitus as monotherapy or combination therapy with Metformin [3].

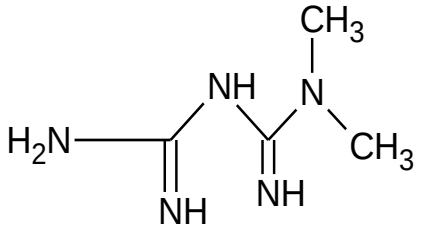


Figure 1: structure of Metformin

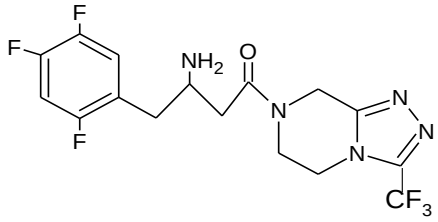


Figure 2: structure of Sitagliptin

Drug Dissolution study is an integral part of pharmaceutical development and routine quality control monitoring of drug release characteristics. The purpose of in vitro dissolution studies in QC is to check batch to batch consistency and detection

of manufacturing deviation while in Research and Development, the focus is to provide some predictive estimate of the drug release in respect to the in vivo performance of a drug product. Tablets or capsules taken orally remain one of the most effective means of treatment available. The effectiveness of such dosage forms relies on the drug dissolving in the fluids of the gastrointestinal tract prior to absorption into the systemic circulation. The rate of dissolution of the tablet or capsule is therefore crucial. Drug release in the human body can be measured in-vivo by measuring the plasma or urine concentrations in the subject concerned. However, there are certain obvious impracticalities involved in employing such techniques on a routine basis. These difficulties have led to the introduction of official in-vitro tests which are now rigorously and comprehensively defined in the respective Pharmacopoeia. The literature reveals that some methods have been reported for Metformin. Few UV spectrophotometric methods [4], HPLC [5-7], ion-pair HPLC [8], UPLC [9] method have been reported for the estimation of Metformin Hydrochloride. Literature survey reveals that UV method [10], liquid chromatographic methods for the determination of Sitagliptin in biological fluids and estimation of Sitagliptin in combination with other drugs [11-13].

None of the method provides their application in dissolution study of the tablet containing mixtures of Metformin Hydrochloride and Sitagliptin. Therefore, it was thought worthwhile to develop a simple, precise, accurate reverse phase high performance liquid chromatographic method for the simultaneous estimation of Metformin Hydrochloride and Sitagliptin in combined tablet dosage form and to study their drug release using dissolution study. The method was validated as per ICH Guidelines [14, 15].

MATERIALS AND METHODS

Materials and Reagents

Metformin hydrochloride and Sitagliptin were provided from Hetero labs Hyderabad, (India). HPLC grade methanol, Acetonitrile, water was purchased from Merck, Mumbai. A high performance liquid chromatography system consisting of Agilent LC 1100 Module with UV-VWD detector was used with data handling system EZ chrome elite software, with 20µl loop, Rheodyne manual injector. Chemicals were weighed using Analytical balance Axis LC GC. All pH measurements were done on pH meter Systronics, Digital pH meter 802. Chromatographic analysis was performed on a reverse phase C8 (250 mm×4.6 mm i.d. ×5 µm) column. The mobile phase consisted of 40 Acetonitrile: 60 Phosphate Buffer (pH 6.8).contents of the mobile phase were filtered through 0.45µ membrane filter and degassed for 15 minutes. The mobile phase was pumped from the solvent reservoir to the column at the flow rate of 1.0 ml min^{-1} with injection volume of 20 µl. The eluents were monitored at 257 nm. USP Type II (Paddle) dissolution, LAB-INDIA 800, was selected for dissolution study and the dissolution medium was maintained at temperature of 37^{o}C and was set at 75 RPM.

RP-HPLC method development for Metformin hydrochloride and Sitagliptin

Preparation of standard drug solutions

Stock solution of Metformin and Sitagliptin was prepared by dissolving 10 mg of each in separate 10 ml of volumetric flask with small quantity of water. The mixture was sonicated for about 10 minutes and then made up to volume with water. From the stock solution final concentrations was prepared.

Chromatographic conditions

The mobile phase consisted of Acetonitrile and Phosphate buffer of pH 6.8 in ratio 40:60. The contents of the mobile phase were filtered before use, through a 0.45 micron membrane filter and degassed for 10 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 ml min^{-1} and the injection volume was 20 µl. The column temperature was maintained at ambient temperature. The eluents were monitored at 257 nm.

Calibration of standards

The standard calibration line was constructed for Metformin and Sitagliptin. Different volumes of stock solutions of each were accurately transferred in to 10 ml volumetric flasks and diluted to mark to yield a concentration range of 1-8 µg ml^{-1} for Sitagliptin and 10-80 µg ml^{-1} for Metformin. The

calibration line was obtained by plotting the peak area against concentration of drug.

Method Validation for Metformin hydrochloride and Sitagliptin

The method was validated according to the ICH guidelines and the parameters like Specificity, Linearity, Accuracy, Precision, Limits of Detection and Limits of Quantification, Robustness, System suitability were addressed:

RESULTS

Method development

Optimization of the chromatographic conditions

In preliminary experiments, Metformin hydrochloride and Sitagliptin was subjected for separation by different trials by reversed phase HPLC using decreasing ratios of Buffer in mobile phase composition using Methanol as organic phase, but one of the drugs was not eluted with this composition (table 1). Hence the elution strength of mobile phase was enhanced by replacing organic solvent Methanol with Acetonitrile with increase in % of Aqueous in the Mobile phase.. The optimized chromatographic condition is shown in table 2. The chromatogram obtained was better than all other trials and shown in Figure 3.

Table 1: Trails with different ratios of Mobile phase during Method development

Trail No.	Mobile phase Composition	Flow Rate 1 ml min^{-1}	R_t		Theoretical plates		Remarks
			MET	SIT	MET	SIT	
1	60 : 40 (MeOH :Buffer)	1.0	4.32	-	183301	-	SIT no retention
2	50:50 (MeOH :Buffer)	1.0	2.52	-	905512		SIT no retention,
3	70 : 30 (ACN :Buffer)	1.0	2.08	2.90	6244403	188675	Less Resolution
4	60 : 40 (ACN :Buffer)	1.0	2.09	3.32	1464881	81707	MET Less retention
5	50 : 50 (ACN :Buffer)	1.0	2.03	3.75	5352063	182227	MET-Less retention
6	40 : 60* (ACN :Buffer)	1.0	2.11	5.28	7757759	298935	Good

MET=Metformin, SIT=Sitagliptin

Table 2: Optimized Chromatographic Conditions of Metformin and Sitagliptin

Parameter	Optimized Condition
Mobile Phase composition	40 Acetonitrile :60 Phosphate Buffer (pH 6.8)
Column	C_8 (250 X 4.6 mm i.d., 5µm)
Flow Rate	1 ml min^{-1}
Detection Wavelength	257 nm with DAD
Run Time	10 minutes
Elution mode	Isocratic elution

i.d= Internal diameter, mm= millimetre, µm=micro meter

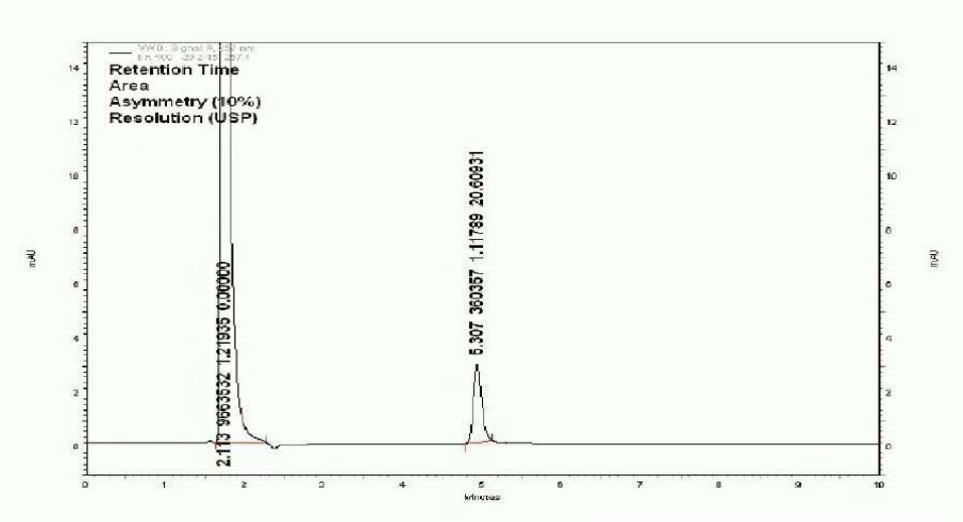


Figure 3: Optimized chromatogram for Metformin HCL and Sitagliptin

Dissolution studies of Metformin and Sitagliptin for marketed formulation

Two tablets of marketed formulation Janumet^{TM} tablet (Merck & Co., India) containing Sitagliptin 50 mg and Metformin 500 mg were taken in two different dissolution chambers which were filled with 900 ml of dissolution medium i.e., Phosphate buffer of pH 6.8. USP Type II (Paddle) dissolution apparatus was selected for dissolution study and the dissolution medium was maintained at temperature of 37^{o}C and was set at 75 RPM. 5ml of the sample was withdrawn at 0, 10, 20, 30, 45 and 60 min and the samples were filtered through the 0.45 micron syringe filter. Then 1 ml of the filtrate was made up to 10 ml with the mobile Phase i.e., Acetonitrile and Phosphate buffer (40:60) the resulting solution was injected into the HPLC under Optimized conditions and chromatograms were recorded, as shown in fig. 4-9. The % drug release was calculated from the peak areas of injected solutions using regression equations obtained from the calibration curves of Metformin and Sitagliptin as shown in table 3.

Table 3: Dissolution Studies of Metformin and Sitagliptin

Time	Peak area Milli absorbance unit (mAU)		Amount of Drug released (µg ml^{-1})		% Drug released	
	MET	SIT	MET	SIT	MET	SIT
0 min	-	-	-	-	0	0
10 min	3019641	142149	275.4	33.20	55.08	55.08
20 min	4855965	240017	447.36	49.92	89.47	99.84
30 min	5272035	207780	486.31	49.37	97.26	98.74
40min	5269885	201058	486.11	47.71	97.22	95.42
50min	5216963	208357	481.15	51.32	96.23	102.64

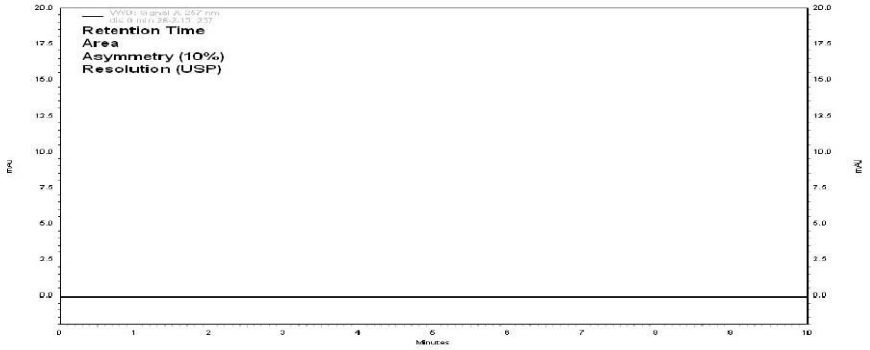


Figure 4: Dissolution at 0 min

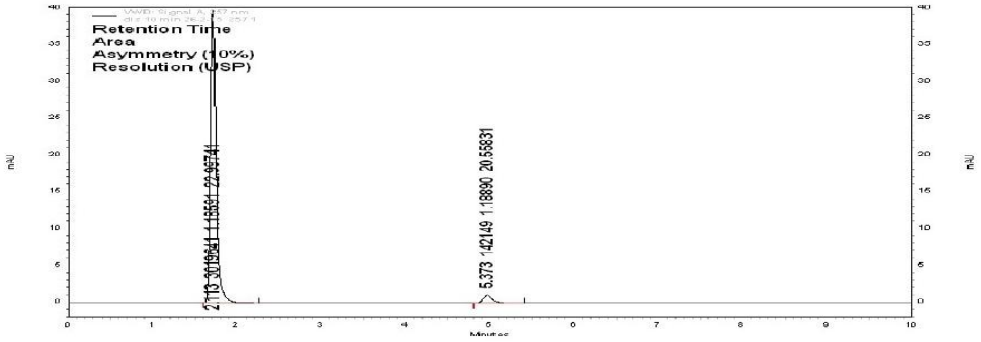


Figure 5: Dissolution at 10 min

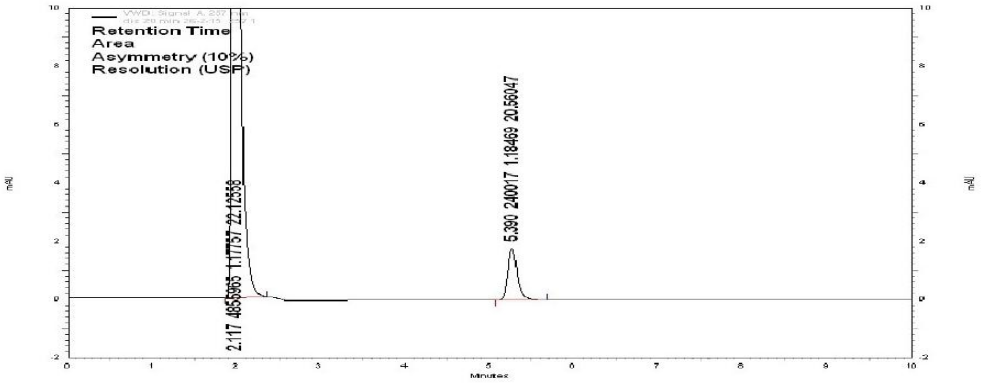


Figure 6: Dissolution at 20 min

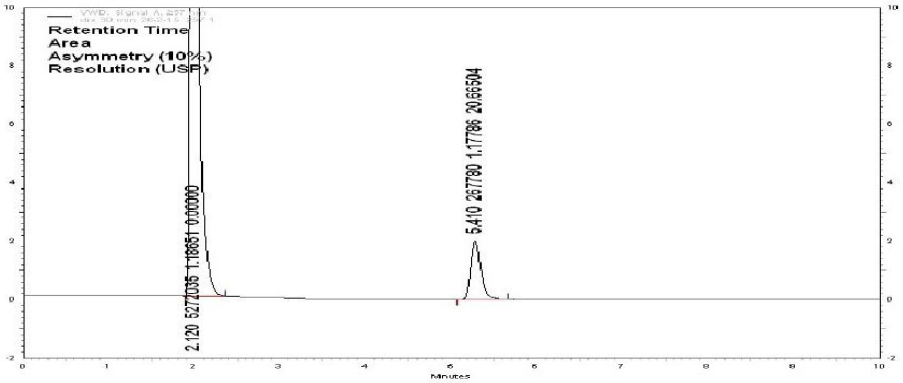


Figure 7: Dissolution at 30 min

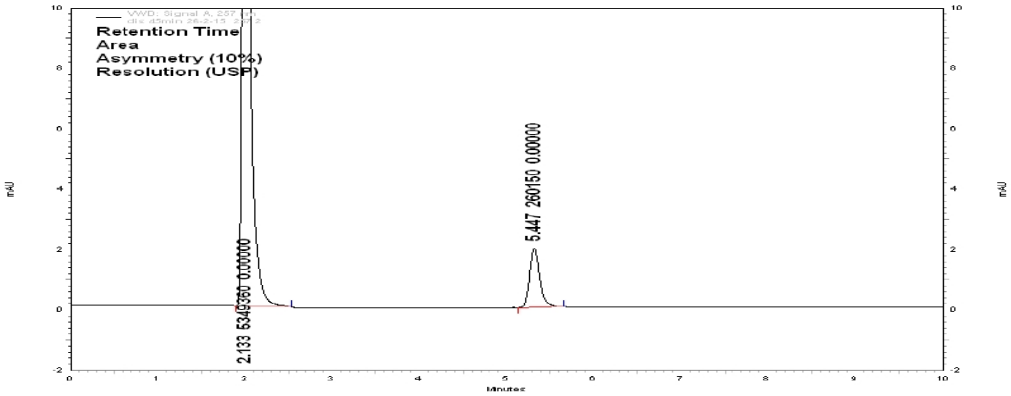


Figure 8: Dissolution at 40 min

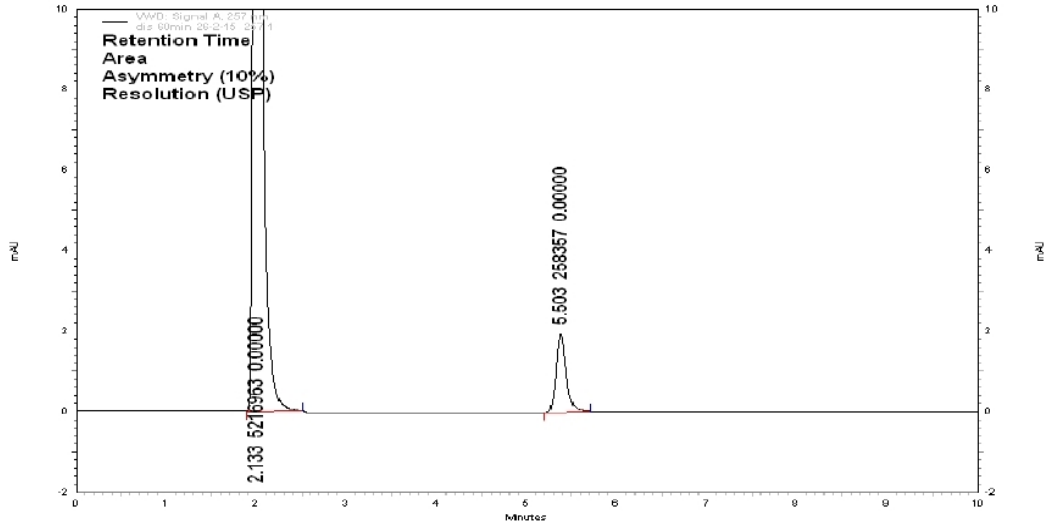


Figure 9: Dissolution at 50 min

Validation of Analytical method for the Assay of Metformin and Sitagliptin:

Validation of an analytical method is process to establish that the performance characteristics of the developed method meet the requirements of the intended analytical application.

Specificity

The specificity of the method was evaluated with regard to interference due to presence of any other excipients. Volume of 20 µl of working placebo sample solution was injected into the chromatograph and the chromatogram was recorded and presented below. No peaks were found at retention time of 2.10 minutes and 5.30 minutes; hence the proposed method was specific for the detection of Metformin and Sitagliptin respectively. The results are shown in table 4.

Table 4: Specificity

Injection	R_t of Analyte (in minutes)		Degradation peak R_t (in minutes)	Remarks
Blank	---	---	---	No component found in blank
Control	2.10	5.30	---	Method is specific

$R_{t=}$ Retention time

Linearity

The linearity of calibration curves (peak area Vs concentration) in pure solution was checked over the concentration range of 1 µg ml^{-1} to 8 µg ml^{-1} for Sitagliptin and from 10 µg ml^{-1} to 80 µg ml^{-1} for Metformin. The total eluting time was less than 15 min. The regression line relating standard concentrations of drug using regression analysis, the calibration curves were linear in the studied range and equations of the regression analysis were obtained y=36534x+7361, R^2=0.9976 for Sitagliptin and Y=96109x+78701, R^2=0.9998 for Metformin. The mean ± standard deviation (SD) for the slope, intercept and correlation coefficient of standard curves (n=3) were calculated. The represented data are shown in tables 5**.**

Table 5: Linearity Range Data for Metformin and Sitagliptin

Sr. No.	Concentration (µg ml^{-1})		Peak area mean ± SD (n=3)		%RSD	
	MET	SIT	MET	SIT	MET	SIT
1	10	1	1120535± 5231	49502 ± 596	1..10	1.41
2	20	2	1951505 ± 3093	79277 ± 465	0.77	0.99
3	30	3	2863732 ± 4045	109943 ± 530	0.86	0.75
4	40	4	3970001± 5179	156007 ± 962	0.86	0.62
5	50	5	4859168± 9327	184835 ± 1605	1.08	0.74
6	60	6	5910018± 6958	228935 ± 1504	0.61	0.58
7	70	7	6786596±9528	266679 ± 1961	0.67	0.61
8	80	8	7757759±8562	298935±1732	0.71	0.92

SD= Standard Deviation, RSD= Relative Standard Deviation

Precision

The data for method precision was obtained by injecting 50 µg ml^{-1} solution in the linearity. The %RSD values for method precision were less than 2 and the result is shown in Table 6. The data for intra-day and inter-day precision studies were obtained for three different concentrations (20, 40 and 80 µg ml^{-1} for Metformin and 2, 4, 6 µg ml^{-1} for Sitagliptin) in the linearity. The %RSD values for intra-day and inter-day precision were less than 2 and the result is shown in Table 7.

Table 6: Method Precision for Metformin and Sitagliptin

Injection No.	Concentration (µg ml^{-1})		Area (n = 6)		R_t (n = 6)	
	MET	SIT	MET	SIT	MET	SIT
1	50	5	5029538	228523	2.12	5.49
2	50	5	5031268	232468	2.11	5.47
3	50	5	5027436	227324	2.10	5.50
4	50	5	5130682	225368	2.12	5.49
5	50	5	5034589	221862	2.10	5.48
6	50	5	5029362	225468	2.11	5.39
Mean ± SD			5047145 ±40994.7	226835 ±3565.68	2.11± 0.0089	5.47± 0.04
% RSD			0.81	1.57	0.42	0.73

Table 7: Intermediate Precision

Drug	Amount (µg ml^{-1})	Intraday (n = 3)		Interday (n = 3)	
		Amount found Mean ± SD	% RSD	Amount found Mean ± SD	% RSD
MET	20	20.10±0.10	0.52	20.33±0.34	1.67
	40	40.33±0.73	1.81	40.22±0.79	1.98
	60	60.22±0.45	0.76	59.88±0.18	0.31
SIT	2	1.96±0.01	0.73	2.00±0.02	1.19
	4	4.04±0.05	1.28	4.04±0.07	1.75
	6	5.98±0.09	1.65	5.95±0.08	1.47

Accuracy

The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 80%, 100% and 120% of target test concentration (5 µg ml^{-1} of Sitagliptin and 50 µg ml^{-1} of Metformin) in tablets. The percentages of recoveries were calculated and a result is presented in table 8.

Table 8: Accuracy Report of Tablet Dosage Form of Metformin and Sitagliptin

Drug	Amount (µg ml^{-1})	Recovery Level	Amount added (µg ml^{-1})	Amount recovered (µg ml^{-1}) (Mean±SD)	% Recovery (n = 3)	Acceptance Criteria
MET	50	80 %	40	88.44 ±0.253	99.38	98 – 102%
	50	100 %	50	99.930±0.967	101.40	98 – 102%
	50	120 %	60	110.381±0.861	100.34	98 – 102%
SIT	5	80 %	4	8.81±0.198	99.36	98 – 102%
	5	100 %	5	9.93±0.048	99.32	98 – 102%
	5	120 %	6	10.95±0.199	99.60	98 – 102%

Limits of Detection and Quantitation

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for the procedure were performed on samples containing very low concentrations of analytes under the ICH guidelines. By applying the mathematical formula method, LOD was expressed by establishing the minimum level at which the analyte can be reliably detected. LOQ was considered as the lowest concentration of analytes in standards that can be reproducibly measured with acceptable precision. The LOD and LOQ value for Metformin and Sitagliptin is shown in the table 9.

Table 9: LOD and LOQ Report of Metformin and Sitagliptin

S. No.	Drug	LOD	LOQ
1	Metformin	0.4308 µg ml^{-1}	1.4217 µg ml^{-1}
2	Sitagliptin	0.083 µg ml^{-1}	0.2766 µg ml^{-1}

Robustness

The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying the HPLC pump flow rate(±0.2 ml) and organic solvent content (±2 ml) and wavelength (±5 nm). None of the alterations caused a significant change in peak area R.S.D., tailing factor, assay and theoretical plates, except pH condition variation. Although, the change in retention times were more significant, quantitation was still possible. Results are presented in table 10.

Table 10: Robustness Studies of Metformin and Sitagliptin

Parameter	Condition	Retention Time		Area ± SD (n = 3)		% Assay		Remarks
		MET	SIT	MET	SIT	MET	SIT	
Optimized	1 ml min^{-1} 40:60 pH 6.8	2.10	5.30	4834824 ± 345	189191± 5749	100%	100%	-------
Flow rate (1 ml min^{-1})	0.8	2.66	6.99	6322113 ± 14166	235842 ± 5833	129.92	125.07	Not robust
	1.2	3.98	17.04	4163823 ± 79480	165765± 756	85.01	86.71	Not robust
Mobile phase	38:62	2.16	6.31	4937723 ± 879	189146± 67465	101.11	95.57	Not robust
	42:58	2.13	5.23	4991503 ± 879	179843 ± 67465	102.23	94.42	Not robust
Wavelength	252	2.12	5.45	16764950	130009	33.24	67.14	Not robust
	262	2.12	5.45	1619087	308521	31.48	151.40	Not robust

System suitability

It is defined as tests to measure the method that can generate result of acceptable accuracy and precision. The system suitability was carried out after the method development and validation have been completed. The system suitability was assessed by five replicate analyses of the drugs at concentrations of 5 µg ml^{-1} of Sitagliptin and 50 µg ml^{-1} of Metformin and for this, parameters like plate number (n), tailing factor, HETP, peak asymmetry of samples were measured, and shown in table 11.

Table 11: System Suitability Parameters for Metformin and Sitagliptin

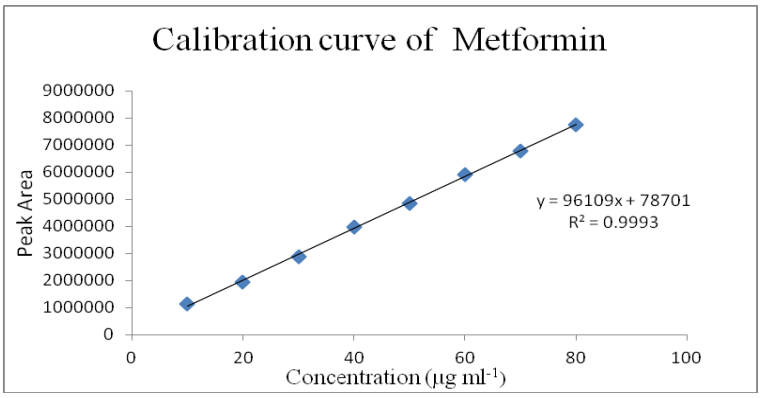
Sr. No.	Parameter	Values obtained		Acceptance criteria
		Metformin	Sitagliptin	
1	Plate count	4859168 ± 215	184835 ± 151	>2000
2	Tailing Factor	1.1 ± 0.02	1.5 ± 0.04	≤ 2.0
3	Asymmetry (10%)	1.101	0.998	0.9-1.2
4	Capacity factor	0.888	7.424	0.5-10

Application of the proposed method for the determination of Metformin and Sitagliptin in Tablets (ASSAY)

Twenty tablets of marketed formulation Janumet™ tablet (Merck & Co., India) containing Sitagliptin 50 mg and Metformin 500 mg were weighed, and finely powdered and transferred to a 100 ml volumetric flask, extracted for 30 min with water and volume was made up to 100 ml with water. 1.0 ml of above solution was transferred into a 10 ml volumetric flask and the volume was made up to 10 ml with mobile phase to obtain 10 µg ml^{-1} of Sitagliptin and 100 µg ml^{-1} of Metformin. The final solution was filtered through 0.45 micron syringe, filters and injected under above chromatographic conditions and peak area was measured. The assay procedure was made triplicate and weight of sample taken for assay was calculated. The percentage of drug found in formulation, mean and standard deviation in formulation were calculated and shown in table 12.

Table 12: Assay Report of Tablet Dosage form of Metformin and Sitagliptin

Formulation	Drugs	Labelled Claim (in mg)	Amount Found (Mean ±SD)	Assay (%)
Janumet	METFORMIN SITAGLIPTIN	500 50	494.86±1.75 49.77±0.97	98.97 99.54



S1: Calibration plot of Metformin

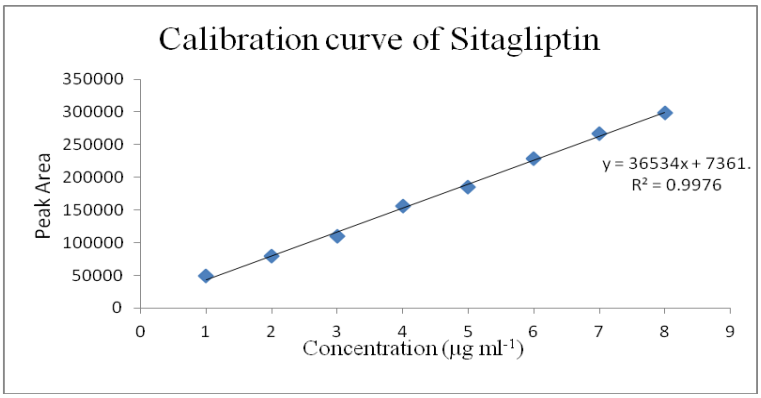


Figure S2: Calibration plot of Sitagliptin

The absorption maxima for Metformin and Sitagliptin were found to be (Water) 232 nm and (Water) 266 nm respectively. By overlaying spectrums, 257nm was selected for the estimation of both drugs.

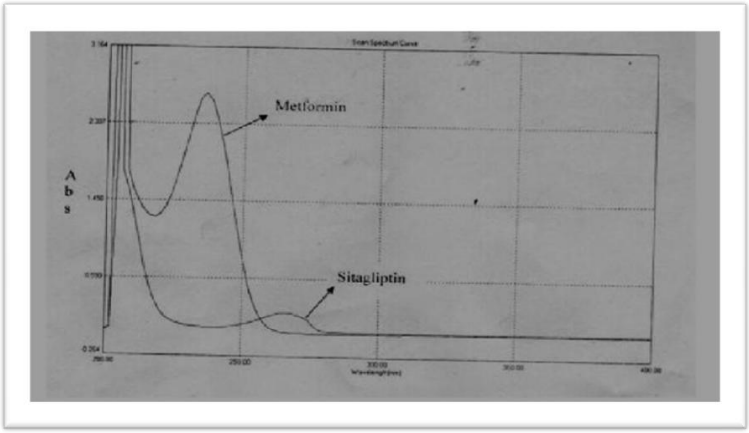


Figure S3. UV spectrum of Metformin and Sitagliptin

General Information of FT-IR (KBR) for Metformin: νcm^{-1} was found as 3365,3285,3134,2317,1622,1544,1472,1432,1409,1260,1206,1161,1034,929,795,731,696, 635, and 577.

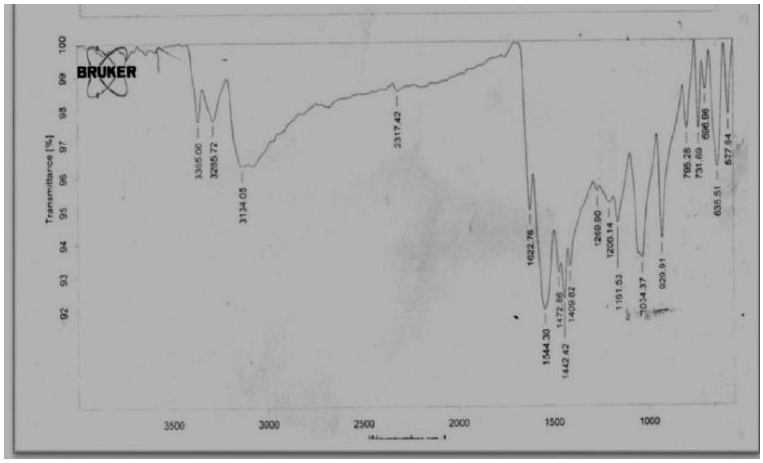


Figure S4. IR spectrum of Metformin

General Information of FT-IR (KBR) for Sitagliptin: νcm^{-1} was found as 3308,3046,2562,1634,1591,1515,1427,1368,1332,1268,1210,1158,1126,1062,1010,978,927,851,751,717,686.

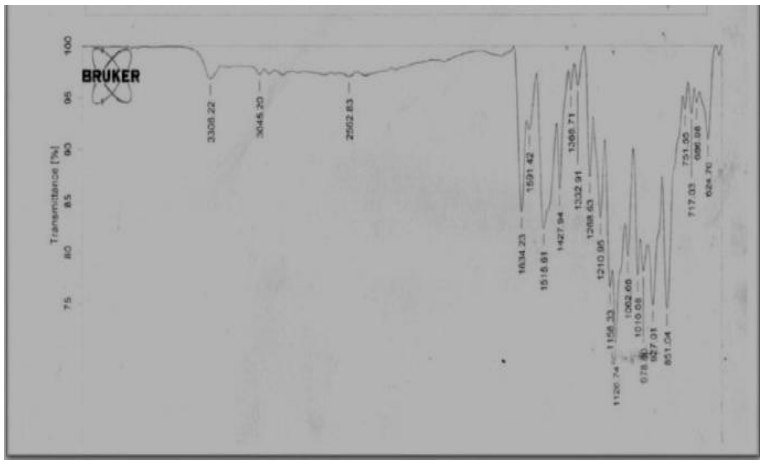


Figure S5. IR spectrum of Sitagliptin

DISCUSSION

The literature reveals that some methods have been reported for Metformin. Few UV spectrophotometric methods [5], HPLC [6-8], ion-pair HPLC [9], UPLC [10] method have been reported for the estimation of METFORMIN, liquid chromatographic methods have been developed for the determination of STG in biological fluids [11-13]. The development of this new method provides their application in dissolution study of the tablet containing mixture of Metformin Hydrochloride and Sitagliptin. Therefore, it was thought worthwhile to develop a simple, precise, accurate reverse phase high performance liquid chromatographic method for the simultaneous estimation of Metformin Hydrochloride and Sitagliptin in combined tablet dosage form and to study their drug release using dissolution study. The method was validated for all validation parameters as per ICH guidelines. The linearity range for Metformin and Sitagliptin was 20-80 µg ml^{-1} and 2-8 µg ml^{-1} with R^2 value of 0.9993 and 0.9976 respectively. The %RSD for intra-day precision was < 2%. The method has been validated in assay of tablet dosage forms. The method was also passes the specifications for robustness parameters. In present work HPLC method has been developed for the estimation of Metformin and Sitagliptin marketed tablet formulation and it is applied to determine the amount of drug released in dissolution studies. HPLC method was developed with the mobile phase system of Acetonitrile: Phosphate Buffer (pH 6.8) in the ratio of 40: 60 v/v. The flow rate of 1ml/min was used on C_8 column (250×4.6 mm, 5 µm particle size). The retention time of Metformin and Sitagliptin was observed at

2.10 ± 0.15 min and 5.30 ± 0.80 min respectively. The method was validated for all validation parameters as per ICH guidelines. The linearity range for Metformin and Sitagliptin was 20-80 µg ml^{-1} and 2-8 µg ml^{-1} with R^2 value of 0.9993 and 0.9976 respectively. The % RSD for intra-day precision was < 2%. The method has been validated in assay of tablet dosage forms. The method also passed the specifications for robustness parameters.

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