Article

Validated Stability Indicating RP-HPLC Method for Estimation of Sitagliptin and Metformin HCL in their Tablet Dosage form

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ABSTRACT

A simple isocratic and rapid reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and successively validated stability for the estimation of Sitagliptin and Metformin HCL. Sitagliptin and Metformin HCL were resolved on an isocratic method, C8 Column, mobile phase utilizing a composition of 2.34g of Sodium 1-Octane Sulfonate Monohydrate : Acetonitrile(67:33 v/v pH 3.0) at a flow rate of 1.0 mL/min with UV detection at 210 nm. Retention Time of Sitagliptin and Metformin HCL were 13 min and 3.5 min respectively. RP-HPLC method for determination as well as force degradation study of Sitagliptin and Metformin HCl was Validated.The results of specific ,precise, accurate, linear, robust, rugged were proved within the limits as per ICH guidelines. The method could be used to select the storage condition improving the manufacturing process and for routine analysis of Sitagliptin and Metformin HCL in their combined dosage forms.

Keywords: Sitagliptin, Metformin HCL, Validation, degradation, HPLC.

INTRODUCTION

An antidiabetic drug Sitagliptin is chemically (R)-4-oxo-4-[3-(trifluromethyl)-5,6-dihydro [1,2,4] triaz-olo [4,3-a] pyrazin 7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine having a molecular formula of $C_{16}H_{15}F_6N_5O$ with a Molecular weight of 407.32 g/mol, soluble in water, ethanol and acetonitrile slightly soluble in methanol and insoluble in isopropyl acetate. It is used to treat type-2 diabetes with the class of dipeptidyl peptidase-4(DPP-4) inhibitor. It increases insulin production decreases glucose overproduction by the pancreas. Metformin HCl is chemically [N,N-dimethylimidodicarbonimidic diamide hydrochloride having a molecular formula of $C_4H_{11}N_5$ • HCl with a Molecular weight of 165.6 g/mol,freely soluble in water, slightly soluble in alcohol, insoluble in acetone and methylene chloride.It is basically used as first line medication to lower the blood sugar for the treatment of type-2 diabetes,mainly in people who are overweight and also in the treatment of

polycystic ovary syndrome. It decreases glucose production by the liver and increasing the insulin sensitivity of body tissue.

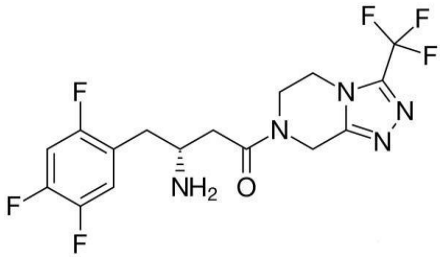


Fig1. SITAGLIPTIN

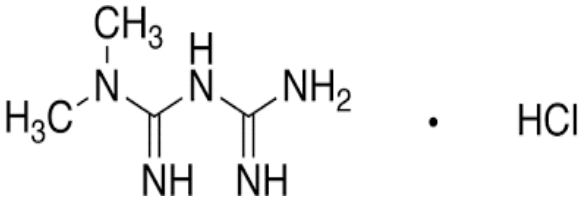


Fig2. METFORMIN

Material and Method:

Sitagliptin-Active pharmaceutical Ingredient (API) and Metformin HCl- Enaltec Pharma Pvt Ltd Mumbai.HPLC grade methanol, Ortho phosphoric acid 88% Sodium 1-octonate sulfonate monohydrate,water, Acetonitrile were used.

Instrumentation :

A Make-Shimadzu (Japan) model plus HPLC system equipped with a pump PU-2080 plus programmable UV detector 2075 and Borwin software package were used.

Preparation of Buffer solution:

Dissolve 2.34g of Sodium 1-Octane Sulfonate Monohydrate in 1000 mL of water and adjust the pH of solution to 3.0 ± 0.05 with dilute Ortho-phosphoric acid. Filter through 0.45µ Nylon membrane filter.

Preparation of Mobile Phase:

Prepare a mixture of Buffer pH 3.0: Acetonitrile in the ratio 67:33 v/v respectively. Mix and degas

Preparation of Standard stock solution:

A. For Sitagliptin:

Weigh accurately about 50 mg of Sitagliptin phosphate monohydrate standard and transfer into a 100 mL of volumetric flask. Add about 70 mL diluent, sonicate to dissolve and make up to volume with diluent and mix. (500mcg/mL)

B. For Metformin HCL:

Weigh accurately about 50 mg of Metformin HCl standard and transfer into a 100 mL of volumetric flask. Add about 70 mL of diluent, sonicate to dissolve and make up to volume with diluent and mix. (500mcg/mL)

Preparation of Sample Stock solution (100/1000 mg):

Weigh and transfer 5 tablets into a 500 mL volumetric flask. Add about 300 mL of diluent, sonicate for 20 minutes with intermittent shaking (Ensure all the tablets have disintegrated).Add about 700 mL of diluent and sonicate for 15 minutes with intermittent shaking. Allow it to cool to room temperature and make up to volume with diluent and mix. Centrifuge the sample at 3000 rpm for 10 min.

For Sitagliptin:

Further dilute 5 mL of sample stock solution to 50 mL with water and mix. Filter the sample solution

through 0.45µ nylon syringe filter. Discard first 2 mL of filtrate. (100 µg/ml).

For Metformin HCl:

Further dilute 2 mL of sample stock solution to 200 mL with diluent and mix. Filter the sample solution through 0.45µ nylon syringe filter. Discard first 2 mL of filtrate. (100 µg/ml).

Chromatographic Conditions:

Chromatographic separation of Sitagliptin and metformin HCL was carried on Zorbax SB C8 column.The mobile phase was composed of Buffer pH 3.0: Acetonitrile in the ratio 67:33 v/v respectively. Mix and degas. Filter through 0.45µ Nylon membrane filter. The flow rate wasof mobile phase was maintained at 1.0 mL/min.UV detection was carried out at 210 nm.The column and sample temp was carried out 25°C and 5°C.Run time was 20 minutes.

Identification by IR-Spectroscopy:

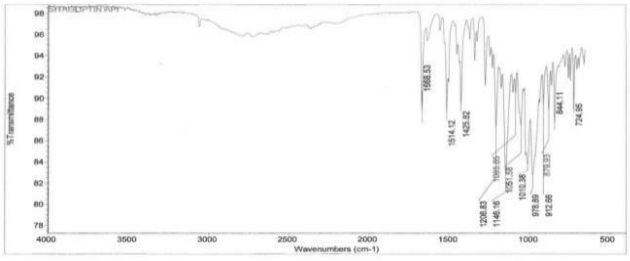


Fig 3: FT-IR spectrum of Sitagliptin

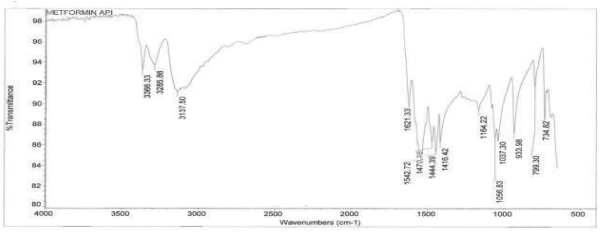


Fig 4: FT-IR spectrum of Metformin

UV Spectrophotometric Method:

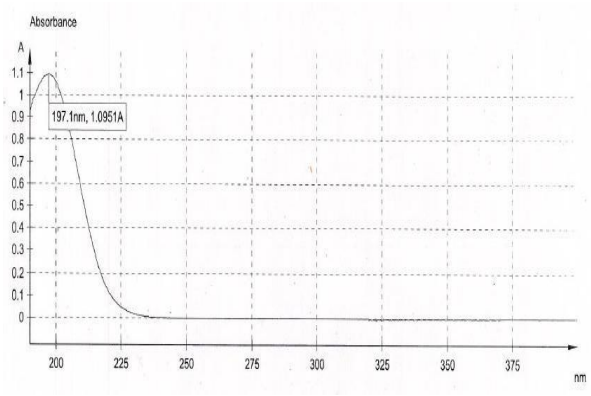


Fig 5: UV Spectra of Sitagliptin

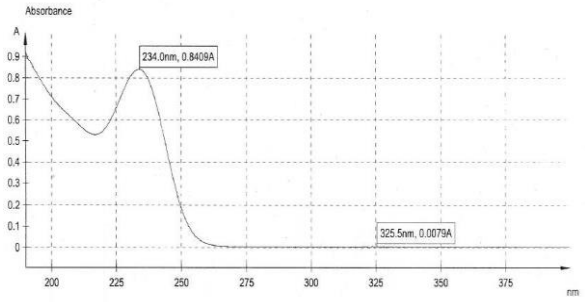


Fig 6: UV Spectra of Metformin HCl

Drug	Max Abs at wavelength 210nm
Sitagliptin	0.5860
Metformin HCl	0.5907

Final wavelength: - 210nm

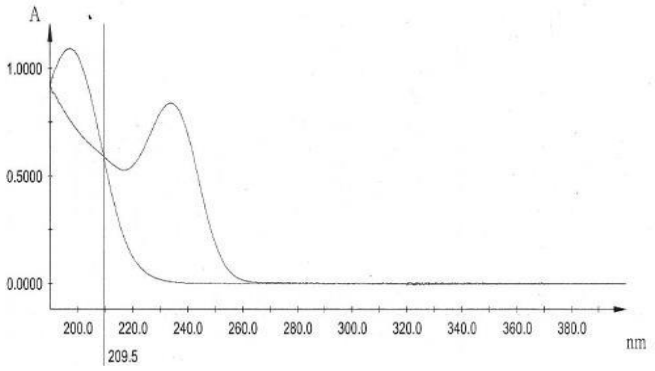


Fig7: Overlay Spectra of Sitagliptin and Metformin HCl

HPLC Assay Method Development:

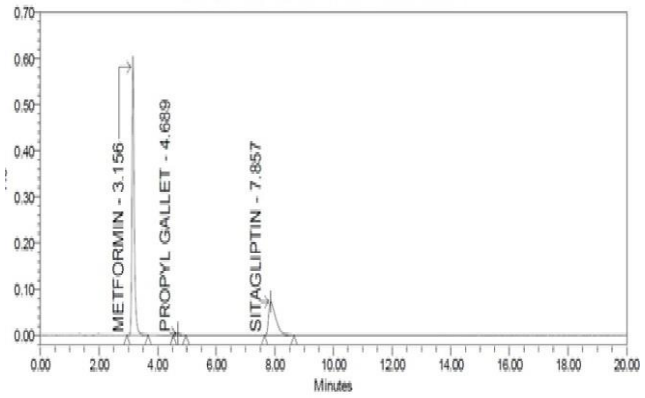


Fig 8: Chromatogram of trial-1

Chromagraphic Condition	
Column	ACE 5 C8, 250 x 4.6 mm, 5 µm
Flow Rate	1.0 mL/min
Injection Volume	10 µL
Wavelength	210nm&234nm
Column Temp	25°C
Sample Temp	5°C
Run Time	20 minutes

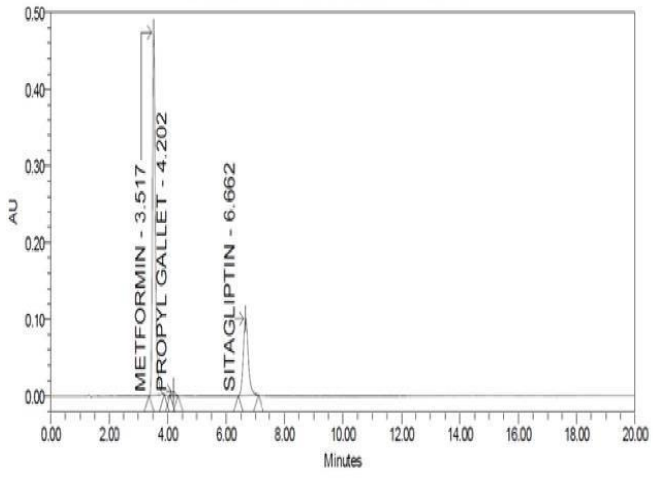


Fig9: Chromatogram of trial-2

Chromagraphic Condition	
Column	ACE 5 C8, 250 x 4.6 mm, 5 µm
Flow Rate	1.0 mL/min
Injection Volume	10 µL
Wavelength	210nm
Column Temp	40°C
Sample Temp	25°C
Run Time	20 minutes

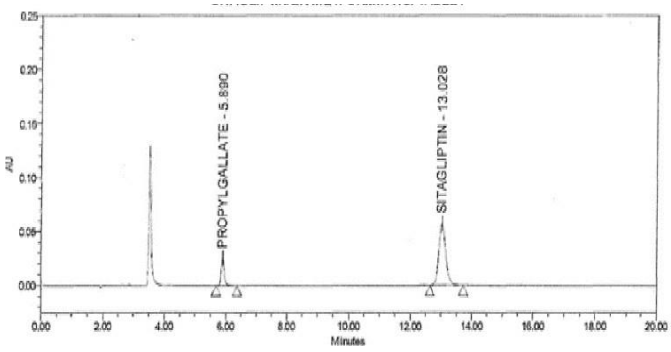


Fig10: Chromatogram of finalized trial

Chromagraphic Condition	
Column	Zorbax SB C8, 250 x 4.6 mm
Flow Rate	1.0 mL/min
Injection Volume	5 µL
Wavelength	210nm & 234nm
Column Temp	25°C
Sample Temp	5°C
Run Time	20 minutes

RESULT: Stability in Analytical solution

System suitability: System suitability test is a Pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis

to be done. Single injection of Blank (Diluent), six replicate of Standard solution was injected on the system. The data obtained is summarized in Table.

	Sitagliptin	Metformin HCl	Propylgallate
USP tailing factor	1.11	1.06	1.02
USP theoretical plates	16925	9079	14231
S. No.	**Area**		
1	896027	867346	163701
2	893571	862386	163321
3	892125	862950	163050
4	891155	861417	162836
5	883273	851442	161753
6	894447	863704	163697
Mean	**891766**	**861541**	**163060**
%RSD	**0.50**	**0.62**	**0.45**

Specificity:

The analytes should have no interference from other extraneous components and be well resolved from them.

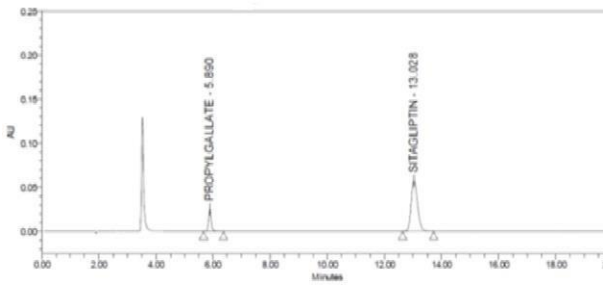


Fig11: Typical Chromatogram of Standard

Discussion:

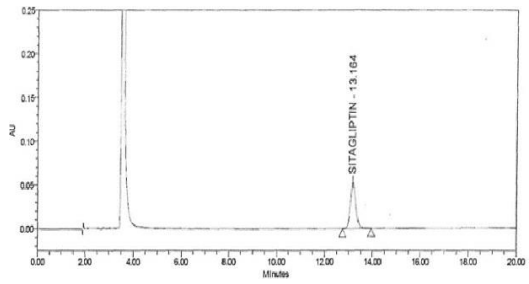
The data demonstrates that there is no interference of Blank and Placebo at the retention time of Sitagliptin, Metformin HCl and Propylgallate peak.

Sr. No.	Sample name	Observation
1	Blank	No interference
2	Placebo	No interference

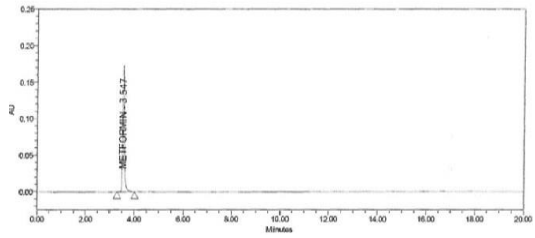
Forced degradation:

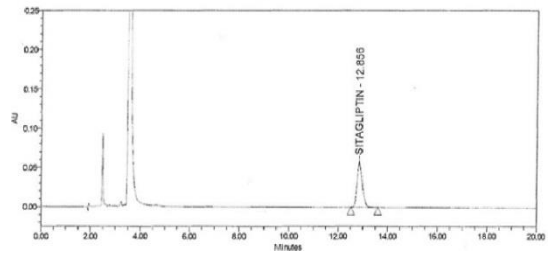
The study was performed to demonstrate the selectivity of the method in presence of degradation products. Sample and Placebo (Sitagliptin and Metformin HCl) were exposed to different stress conditions.

Acid degradation:

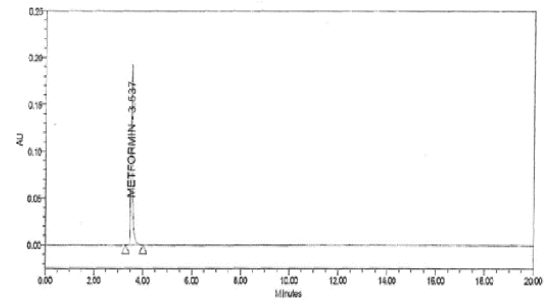


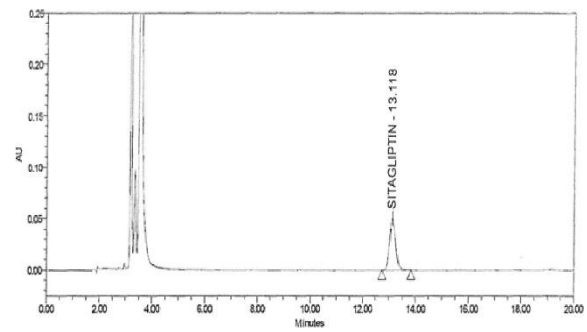
Base degradation:



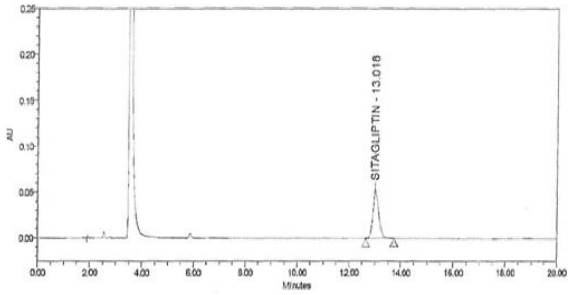


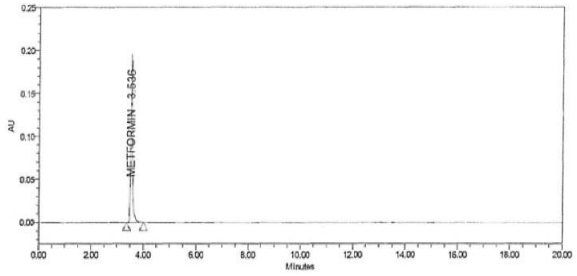
Photolytic degradation:



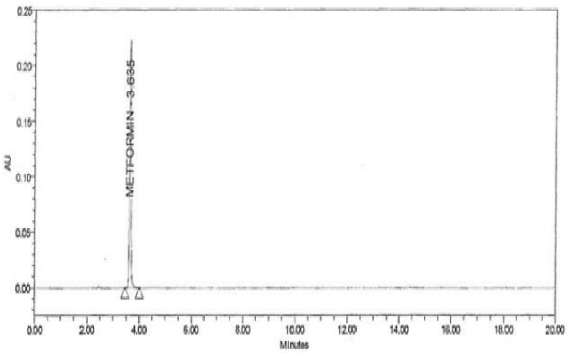


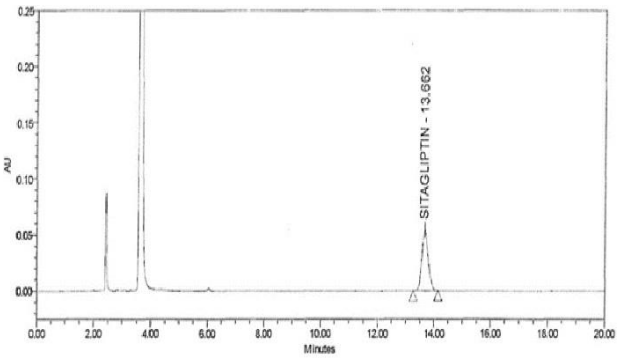
Heat degradation:



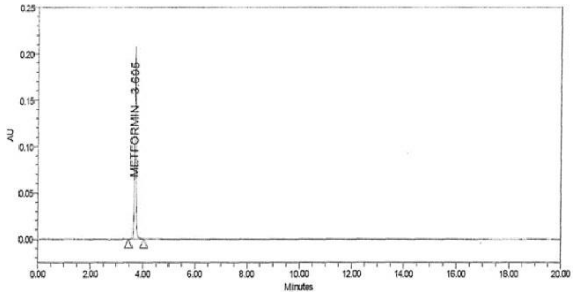


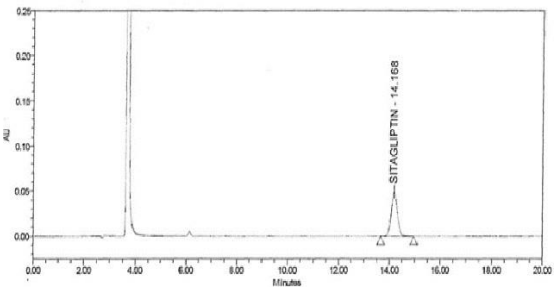
Peroxide degradation:





Humidity degradation:





Forced degradation:

Conditions	% Assay	Purity angle	Purity threshold
Sitagliptin			
Acid degradation 10 mL 5N HCl_24 hrs_RT	98.1	0.372	0.490
Base degradation 10 mL 5N NaOH_24 hrs_RT	103.1	0.289	0.457
Peroxide degradation 10 mL 15% H_2O_2_24 hrs_RT	101.2	0.369	0.608
Heat 24 hrs	98.7	0.249	0.522
Humidity	99.3	0.728	0.835
Amber flask + Aluminum Covered	98.5	0.214	0.535
Amber flask	98.0	0.373	0.458
Metformin HCl			
Acid degradation 10 mL 5N HCl_24 hrs_RT	92.8	0.054	0.253
Base degradation 10 mL 5N NaOH_12 hrs_RT	83.8	0.177	0.267
Peroxide degradation 10 mL 15% H_2O_2_24 hrs RT	97.1	0.305	0.292
Heat 24 hrs	95.9	0.055	0.256
Humidity	97.9	0.202	0.307
Amber flask + Aluminum Covered	97.2	0.083	0.251
Amber flask	97.9	0.080	0.250

Linearity and Range:

a) Sitagliptin

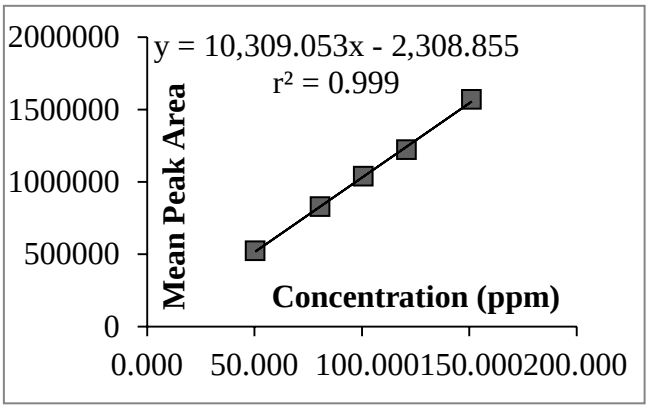
Concentration (ppm)	Response		
	1	2	Mean
50.376	518356	526740	522548
80.601	824064	831701	827883
100.752	1027641	1050279	1038960
120.902	1218287	1227707	1222997

151.128	1571418	1567273	1569346
Co-relation coefficient (R)			0.999
SLOPE			10309.053
Y-INTERCEPT			-2308.855
WORKING LEVEL AREA			1038960.0
%LIMIT OF Y-INTERCEPT			-0.22

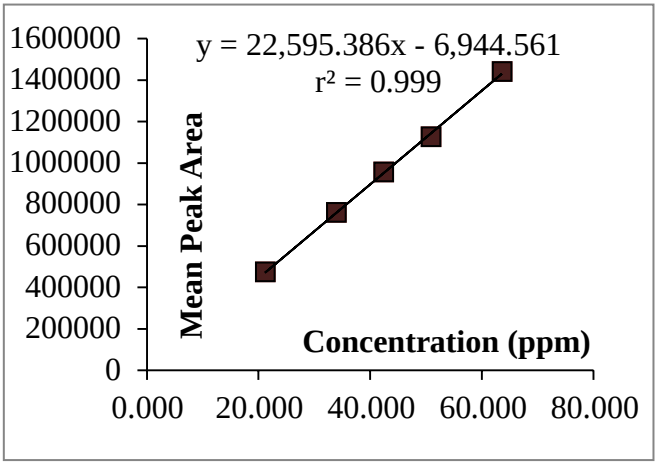
b) Metformin HCL

Concentration (ppm)	Response		
	1	2	Mean
21.210	466786	482662	474724
33.937	760075	763015	761545
42.421	936676	973922	955299
50.905	1123677	1126525	1125101
63.631	1434975	1447385	1441180
Co-relation coefficient (R)			1.000
SLOPE			22595.386
Y-INTERCEPT			-6944.561
WORKING LEVEL AREA			955399.0
%LIMIT OF Y-INTERCEPT			-0.73

Calibration Curve of Sitagliptin:



Calibration Curve of Metformin HCl:



Accuracy (Recovery): Sitagliptin

Level (%)	Mean response	% Recovery	Mean recovery %
50	440145	102.0	101.8
	440233	101.8	
	439563	101.7	
100	869242	100.7	99.2
	847339	98.2	
	852380	98.7	
150	1291749	99.4	100.1
	1301260	100.2	
	1306241	100.7	

Accuracy (Recovery): Metformin HCl

Level (%)	Mean response	% Recovery	Mean recovery %

50	443986	101.7	100.9
	444162	101.8	
	432643	99.1	
100	855322	98.0	98.3
	859149	98.4	
	860150	98.6	
150	1315305	100.5	100.1
	1297445	99.1	
	1319231	100.8	

Precision: Precision data from Analyst-1 & Analyst-2: Sitagliptin

Parameter	**% Assay**	
Sample	**Precision(Analyst-1)**	**Intermediate Precision (Analyst-2)**
1	98.4	98.4
2	99.0	99.0
3	100.3	100.3
4	100.2	100.2
5	100.0	101.0
6	100.6	100.6
Mean	**99.9**	
%RSD	**1.00**	

Precision: Precision data from Analyst-1 & Analyst-2: Metformin HCl

Parameter	**% Assay**	
Sample	**Precision(Analyst-1)**	**Intermediate Precision (Analyst-2)**
1	97.2	100.6
2	96.7	98.7
3	98.1	98.6
4	98.3	97.9

5	98.1	97.4
6	98.5	97.6
Mean	**98.1**	
%RSD	**1.0021**	

Robustness: Sitagliptin

Changes in parameters	**Values**	**Retention Time of Sitagliptin peak**	**USP tailing factor**	**USP theoretical plates**	**% RSD of standard area**	**% Assay**	**Absolute difference**
Flow rate (± 0.1 mL/min)	0.9 mL/min	14.799	1.14	17626	0.45	101.6	1.0
	1.1 mL/min	12.158	1.13	15851	0.27	101.0	0.4
Buffer pH (±0.2 unit)	pH-2.8	13.079	1.13	16664	0.27	100.6	0.0
	pH-3.2	12.830	1.14	16579	0.75	100.1	0.5
Column temperature (± 5°C)	20°C	13.662	1.15	15823	0.61	100.8	0.2
	30°C	12.964	1.13	17451	0.33	99.1	1.5
Change in Mobile phase composition (2% absolute)	-2% (69:31)	16.843	1.12	17269	0.25	99.6	1.0
Change in Mobile phase composition (2% absolute)	+2% (65:35)	10.148	1.12	15514	0.63	98.1	1.4
Change in Wavelength (±5 nm)	205 nm	13.505	1.14	18406	0.56	104.3	1.1
	215 nm	13.503	1.16	18225	0.90	105.5	0.1

Robustness: Metformin HCl

Changes in parameters	Values	Retention Time of Metformin HCl peak	USP tailing factor	USP theoretical plates	% RSD of standard area	% Assay	Absolute difference
Flow rate (± 0.1 mL/min)	0.9 mL/min	4.037	1.11	9821	0.46	97.2	0.9
	1.1 mL/min	3.314	1.08	7767	0.34	96.2	0.1
Buffer pH (±0.2 unit)	pH-2.8	3.651	1.10	8815	0.20	97.1	0.8
	pH-3.2	3.573	1.11	8659	0.67	97.8	1.5
Column temperature (± 5°C)	20°C	3.660	1.10	8502	0.57	96.0	0.3
	30°C	3.608	1.11	8982	0.30	96.2	0.1
Change in Mobile phase composition (2% absolute)	-2% (69:31)	3.931	1.09	9493	0.11	94.3	2.0
Change in Mobile phase composition (2% absolute)	+2% (65:35)	3.363	1.07	8290	1.11	93.4	1.3
Change in Wavelength (±5 nm)	229 nm	3.620	1.16	18735	0.88	96.9	0.0
	239 nm	3.621	1.17	19075	0.91	97.1	0.2

CONCLUSION:

The RP-HPLC method for determination as well as degradation study of Sitagliptin and Metformin HCl was Validated.The results of specific, precise, accurate, linear, robust, rugged were proved within the limits. The major advantage of this technique is less time consuming and also eco-friendly because of its low consumption of Organic solvents as compared to other analytical technique. Combos of Sitagliptin and Metformin in pharmaceutical market used for the treatment of type2 diabetes specially in Janumet, Glucophage as a novel technique without interference of degradants product from excipients and also helpful in monitoring drug stability. Method was validated as per ICH guidelines. Therefore, this method can be employed for the routine analysis.

CONFLICT OF INTEREST

Authors don't have any conflict of interest

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