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Method Development and Validation for Simultaneous Estimation of Sitagliptin and Metformin Hcl by RP-HPLC

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ABSTRACT

This present work is concerned with the application of simple, accurate, precise and highly selective HPLC method for simultaneous estimation of sitagliptin and metformin hcl in Bulk drugs. The developed method was validated for linearity, accuracy, precision, limit of detection, limit of quantification, robustness parameters and found to be in good accordance with the prescribed values. Thus the proposed method can be successfully applied for simultaneous determination of sitagliptin and metformin hcl in routine bulk drug analysis.

Keywords: *sitagliptin and metformin hcl ,Validation, Bulk drugs.*

INTRODUCTION

Sitagliptin is anti-diabetic drug.It is mainly used in treatment of diabetics. Chemically it is 7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine phosphate monohydrate (**Fig. 1**). It is a white to offwhite crystalline powder which is odourless and freely soluble in water. Numerous authors have reported CTZ detection methods in biological fluids and pharmaceutical formulations [2-6].

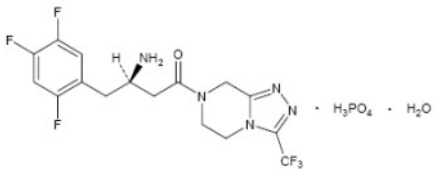

Fig. 1: Structure of Sitagliptin

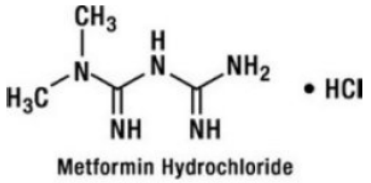

Fig. 2: Structure of Metformin

Metformin is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function. It is also used in the treatment of polycystic ovary syndrome, Metformin is the only antidiabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. It helps reduce LDL cholesterol and triglyceride levels, and is not associated with weight gain.Chemically it is *N,N*-Dimethylimidodicarbonimidic diamide (**Fig. 2**) It is a white crystalline powder which is odourless and freely soluble in water [2-6].

The aim of this work is to develop accurate, specific, cost effective, repeatable and validated HPLC method for the simultaneous estimation of sitagliptin and metformin hcl in the bulk drug samples.

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EXPERIMENTAL

An UV spectrum of 10µg/ml, of in diluents was recorded by scanning in the wavelength range of 200nm to 400nm. From the overlein spectrum the absorption wavelength was found at 336nm for 10µg/ml solution. Initially the mobile phase tried was methanol: acetonitrile and acetonitrile: phosphate buffer and finally phosphate buffer : methonol with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate buffer (pH 3): methonol in the ratio of 50:50 respectively

Preparation of standard solution:

Accurately Weighed and transferred 100mg of Metformin and 10mg of Sitagliptin working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent , sonicated for 5 minutes and make up to the final volume with diluent(standard stock).

Preparation of sample solution:

20 tablets were weighed and calculated the average weight of tablets .then the weight equivalent to 5 tablets was transferred into a 100 mL volumetric flask, 70mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.4ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Validation Parameters:

Validation experiments were performed to demonstrate accuracy, precision, intermediate precision, linearity, specificity, LOD, LOQ, robustness.

A) Accuracy:

Preparation of stock solution containing sitaglipten and metformin:

Accurately weighed and transferred 5 mg of sitaglipten and 50 mg of metformin working standards into 10mL clean and dry volumetric flask and add ¾ th of milli Q water sonicated to dissolve completely and adjusted the volume upto the mark with the mill Q water. Further pipetted following concentrations from stock solution.

Preparation of Level – I (50%):

0.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (100%):

1.0ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (150%):

1.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluents.

Procedure:

The 50% level, 100% level and 150% level solutions were injected into the HPLC system and run the chromatograms and the peak areas were noted and then calculated the percentage recovery of each standard drug added and % RSD was calculated.

B) Precision:

Preparation of stock solution containing sitaglipten and metformin:

Accurately weighed and transferred 5 mg of sitaglipten and 50 mg of metformin working standards into 10mL clean and dry volumetric flask and add ¾ th of milli Q water sonicated to dissolve completely and adjusted the volume upto the mark with the mill Q water. Further pipetted following concentrations from stock solution.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

C) Linearity:

Preparation of stock solution containing sitaglipten and metformin:

Accurately weighed and transferred 5 mg of sitaglipten and 50 mg of metformin working standards into 10mL clean and dry volumetric flask and add ¾ th of milli Q water sonicated to dissolve completely and adjusted the volume upto the mark with the mill Q water. Further pipetted following concentrations from stock solution.

Preparation of Level – I (50ppm):

0.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (70ppm):

0.7ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (100ppm):

1.0ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV 1(120ppm):

1.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (150ppm):

1.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluents.

Procedure:

Injected each level standard solution into the chromatographic system and measured the peak area. Plotted a standard graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculated the correlation coefficient.

D) Limit of Detection:

$$\text{LOD of metformin} = 3.3 \times \frac{\text{Standard Deviation}}{\text{Slope}}$$

E) Limit of Quantification:

$$\text{LOQ of metformin} = 10 \times \frac{\text{Standard Deviation}}{\text{Slope}}$$

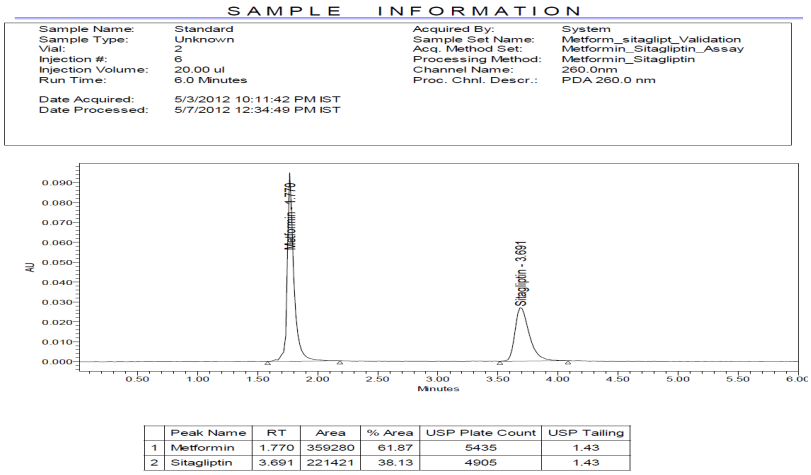
F) Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

G) System Suitability Parameters:

System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factors, resolution, and precision of standard peak areas, and comparison to a confirmation standard, capacity factors, retention times, and theoretical plates.

RESULTS AND DISCUSSION



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.770	359280	61.87	5435	1.43
2	Sitagliptin	3.691	221421	38.13	4905	1.43

Fig. 3: Optimised Chromatogram

Validation Parameters:

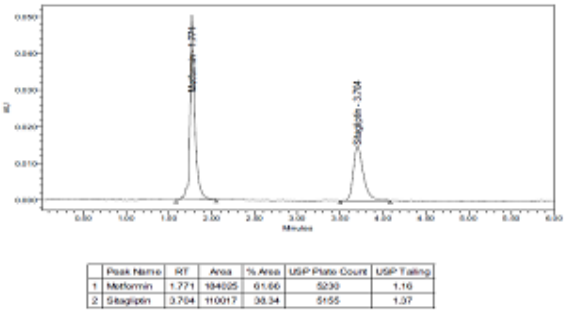
Table No. 1: Accuracy of Metformin

Sample ID	Conc.	% Recovery	Mean % Recovery	SD	% RSD
1	50%	101.2953			
2	50%	101.536	**100.373**	**1.807**	**1.804**
3	50%	98.287			

4	100%	99.2998			
5	100%	101.101	**100.263**	**1.19**	**1.19**
6	100%	101.564			
7	150%	98.333			
8	150%	98.36	**98.39**	**0.081**	**0.082**
9	150%	99.93			

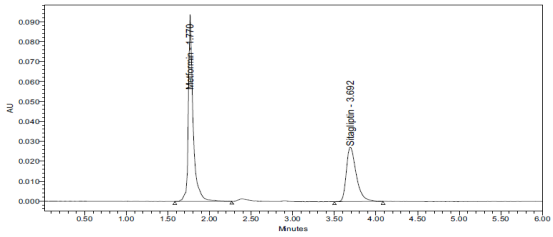
Table No. 2: Accuracy of Sitagliptin

Sample ID	Conc.	% Recovery	Mean % Recovery	SD	% RSD
1	50%	98.7905			
2	50%	99.3374	**98.97**	**0.3159**	**0.31**
3	50%	98.7923			
4	100%	101.387			
5	100%	100.582	**101.74**	**1.36**	**1.34**
6	100%	103.24			
7	150%	98.623			
8	150%	99.06	**98.94508**	**0.274**	**0.277**
9	150%	99.15			



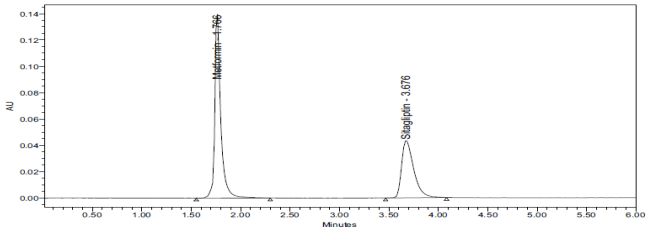
	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.771	184025	61.66	5230	1.16
2	Sitagliptin	3.704	110017	38.34	5155	1.37

Fig. 4: Chromatogram of the first injection of 50% level solution



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.770	360796	62.15	5448	1.62
2	Sitagliptin	3.692	225816	37.85	4895	1.43

Fig. 5: Chromatogram of the first injection of 100% level solution



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.766	536753	61.36	5062	1.33
2	Sitagliptin	3.676	329523	38.64	4508	1.54

Fig. 6: Chromatogram of the first injection of 150% level solutions

Precision:

Table No. 3: Precision for Metformin

Injection No.	Sample area	% Assay
1	357969	99.1887
2	361586	99.68
3	363182	98.76
4	361588	99.19
5	359015	99.18
6	359280	100.01
Average	360437	99.33
Std. Dev.	0.44136	0.44
% RSD	0.44345	0.443

Table No. 4: Precision for Sitagliptin

Injection No.	Sample area	% Assay
1	219848	99.35
2	221565	99.96
3	219812	100.47
4	221878	100.72
5	221152	100.12
6	221421	99.98
Average	220946	100.01

Std. Dev.	0.4728	0.47
% RSD	0.47238	0.47

Table No. 5: Linearity of Metformin

Conc. of Metformin (ppm)	Peak Area
0	0
50	194715
70	265641
100	372832
130	488901
150	572973
Correlation Coefficient	0.9995

Table No. 6: Linearity of Sitagliptin

Conc. of Metformin (ppm)	Peak Area
0	0
50	119633
70	160921
100	223536
130	301128
150	346153
Correlation Coefficient	0.9995

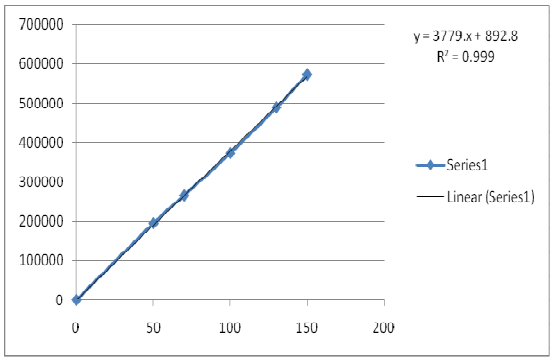


Fig. 7: Calibration curve of Metformin

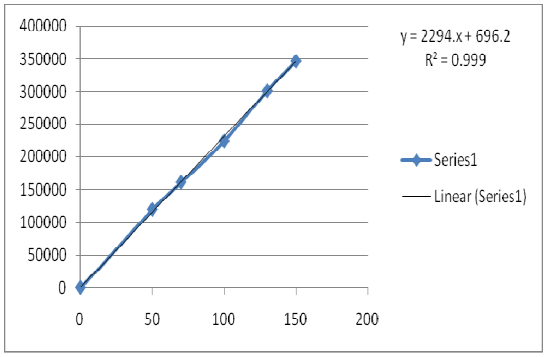


Fig. 8: Calibration curve of Sitagliptin

Limit of Detection and Limit of Quantitation for Metformin:

$$\text{LOD of metformin} = 3.3 \times \frac{\text{Standard Deviation}}{\text{Slope}}$$

$$\text{LOQ of metformin} = 10 \times \frac{\text{Standard Deviation}}{\text{Slope}}$$

Table No. 7: Slope Intercept Results for Metformin

x	Y	M	C	Yint	Resi
50	194715	3766.279	2384.459	190698.4	4016.571
70	265641	3766.279	2384.459	266024	-383.018
100	372832	3766.279	2384.459	379012.4	-6180.4
130	488901	3766.279	2384.459	492000.8	-3099.78
150	572973	3766.279	2384.459	567326.4	5646.629
		SD			4898.213
		LOD			4.291796
		LOQ			13.00544

For Sitagliptin:

$$\text{LOD of sitagliptne} = 3.3 \times \frac{\text{Standard Deviation}}{\text{Slope}}$$

$$\text{LOQ of sitagliptne} = 10 \times \frac{\text{Standard Deviation}}{\text{Slope}}$$

Table No. 8: Slope Intercept Results for Sitagliptin

x	y	M	C	Yint	Resi
50	119633	2284.149	1859.347	116066.8	3566.226
70	160921	2284.149	1859.347	161749.7	-828.744
100	223536	2284.149	1859.347	230274.2	-6738.2
130	301128	2284.149	1859.347	298798.7	2329.344
150	346153	2284.149	1859.347	344481.6	1671.374
		SD			4093.515
		LOD			5.914064
		LOQ			17.9214

Robustness:

Table No. 9: Robustness Parameters

Parameters	Optimum range	Conditions in procedure	Remarks
Mobile Phase variation	60:40-70:30	50:50	Retention time changed
Flow rate ml/min	0.9-1.1	1.0	At higher flow rates the relative retentions was decreased
Temperature	25-35°C	Ambient	Beyond the optimum range peak shape and symmetry was lost

System Suitability:

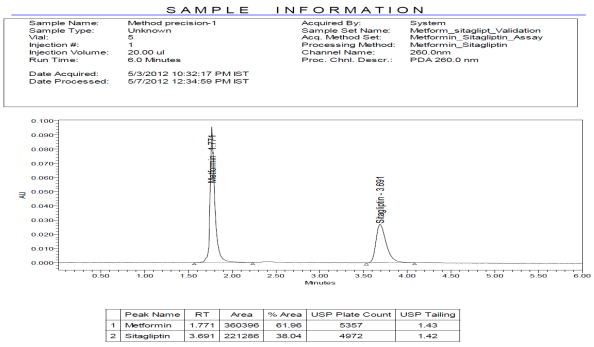
Table No. 10: System suitability for Sitagliptin

S. No.	RT	Area	USP Plate count	USP Tailing
1	3.683	219848	4902	1.43
2	3.673	221565	4926	1.44
3	3.675	219812	4915	1.41
4	3.681	221878	4790	1.43
5	3.686	221152	4856	1.43
6	3.691	221421	4905	1.42
Mean	3.6815	220946	4882	1.43

Table No. 11: System suitability for Metformin

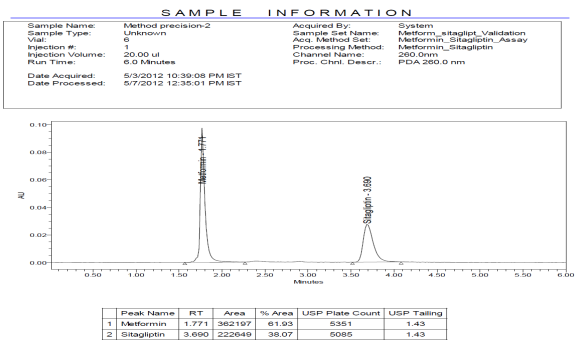
S. No.	RT	Area	USP Plate count	USP Tailing
1	1.774	357969	6289	1.48
2	1.760	361586	5360	1.42
3	1.759	363182	5429	1.43
4	1.759	361588	5324	1.43
5	1.766	359015	5459	1.44
6	1.770	359280	5435	1.43
Mean	1.764	360436.6	5549.3	1.438

Ruggedness:



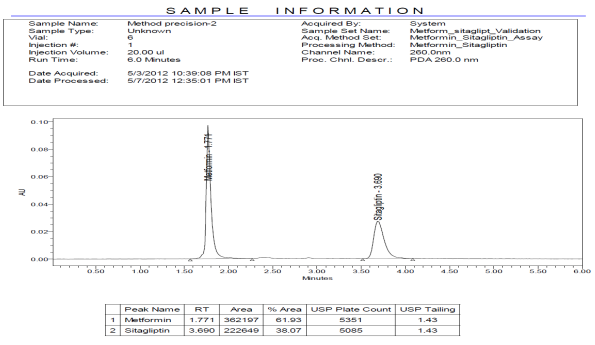
	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.771	360396	61.96	5357	1.43
2	Sitagliptin	3.691	221286	38.04	4972	1.42

Chromatogram for sample injection-1



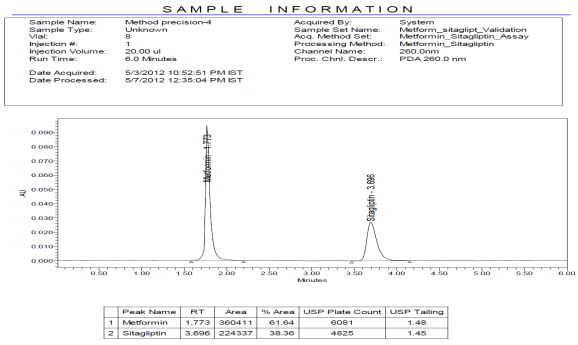
	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.771	362197	61.93	5351	1.43
2	Sitagliptin	3.690	222649	38.07	5085	1.43

Chromatogram for sample injection-2



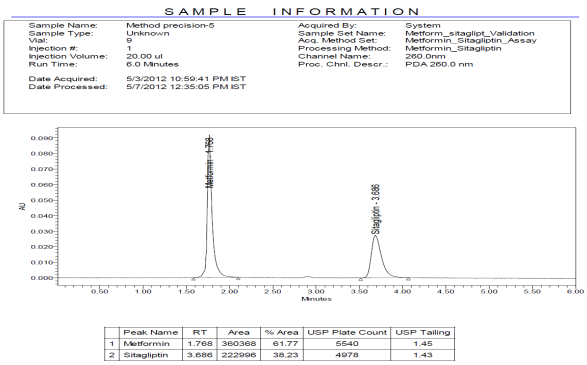
	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.771	362197	61.93	5351	1.43
2	Sitagliptin	3.690	222649	38.07	5085	1.43

Chromatogram for sample injection-3



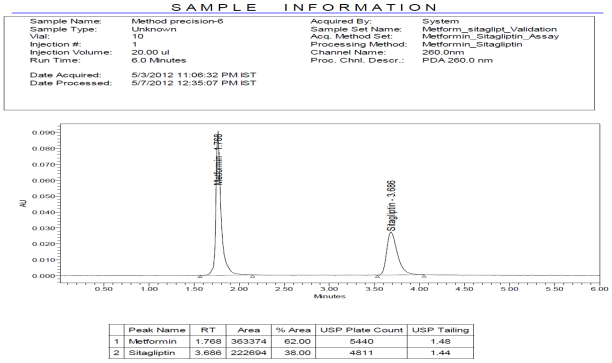
	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.773	360411	61.64	6081	1.48
2	Sitagliptin	3.696	224337	38.36	4825	1.45

Chromatogram for sample injection-4



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.768	360368	61.77	5540	1.45
2	Sitagliptin	3.686	222996	38.23	4978	1.43

Chromatogram for sample injection-5



	Peak Name	RT	Area	% Area	USP Plate Count	USP Tailing
1	Metformin	1.768	363374	62.00	5440	1.48
2	Sitagliptin	3.686	222694	38.00	4811	1.44

Chromatogram for sample injection-6

CONCULSION

As there are very few reports on the simultaneous estimation of Metformin and Sitagliptin a new RP-HPLC method was developed and validated. It was found from the results presented above the proposed method has good sensitivity, precision and accuracy. As chromatographic run time is 4.5mints it allows the analysis of a large number of samples in a short period of time. The percentage recovery of the combination of Metformin and Sitagliptin was found to be in the range of 98.7%-100.01%. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in flow rate and composition of mobile phase. Therefore it is suitable for the routine analysis of in pharmaceutical dosage forms. The present method succeeded in adopting a simple sample preparation that achieved satisfactory extraction recovery and facilitated its application in co formulated formulation.

The results of our study indicate that the proposed RP-HPLC method was simple, rapid, precise and accurate. Statistical analysis proves that, this method was reproducible and selective for the combination analysis of Sitagliptin and Metformin. It can therefore be concluded that use of this method can save much time, very economic and that can be with accuracy.

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