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THE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHOLRIDE & SITAGLIPTIN BY USING RP- HPLC

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Abstract:

A new method was established for simultaneous estimation of metformin and sitagliptin by RP-HPLC method. The chromatographic conditions were successfully developed for the proportion of metformin and sitagliptin by utilizing SYMMETRYC18 column (4.6×150mm)5µ, flow rate were1ml/min, mobile phase proportion were (70:30 v/v)methanol : phosphate buffer($KH_2PO_4$and K_2HPO_4) pH 3 (pH were adjusted with orthophosphoric acid),detection wave length were 258nm. The instrument used were WATERS HPLC Auto Sampler, Sepaproportionn module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 1.694mins and 3.334 mins. The % purity of Metformin and sitagliptin were found to be 100.27% and 99.87% individually. The system suitability parameters for metformin and sitagliptin such as theoretical plates and tailing factor were found to be 2294, 1.27 and 4891 and 1.03, the resolution were found to be 8.67. The analytical method were validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study metformin and sitagliptin were found in consent proportion range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) were found to be 0.999 and 0.999, % recovery were found to be 99.56% and 99.48%, %RSD for repeatability were 0.27 and 0.40, % RSD for intermediate precision were 0.27 and 0.94individually. The precision study were precise, robust, and repeatable.LOD value were 2.17 and 6.60, and LOQ value were 0.032 and 0.1125individually.

Keywords: *Metformin , sitagliptin, precise, robust, and repeatable.*

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INTRODUCTION:

Metformin was a white to off-white crystalline compound which is freely soluble in water, Insoluble in acetone, ether, chloroform. which have melting point of 215-218°Cwhich was used for antidiabetic drug and Anti-hypoglycaemic agent. Sitagliptin was a White to off-white crystalline, non-hygroscopic powder which was Soluble in water and N,N-dimethyl formamide, slightly soluble in methanol; very slightly soluble in ethanol, acetone, and acetonitrile; and insoluble in isopropanol and isopropyl acetate. /Phosphate, belongs to Category Anti diabetic agent [1].

The analysis of Metformin and sitagliptin by simultaneous estimation by RP-HPLC. Spectrophotometer, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Metformin and sitagliptin in pharmaceutical dosage form [2].

Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Metformin and sitagliptin. The developed technique determination be validated conferring to ICH guidelines.

Method and methodology:

List of chemicals and standards used

S.No	Chemicals	Manufacturer Name	Grade
1.	Water	Merck	HPLC grade
2.	Methanol	Merck	HPLC grade
3.	Acetonitrile	Merck	HPLC grade
4.	Ortho phosphoric acid	Merck	G.R
5.	KH_2PO_4	Merck	G.R
6.	K_2HPO_4	Merck	G.R
7.	0. 22µ Nylon filter	Advanced lab	HPLC grade
8.	0.45µ filter paper	Millipore	HPLC grade
9.	Tancodep-2	Torrent pharmaceuticals	Tablet form
10.	Metformin and sitagliptin	In - House	In- House

Instruments used

S.No	Instrument name	Model number	Soft ware	Manufacturers Name
1	HPLC-auto sampler – UV detector	Separation module2695, UV.detector2487	Empower-software version-2	Waters
2	U.V double beam spectrometer	UV 3000+	U.V win soft ware	Lab India
3	Digital weighing balance(sensitivity 5mg)	ER 200A	-	Ascoset
4	pH meter	AD 102U	-	ADWA
5	Sonicator	SE60US	-	Enertech

Preparation of phosphate buffer[2]

2.95 grams of $KH_2PO_4$and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with orthophosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation

Mobile phase was used as the diluent.

Preparation of the individual metformin standard preparation

10 mg of metformin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.0 ml from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. The chromatogram is shown in Fig.No.26.

Preparation of the individual sitagliptin standard preparation

1 mg of sitagliptin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate too.

Dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1ml from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. The chromatograms are shown in Fig.

Preparation of the metformin and sitagliptin standard and sample solution

Sample solution preparation:

10 mg of metformin and 1mg sitagliptin tablet powder were accurately weighed and transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent. The chromatograms are shown in Fig. and results are tabulated in Table.

Standard solution preparation

10mg metformin and 1mg sitagliptin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. The chromatograms are shown in Fig. No. 31-33 and results are tabulated in Table.No.14.

Procedure

10µL of the blank, standard and sample were injected into the chromatographic system and areas for the metformin and sitagliptin the peaks were used for calculating the % assay by using the formulae.

System suitability[4]

- Tailing factor for the peaks due to metformin and sitagliptinin standard solution should not be more than 1.5.
- Theoretical plates for the metformin and sitagliptin peaks in standard solution should not be less than 2000.

Assay calculation

$$\textbf{Assay}\ \% = \frac{\textbf{sample area}}{\textbf{Standard area}} \times \frac{\textbf{dilution sample}}{\textbf{dilution of standard}} \times \frac{\textbf{P}}{\textbf{100}} \times \frac{\textbf{Avg.wt}}{\textbf{Lc}} \times \textbf{100}$$

Where:

Avg.wt = average weight of tablets

P= Percentage purity of working standard

LC= Label Claim of metformin mg/ml.

	Trail -1	Trail 2	Trail 3	Trail 4	Trail 5 **optimised**

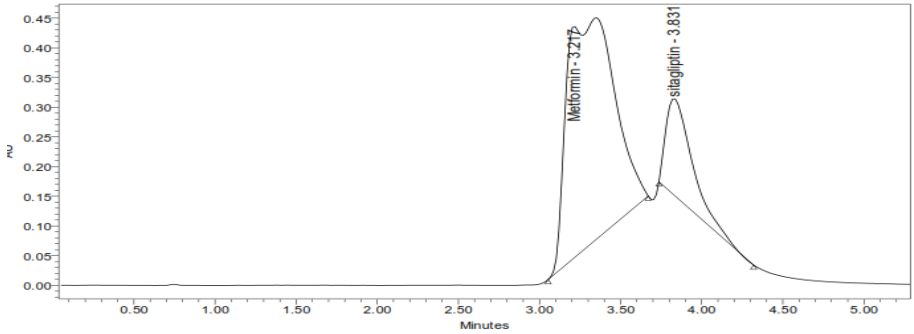
					method
Mobile phase	MeOH: H_2O (50:50%v/v)	ACN: H_2O (50:50%v/v)	ACN: pH 6.8 buffer (50:50 % v/v)	ACN: pH 3 buffer (65:35% v/v)	ACN: pH 3 buffer (70: 30 % v/v)
Diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent
Flow rate	1ml/min	1.0ml per min	1.0 ml per min	1.0 ml per min	1.0 ml per min
Column	symmetry C18 4.6x150mm 5µm	Zodiacsil C18 4.6x150mm 5µm	Hypersil RPC8 4.5×150mm 5.0 µm	Zodiac sil RPC18 4.6×250mm 5µm	zodiac silRP C18 4.6×250mm 3.0µm
Detector wavelength	236nm	271nm	271nm	271 nm	271nm
Column oven	Ambient	Ambient	Ambient	Ambient	Ambient
Injection volume	10µl	20µl	20µl	20µl	20µl
Running time	10min	8.0 min	6 min	10min	10min

Validation parameters such as specificity, Linearity, Range, Accuracy, Precision, Repeatability, Intermediate Precision, Detection Limit, Quantitation Limit, Robustness also studied [5-10].

Results and discussion

Chromatographic trials for simultaneous estimation of metformin and sitagliptin by RP- HPLC.

Chromatogram showing trial-1 injection

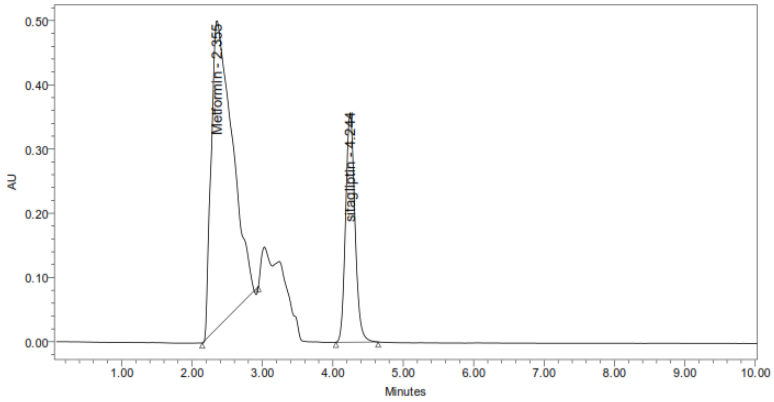


	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing
1	Metformin	3.217	7913799	394185	722		2.21
2	sitagliptin	3.831	1853381	162758	2614	1.52	2.85

	Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing
1	Metformin	3.217	7913799	394185	722		2.21
2	sitagliptin	3.831	1853381	162758	2614	1.52	2.85

Observation:
The trial doesn't show any peaks in the chromatogram, so more trials were required for obtaining peaks.

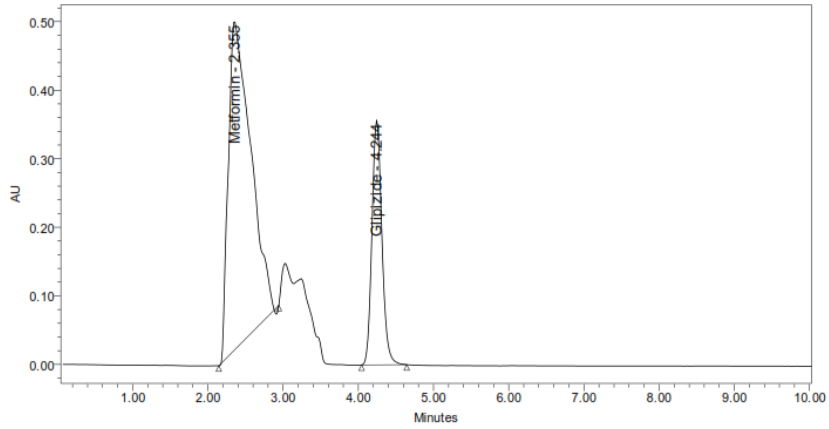
Chromatogram showing trial-2 injection



Observation

In this trial only sitagliptin peak only eluted, still more trials were required for both peaks.

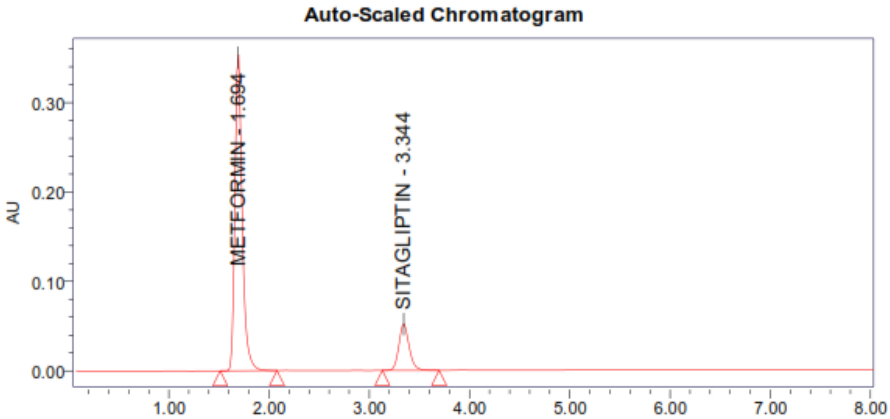
Chromatogram showing trial-3 injection



Observation

In this trial both Metformin and sitagliptin were eluted but there is no proper resolution. Still more trials were required for better resolution in peaks.

Chromatogram showing trial-4 injections

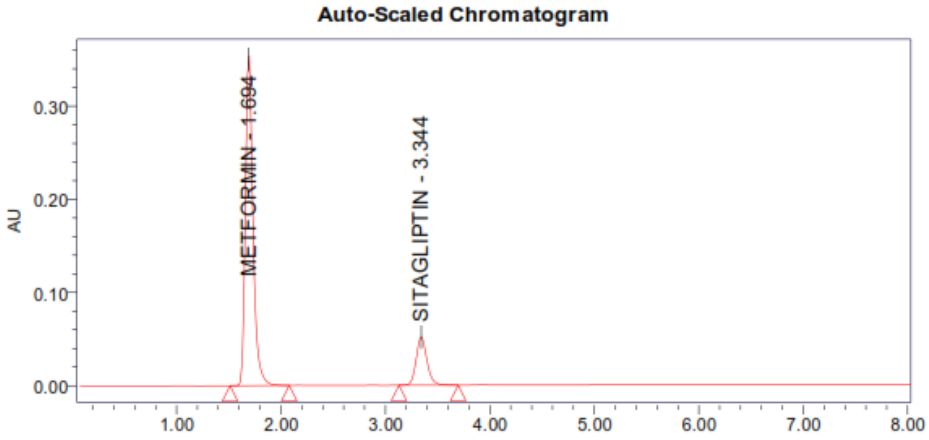


Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	METFORMIN	1.694	1791644	349533		1.29	2761	1
2	SITAGLIPTIN	3.344	371039	51965	10.12	1.15	5267	1

Observation:
The separation was good; peak shape was good, still more trials were required to reduce the retention times of peaks.
Trial -5 (optimised method):

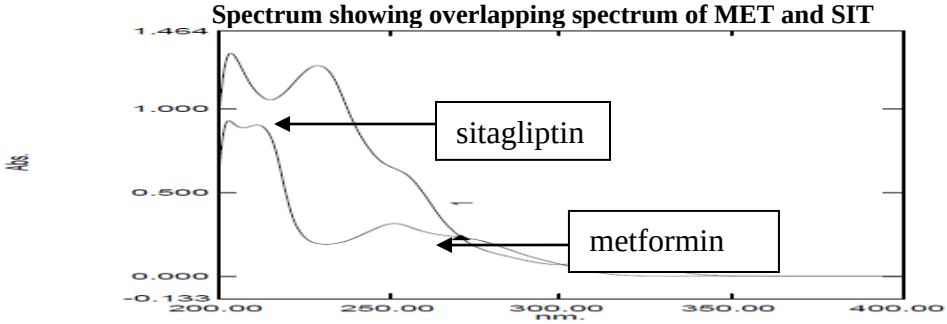
Chromatogram showing trial-5 injection



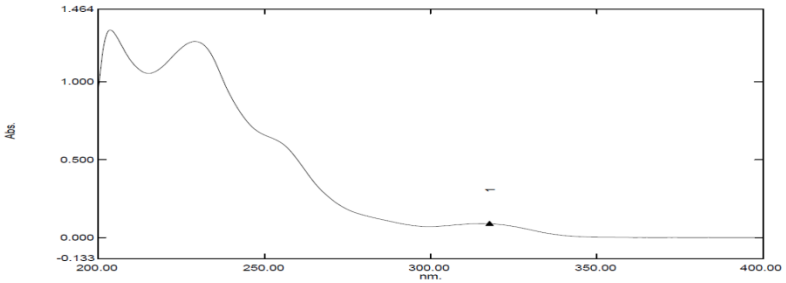
ObservationThe separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks.

Method Development
The identification wavelength were selected by dissolving the drug in mobile phase to get a concentproportionn of 10µg/ml for individual and mixed standards. The resulting solution were scanned in U.V range from 200-400nm. The overlay spectrum of metformin and sitagliptin were obtained and the isobestic point of metformin and sitagliptin showed absorbance's maxima at 271nm. The spectrums are shown in Fig. below

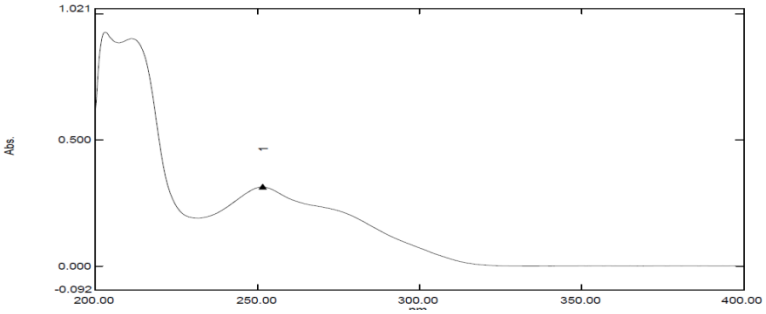
Spectrum showing overlapping spectrum of MET and SIT



Spectrum showing wavelength of metformin



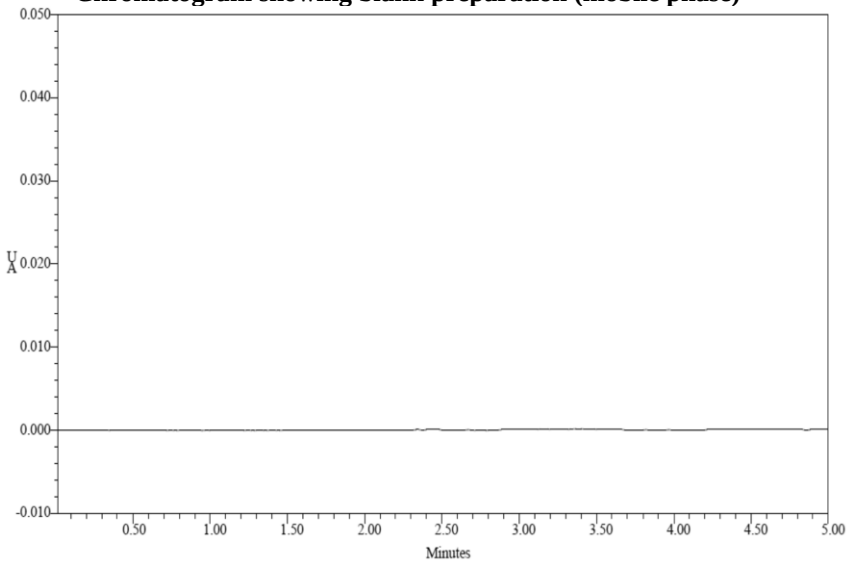
Spectrum showing wavelength of sitagliptin



The chromatographic method development for the simultaneous estimation of metformin and sitagliptin were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method were selected for the sepaproportionn and quantification of metformin and sitagliptin in API and pharmaceutical dosage form by RP-HPLC method.

Optimized chromatographic conditions for simultaneous estimations of Metformin and Sitagliptin by RP-HPLC method

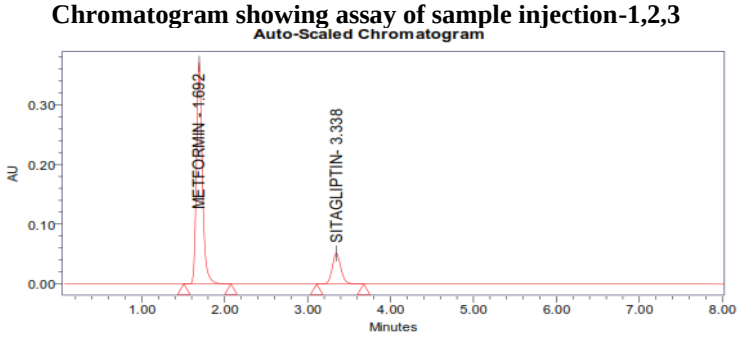
Chromatogram showing blank preparation (mobile phase)

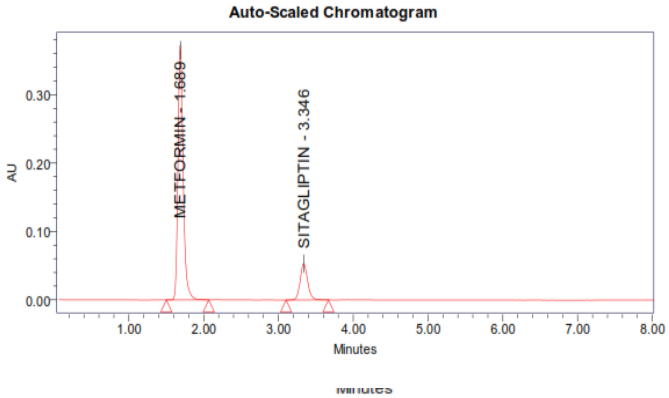


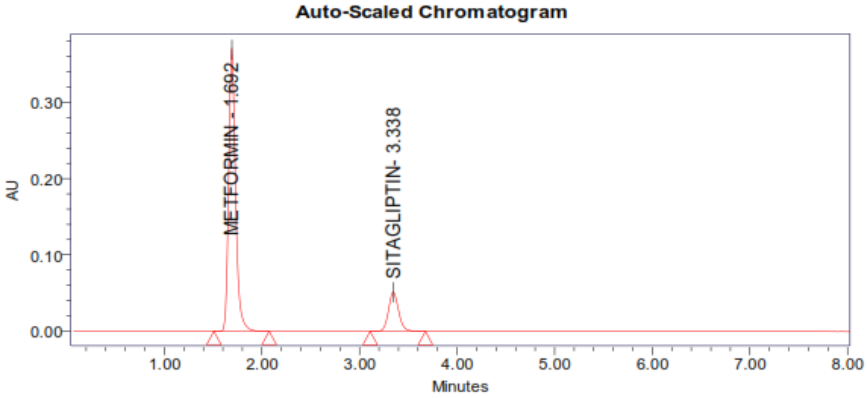
Assay calculation for metformin and sitagliptin:

The assay study were performed for the metformin and sitagliptin. Each three injections of sample and standard were injected into chromatographic system. The chromatograms are shown in Fig. below And results are tabulated in Table.below.

Chromatogram showing assay of sample injection-1,2,3



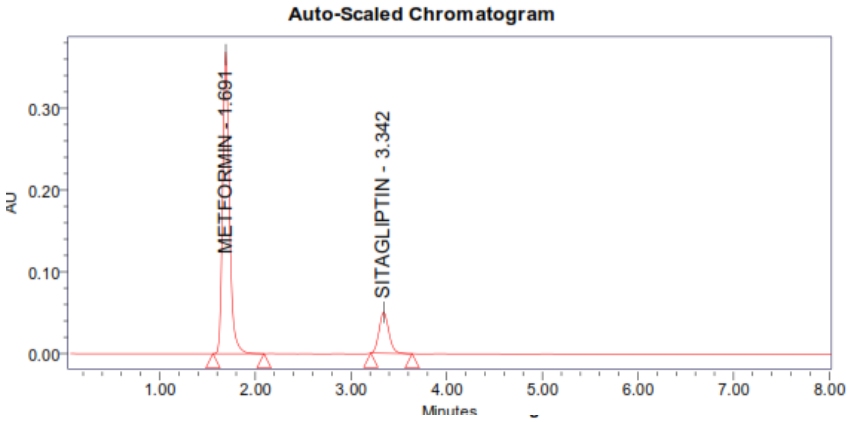


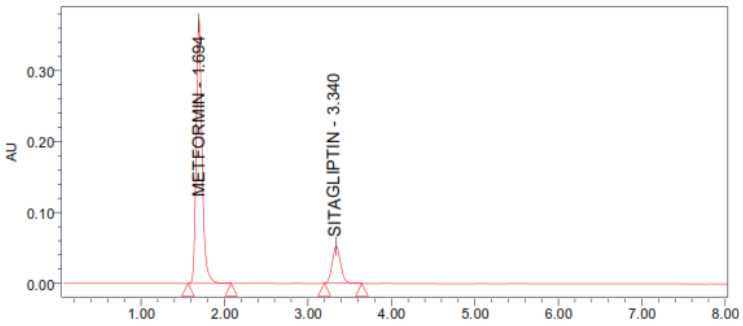


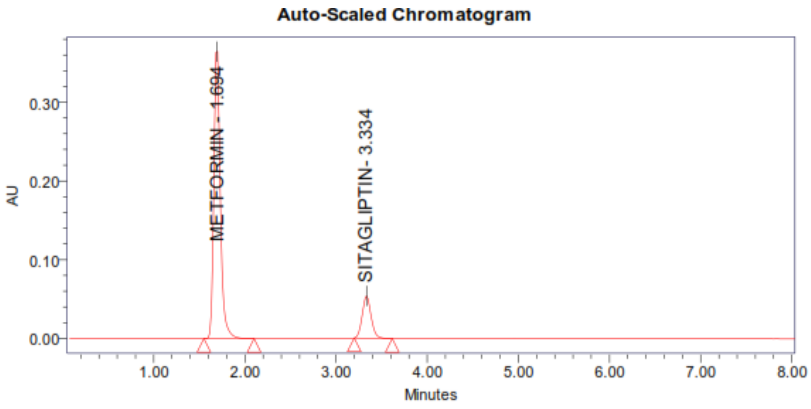
Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	METFORMIN	1.694	1791644	349533		1.29	2761	1
2	SITAGLIPTIN	3.344	371039	51965	10.12	1.15	5267	1
3	METFORMIN	1.689	1801923	367270		1.28	2861	2
4	SITAGLIPTIN	3.338	370058	53393	10.64	1.12	5513	2
5	METFORMIN	1.692	1810366	364326		1.27	2824	3
6	SITAGLIPTIN	3.346	369839	51558	10.28	1.12	5109	3

Chromatogram showing assay of sample injection -1,2,3.







Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	METFORMIN	1.691	1809940	362365		1.27	2784	1
2	SITAGLIPTIN	3.342	358795	50398	10.18	1.15	5077	1
3	METFORMIN	1.694	1816509	365598		1.24	2874	2
4	SITAGLIPTIN	3.340	364637	52531	10.46	1.12	5378	2
5	METFORMIN	1.694	1819524	364752		1.23	2993	3
6	SITAGLIPTIN	3.334	362414	53626	10.69	1.12	5735	3

Showing assay results

S.No	Name of compound	Label claim	Amount taken	%purity
1	Metformin	500	724.5	98.24
2	Sitagliptin	50	724.5	100.27

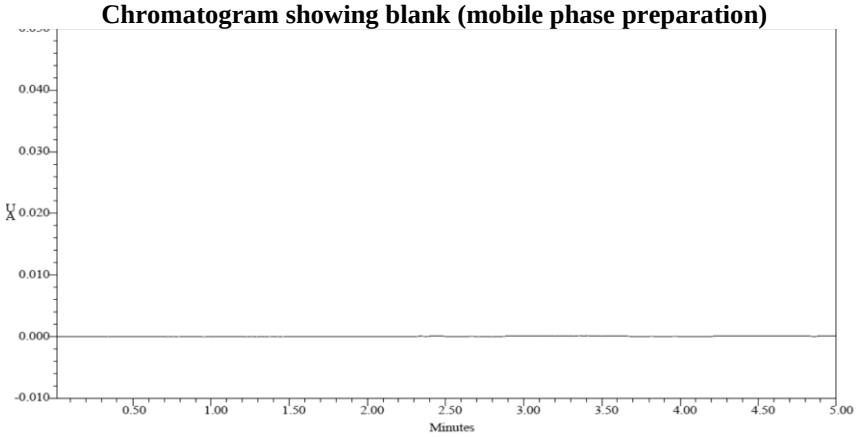
The retention time of metformin and sitagliptin were found to be 1.691mins and 3.344 mins individually. The system suitability parameters for metformin and sitagliptin such as theoretical plates and tailing factor were found to be 2294, 1.27and 4891, 1.03. Resolution were 8.67. The % purity metformin and sitagliptinin pharmaceutical dosage form were found to be 98.24 and 100.27% individually.

VALIDATION REPORT

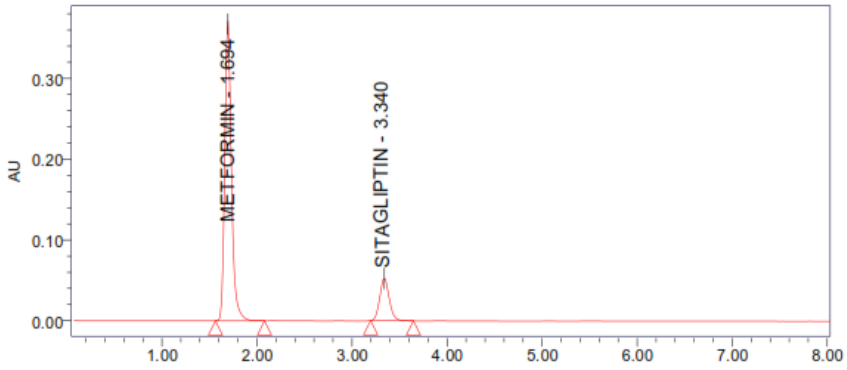
Specificity:

The system suitability for specificity were carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study were performed by injecting blank. The chromatograms are shown in Fig below

Chromatogram showing blank (mobile phase preparation)



Chromatogram showing standard injection

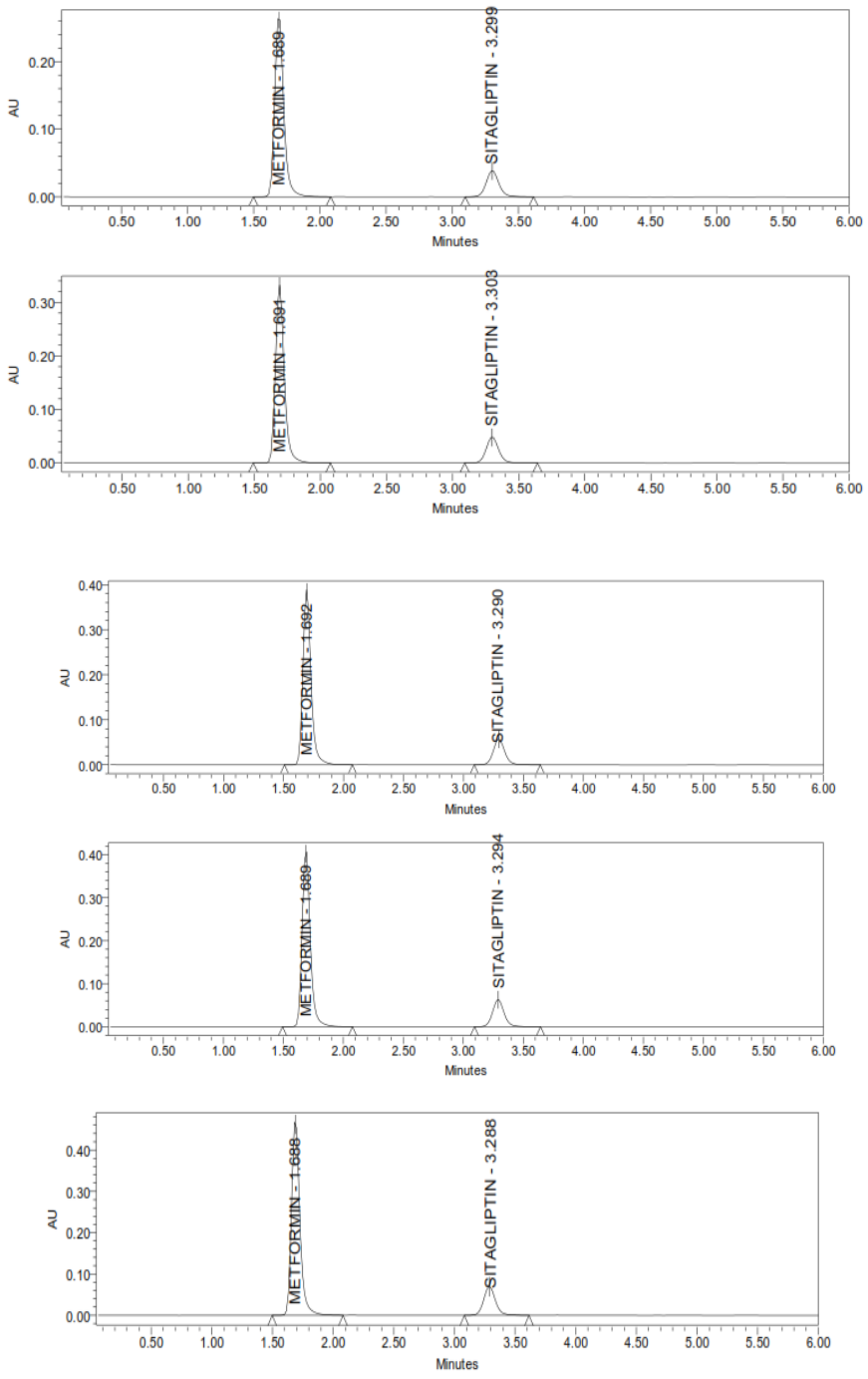


The specificity test were performed for Metformin and Sitagliptin. It were found that there were no interference of impurities in retention time of analytical peak.

Linearity:

The linearity study were performed for the concentproportionn of 50 ppm to 250 ppm and 5ppm to 25 ppm level. Each level were injected into chromatographic system. The area of each level were used for calculation of correlation coefficient. The chromatograms are shown in Fig. below and results are tabulated in Table. below. Calibration graph for MET and SIT are shown in Fig.No. below

Chromatograms showing linearity level-1 (50ppm-250 ppm of MET and 5ppm -50ppm of SIT) injections.

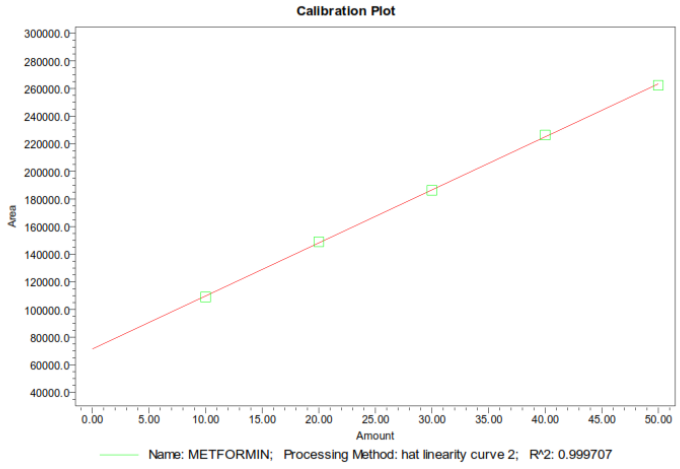


Peak Name: METFORMIN

	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	METFORMIN	1.689	1274954	264679	3109.6	1.2
2	METFORMIN	1.691	1548753	325904	3151.3	1.2
3	METFORMIN	1.692	1796583	381389	3111.1	1.2
4	METFORMIN	1.689	2045498	402853	3060.7	1.2
5	METFORMIN	1.688	2272948	466405	3034.3	1.3
Mean			1773747.3		3093.4	1.2
Std. Dev.			381086.8			
% RSD			21.5			

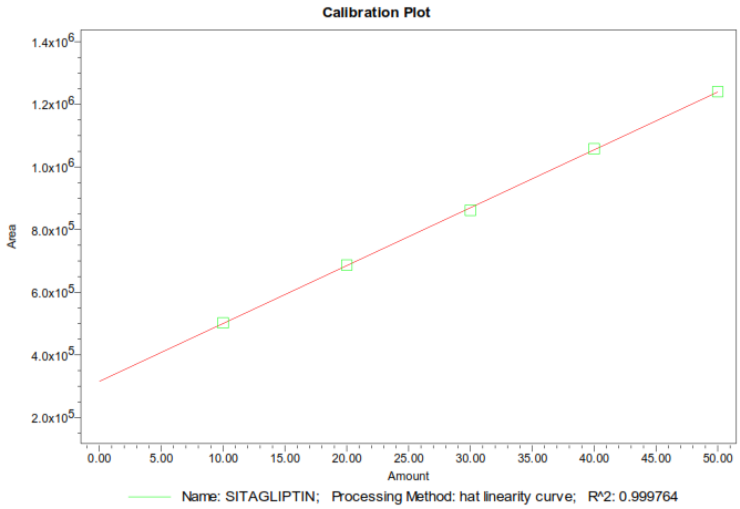
Peak Name: SITAGLIPTIN

	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	USP Resolution
1	SITAGLIPTIN	3.303	257359	38680	5873.9	1.1	10.9
2	SITAGLIPTIN	3.299	311497	48431	5780.7	1.1	10.7
3	SITAGLIPTIN	3.294	369389	57171	5898.2	1.1	10.6
4	SITAGLIPTIN	3.290	418105	62980	5931.1	1.1	10.8
5	SITAGLIPTIN	3.288	470352	69863	5631.3	1.1	10.7
Mean			369540.4		5823.0	1.1	10.7
Std. Dev.			82964.0				
% RSD			22.5				



Metformin $r^2 = 0.999$

Showing calibproportionn graph for Sitagliptin



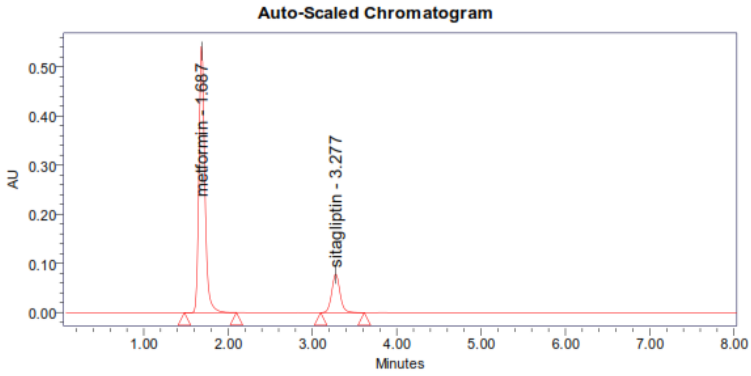
Sitagliptinr^2 = 0.999

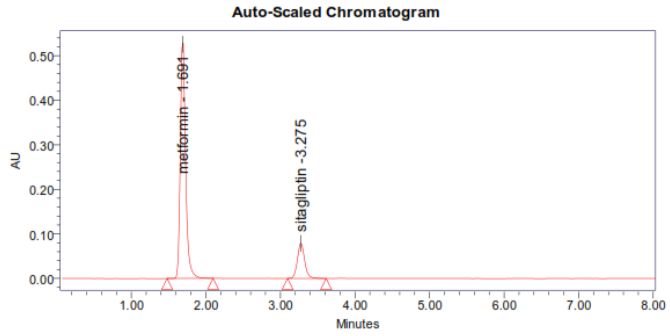
The linearity study were performed for concentproportionn range of 50.µg-250µg and 5µg-50µg of metformin and sitagliptin and the correlation coefficient were found to be 0.999 and 0.999.(NLT 0.999).

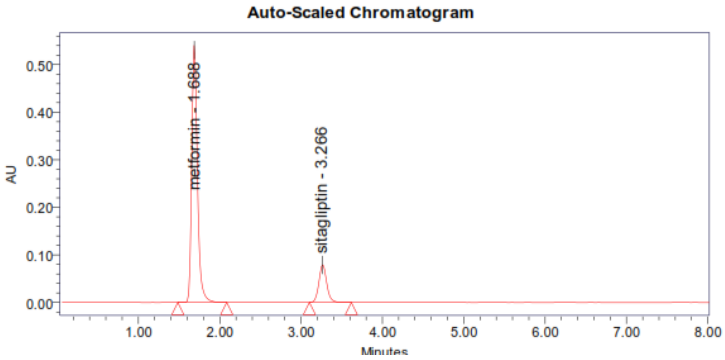
Accuracy

The accuracy study were performed for 50%, 100% and 150 % for metformin and sitagliptin. Each level were injected in triplicate into chromatographic system. The area of each level were used for calculation of % recovery. Chromatograms are shown in Fig below and results are tabulated in below.

Chromatograms showingaccuracy-50% injection-1,2,3.





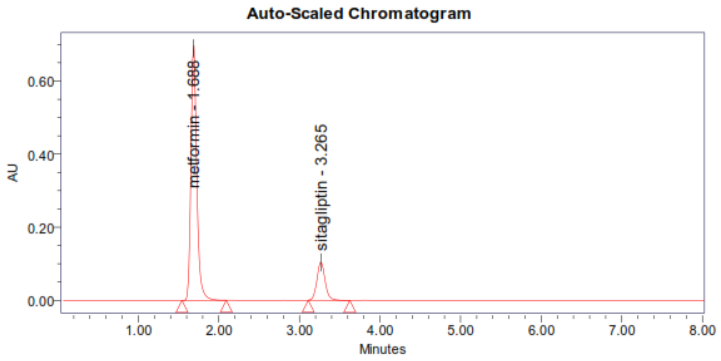


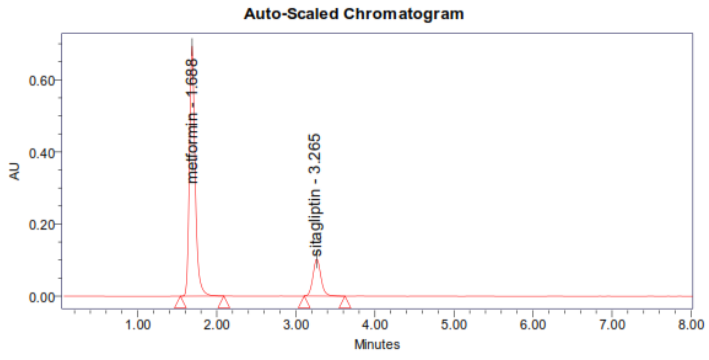
Peak Results

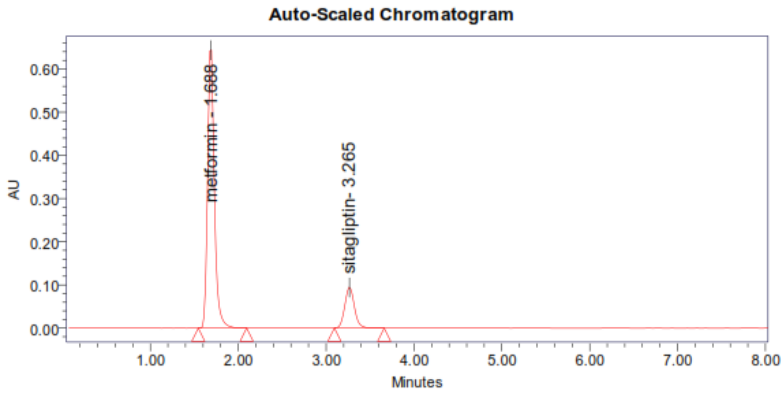
	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	metformin	1.687	2617903	536697		1.25	2962	1
2	sitagliptin	3.277	533081	78282	10.48	1.09	5531	1
3	metformin	1.691	2621619	526582		1.24	2991	2
4	sitagliptin	3.275	533591	79069	10.50	1.11	5624	2
5	metformin	1.688	2610076	531801		1.28	2896	3
6	sitagliptin	3.266	529920	79061	10.36	1.11	5735	3

Accuracy -100%

Chromatogram showing accuracy -100%injection-1,2,3.





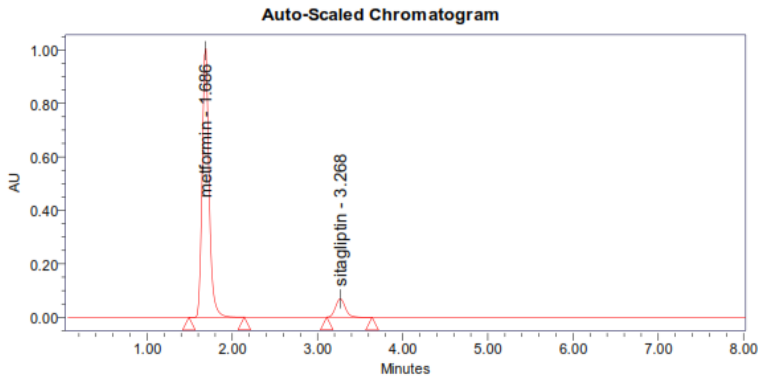


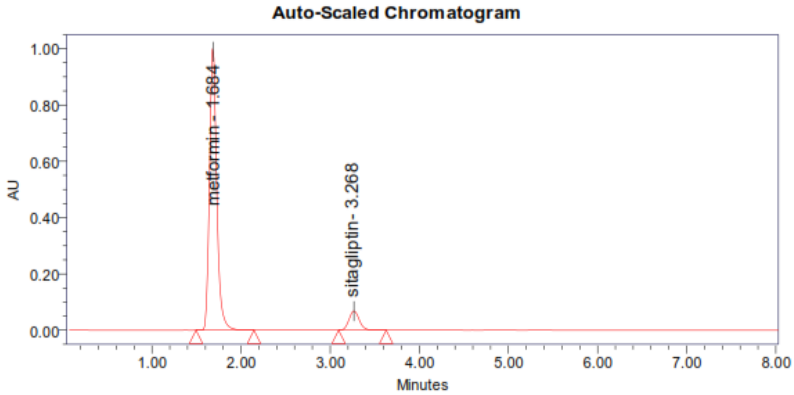
Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	metformin	1.688	3495304	689759		1.22	2808	1
2	sitagliptin	3.265	728605	104588	10.05	1.12	5139	1
3	metformin	1.688	3454965	681690		1.26	2764	2
4	sitagliptin	3.265	720532	102569	9.86	1.12	5099	2
5	metformin	1.688	3452614	645561		1.24	2605	3
6	sitagliptin	3.265	719474	94592	9.34	1.12	4332	3

Accuracy 150%

Chromatogram showing accuracy -150 %injection-1,2,3.





Showing accuracy results for metformin

%Concentproportionn (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	7371253	5	4.96	99.91%	99.56%
100%	14634226.7	10	9.98	99.18%	
150%	2243270.7	15	15.02	99.60%	

Showing accuracy results for sitagliptin

%Concentproportionn (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	484733	0.5	0.99	99.53%	99.47%
100%	967998	1.0	1.05	99.38%	
150%	145437	1.5	1.495	99.52%	

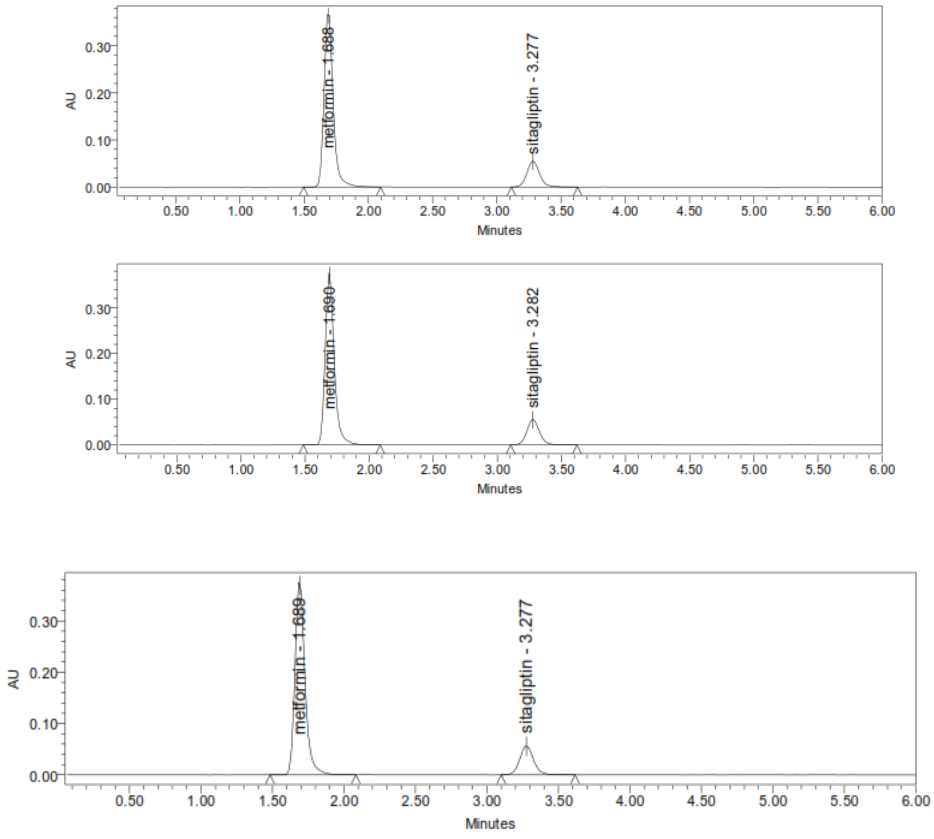
The accuracy study were performed for % recovery of Metformin and sitagliptin. The % recovery were found to be 99.18% and 99.91% individually (NLT 98% and NMT 102%).

Precision

Repeatability

The precision study were performed for five injections of metformin and sitagliptin. Each standard injection were injected in to chromatographic system. The area of each Standard injection were used for calculation of % RSD. The chromatograms are shown in Fig. below and results are tabulated in Table. below

Chromatograms showing precisioninjections-1 to 3



Showing% RSD results for metformin

Peak Name: metformin

	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	metformin	1.688	1817589	368060	3001.1	1.2
2	metformin	1.690	1834970	371075	2862.2	1.3
3	metformin	1.689	1840643	373296	2945.5	1.3
Mean			1831067.5		2936.2	1.3
Std. Dev.			12012.5			
% RSD			0.7			

Showing %RSD results for sitagliptin

Peak Name: sitagliptin

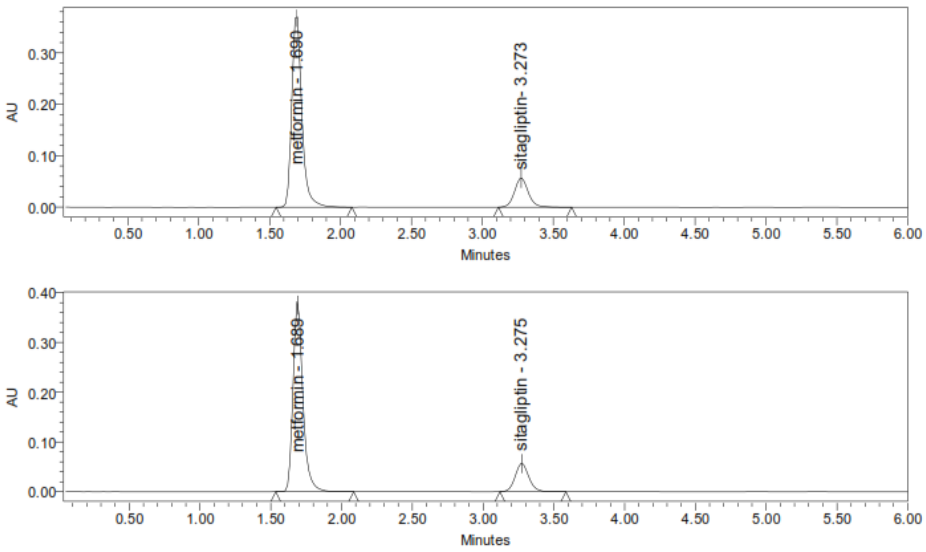
	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	USP Resolution
1	sitagliptin	3.282	376633	54987	5537.9	1.1	10.4
2	sitagliptin	3.277	380765	55523	5345.1	1.1	10.3
3	sitagliptin	3.277	382506	56202	5453.2	1.1	10.5
Mean			379967.9		5445.4	1.1	10.4
Std. Dev.			3016.1				
% RSD			0.8				

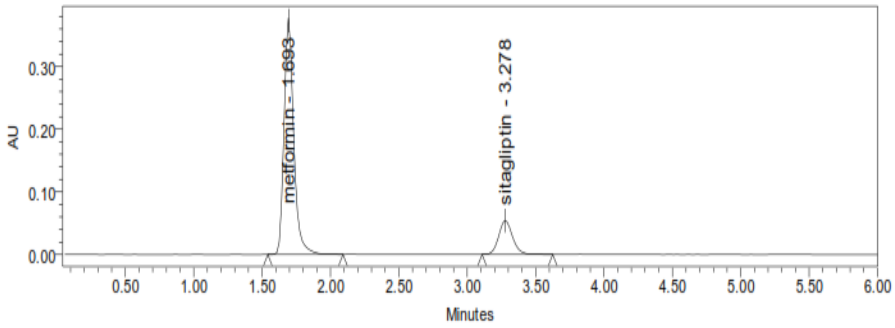
The Method precision study were performed for the %RSD of metformin and sitagliptin were found to be 0.7 and 0.8(NMT 2).

Intermediate precision/Ruggedness

The intermediate precision study were performed for five injections of metformin and sitagliptin. Each standard injection were injected into chromatographic system. The area of each standard injection were used for calculation of % RSD. The chromatograms are shown in below

Chromatogramsshowingintermediate precision injections-1to 3





Peak Name: metformin

	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	metformin	1.690	1837154	369568	3022.6	1.2
2	metformin	1.689	1844969	376764	2910.0	1.3
3	metformin	1.693	1856220	370802	2797.0	1.3
Mean			1846114.3		2909.9	1.3
Std. Dev.			9584.3			
% RSD			0.5			

Showing results for intermediate precision of sitagliptin

Peak Name: sitagliptin

	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	USP Resolution
1	sitagliptin	3.273	380522	56875	5623.5	1.1	10.4
2	sitagliptin	3.275	380632	56469	5600.2	1.1	10.6
3	sitagliptin	3.278	383855	54278	5084.0	1.1	9.9
Mean			381669.4		5435.9	1.1	10.3
Std. Dev.			1893.4				
% RSD			0.5				

The intermediate precision were performed for %RSD of metformin and sitagliptinwere found to be 0.5 and 0.5individually (NMT 2).

Identification limit:

LOD's can be calculated based on the standard deviation of the response (SD)and the slope of the calibproportionn curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:LOD $= 3.3 \text{ X } \frac{\sigma}{s}$

Where σ - Standard deviation (SD) S - Slope

Showing results for Limit of Identification

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Metformin	371827.90	563365963	2.17
Sitagliptin	5401.60	479884400	0.0372

The LOD were performed for metformin and sitagliptin were found to be 2.17and 0.0372individually.

Quantitation limit:

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibproportionn curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:$LOQ = 10 \text{ X } \frac{\sigma}{S}$

Where :σ - Standard deviation S - Slope

Showing results for Limit of Quantitation

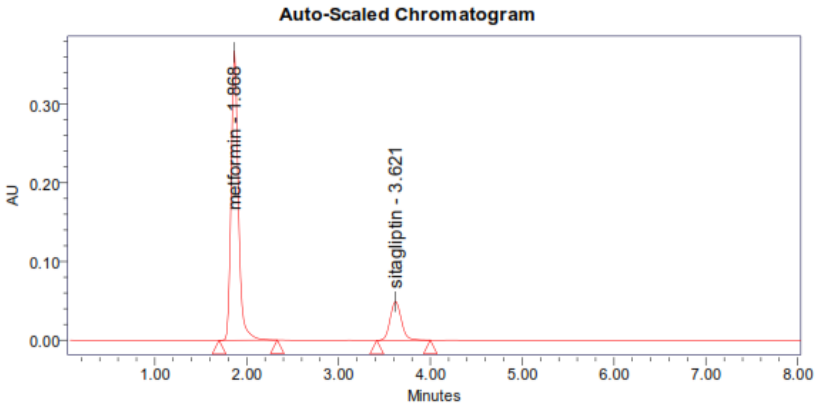
Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Metformin	371827.90	563365963	6.60
Sitagliptin	5401.60	479884400	0.112

The LOQ were performed for metformin and sitagliptin were found to be 6.60 and 0.112individually.

Robustness:

The robustness were performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase proportion variation from more organic phase to less organic phase proportion for metformin and sitagliptin. The method is robust only in less flow condition andthe method is robust even by change in the Mobile phase ±5%.The chromatograms are shown below

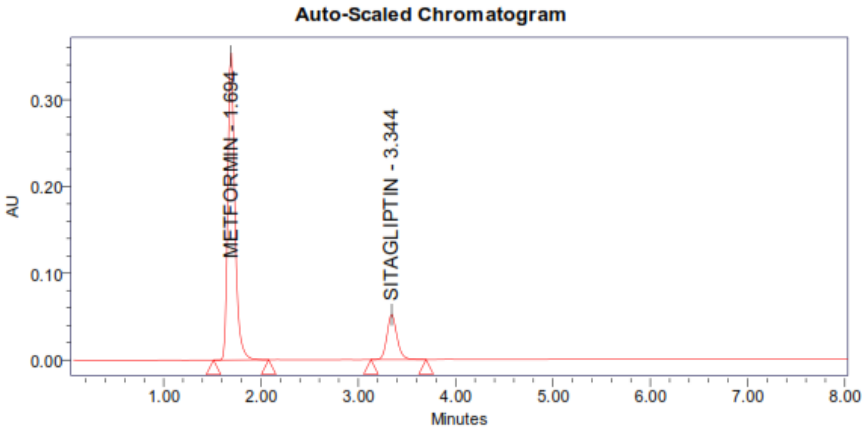
Chromatogram showing more flow rate 0.8ml/min



Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	metformin	1.868	2002121	363410		1.23	2728	1
2	sitagliptin	3.621	415201	49425	9.64	1.11	4402	1

Chromatogram showing less flow rate 1 ml/min



The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min.The method is robust only in less flow condition.

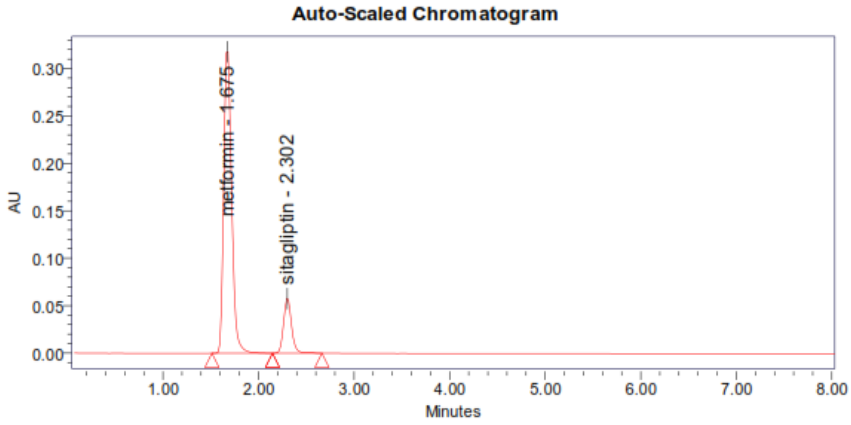
Showing system suitability results for metformin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2590	1.39
2	**1**	**2294**	**1.27**
3	1.2	2146	1.26

Showing system suitability results for sitagliptin

S.No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5435	1.04
2	**1**	**4891**	**1.03**
3	1.2	4781	1.04

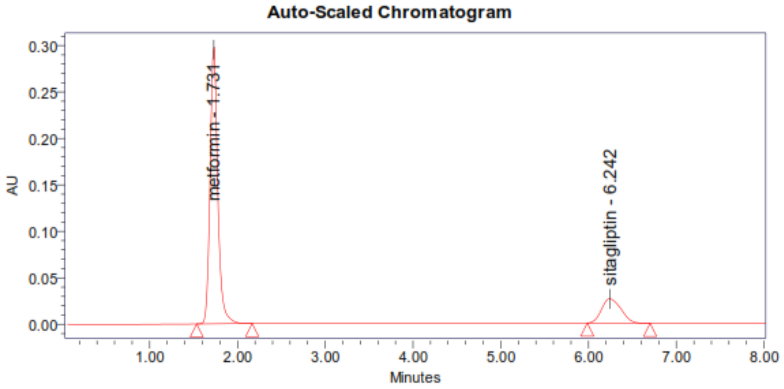
Chromatogram showing more organic phase proportion



Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	metformin	1.675	1829674	319174		1.24	1996	1
2	sitagliptin	2.302	337931	58066	4.12	1.15	3831	1

Chromatogram showing less organic phase proportion



Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	metformin	1.731	1797951	296370		1.18	2056	1
2	sitagliptin	6.242	402586	26209	15.83	1.20	3596	1

On evaluation of the above results, it can be concluded that the variation in±5%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%.

Showing system suitability results for metformin

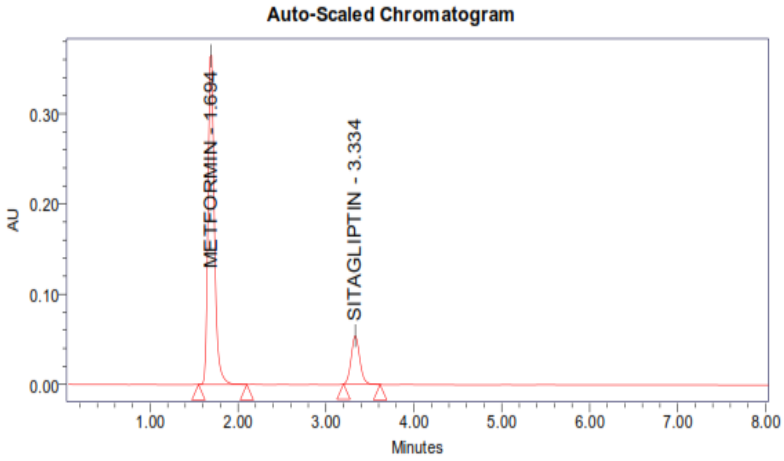
S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	2347	1.44
2	***Actual**	**2294**	**1.27**
3	5 % more	2239	1.13

Showing system suitability results for sitagliptin

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	0.99
2	***Actual**	**4891**	**1.03**
3	5 % more	4817	1.05

System suitability

Chromatogram showing standard injection



Showing system suitability results

Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	METFORMIN	1.694	1819524	364752		1.23	2993	3
2	SITAGLIPTIN	3.334	362414	53626	10.69	1.12	5735	3

The retention time of metformin and sitagliptin were found to be 1.694mins and 3.334 mins individually. The system suitability parameters for metformin and sitagliptin such as theoretical plates and tailing factor were found to be 2294, 1.27 and 4891, 1.03 and the Resolution were found to be 8.67. The % purity of metformin and sitagliptin in pharmaceutical dosage form were found to be 98.24% and 100.27% individually. Overall summarized method validation results are tabulated

CONCLUSION:

A new method were established for simultaneous estimation of metformin and sitagliptin by RP-HPLC method. The chromatographic conditions were successfully developed for the sepaproportionn of metformin and sitagliptinby utilizing SYMMETRYC18 column (4.6×150mm)5µ, flow rate were1ml/min, mobile phase proportion were (70:30 v/v) methanol : phosphate buffer($KH_2PO_4$and K_2HPO_4) pH 3 (pH were adjusted with orthophosphoric acid),detection wave length were 258nm. The instrument used were WATERS HPLC Auto Sampler , Sepaproportionn module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 1.694mins and 3.334 mins. The % purity of Metformin and sitagliptinwere found to be 100.27% and 99.87% individually. The system suitability parameters for metformin and sitagliptin such as theoretical plates and tailing factor were found to be 2294, 1.27 and 4891 and 1.03, the resolution were found to be 8.67. The analytical method were validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study metformin and sitagliptin were found in concentproportionn range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) were found to be 0.999 and 0.999, % recovery were found to be 99.56% and 99.48%, %RSD for repeatability were 0.27 and 0.40, % RSD for intermediate precision were 0.27 and 0.94individually. The precision study were precise, robust, and repeatable. LOD value were 2.17 and 6.60, and LOQ value were 0.032 and 0.1125individually.

Hence the suggested RP-HPLC method can be used for routine analysis of metformin and sitagliptinin API and Pharmaceutical dosage form.

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