RESEARCH ARTICLE

Stability Indicating Assay Method Development and Validation of Sitagliptin and Metformin Combination Tablets by Reverse Phase - HPLC

Gogada V. P. Rao[1], Meka Lingam[1], Bavisetti Lakshmi[2], Anantha L.Vadivelu[2], Duvvuri Suryakala[2*]

[1]*Department of Formulation, R and D, Lee Pharma Limited, Visakhapatnam, Andhra Pradesh, India.*
[2]*Department of Chemistry, GITAM Institute of Science, GITAM University, Visakhapatnam, Andhra Pradesh, India.*

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ABSTRACT

Two pharmaceutical compounds were isolated, separated, and confirmed using chromatographic techniques. The method involved utilizing an inertsil phenyl analytical the analytical column has dimensions of 250 mm x 4.0 mm and contains particles with a size of 5 µm. A solution is filled as a buffer, combined with a mobile phase containing a grid. with a pH of 4.0 made from potassium dihydrogen phosphate, orthophosphoric acid and of acetonitrile in a 70:30 ratio 0.8 mL per minute. The column temperature is maintained at 30°C as part of the setup. The detection of the two drugs is continuously monitored using this setup. A wavelength of 210 nm using a UV detector with injection volume 20 µL. Forced degradation studies are carried out using PDA detector. Peak homogeneity explained as peak purity using lab solution software.

Keywords: Stability indicating, Assay, Sitagliptin, Metformin, Reverse phase-HPLC, Tablet dosage form.

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INTRODUCTION

Sitagliptin and metformin tablets are employed type 2 diabetes for treatment. Changes a partner act as approach. Metformin is often prescribed at its maximum tolerated dose for those inadequately controlling their glycemic levels. Additionally, maintaining glycemic control also involves incorporating exercise into the regimen. Metformin helps by reducing glucose production in the liver and enhancing insulin sensitivity. Whereas sitagliptin inhibits enzyme DPP4 and increases the release of insulin from the pancreas.[1]

Sitagliptin phosphate is chemically termed as the chemical compound is described as 3-Amino-1-(3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]-pyrazin-7(8H)-yl)-4-(2,4,5-trifluorophenyl)butan-1-one.

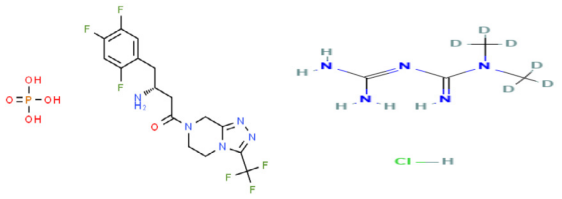
Metformin HCl is chemically termed as 3-(diaminomethylidene)-1,1-bis(trideuteriomethyl) guanidine; hydrochloride.[2]

Simultaneous evaluation of sitagliptin and metformin no pills also official in pharmacy no. that literature revealed Sita/Met either individual or combined with other drugs reported using spectrophotometric methods, HPLC methods. Only few UV, HPLC methods are reported for combination of these products. There are options with lower recovery and linearity levels. The present work designs to evaluate forced degradation studies and validate a simple performing a quantitative determination analyzing sitagliptin and metformin tablets utilizing. Reverse-phase high-performance liquid chromatography (RP-HPLC) is included in forms.[3-5] These methods are evaluated as per ICH guidelines and are specific throughout the product's shelf life. Figure 1 shows chemical structure of sitagliptin and metformin.

MATERIALS AND METHODS

Working standard of sitagliptin and metformin samples are obtained from Lee Pharma Private Limited Vizag. Sitagliptin and metformin tablets containing strengths of 50/1000 mg, 50 mg of sitagliptin and 1000 mg of metformin, respectively and manufactured by Lee Pharma Limited Vizag. Monobasic dihydrogen phosphate, Gradient grade acetonitrile, water, and orthophosphoric acid are procured from Merck, Hyderabad. Samples were analyzed with HPLC system Shimadazu 2030 series UV and PDA detector with Lab solution software. System equipped with autosampler module with degassing pumping system. Also, system equipped with column and sampler compartments to maintain the temperature as per the methodology. Mobile phase column 0.8 contains the flow rate mL per minute and consists of an inertsil phenyl phase. It has

**Author for Correspondence: duvvurisuryakala@gmail.com*



a) Sitagliptin Phosphate b) Metformin HCL

Figure 1: Chemical structure of sitagliptin and metformin

dimensions of 250 mL in length and 4.0 mL in diameter. The injection size is 5 µm. Mobile stage in a ratio of 70:30 (v/v). of acetonitrile. and it contains a phosphate buffer with a pH value of 4.0. 10.0 µL. Both the drugs are monitored at a wavelength of 210 nm. Preparation of buffer is made with we dissolved precisely 2.72 grams in 1000 mL of water potassium dihydrogen phosphate, ensuring accurate measurement. Subsequently, we diluted the resulting solution with orthophosphoric acid and filtered it. We carefully adjusted the pH to be within the range of 4.0 ± 0.05. 0.45 µ membrane filter. Mobile phase preparation is made by preparing of 70:30 v/v buffer in rate and mixture of acetonitrile and sonicated to get homogeneous solution. The diluent is prepared by mixing pH 6.8 buffer and acetonitrile in 70:30 v/v ratio. Sitagliptin standard stock solution is made by weigh and transfer 30 mg. Take a sitagliptin phosphate. The dose is 24.2 mg sitagliptin and equal to place it in a 100 mL volumetric flask. Dilute this solution to a final volume of 50 mL and use sonication to ensure complete dissolution. This prepared solution is then dosed with a dilution of the metformin HCl standard stock solution containing 25 mg of metformin. The metformin HCl standard stock solution was precisely weighed to achieve a volume of 25 mL before being transferred to a volumetric flask. It is diluted with 15 mL of diluent and sonicated further to get a homogeneous solution. Preparation of standard solution is made by transfer 2.0 mL sitagliptin fixed stock solution, 10 mL metformin HCL standard stock. Take the solution and bulk dilute. volume of 50 mL, and then transfer it to a volumetric flask. The solutions are mixed well. The sample solution is made by weighing about 5 tablets and crush them into fine powder. Weigh and transfer 1209.50 mg of tablet powder. Dissolve one tablet's equivalent weight in 250 mL of a volumetric flask, adding 180 mL of diluent. Mix thoroughly until fully dissolved. Minutes to get homogeneous solutions. Cool to room temperature. Centrifuge the solution at 3000 rpm for 10 minutes. Further, 5 mL of supernatant 100 mL of the solution volumetric transfer to flask.

RESULTS AND DISCUSSION

Method development initiated with mobile phase optimization. Since, metformin is highly polar and sitagliptin mid polar. As per the literature, mamdouh R. Rezk reported combination of sitagliptin and metformin tablets in their pure form. Several literatures suggested metformin component alone retained by using ion-pair reagent as a mobile phase buffer in USP monographs and official monographs suggest non-specific methods for the metformin in Ph.Eur.[6-9] The combination of sitagliptin and metformin tablets reported a gradient program and no isocratic methods. Present paper set to design isocratic method to get optimal RT's; hence different trials are opted and the mobile phase is proposed as monobasic in a ratio of 70:30 v/v pH 4.0 and acetonitrile containing phosphate buffer. The column was finalized as inertsil phenyl, (250 X 4.0) mm, 5 µm to get optimum resolution between sitagliptin and metformin and good response obtained with injection volume 10 µL with detection wavelength 210 nm. Finally, run time was finalized as 10 minutes in single chromatographic conditions.[10-12] Table 1 shows the system suitability of sitagliptin and metformin

Table 1: System suitability of sitagliptin and metformin

	Sitagliptin	*Metformin HCl*
No. of Injections	*Areas response for 50/1000 mg*	*Areas response for 50/1000 mg*
1	193165	8227205
2	192530	8267295
3	191629	8223132
4	192727	8270580
5	191917	8237271
Average	192394	8245097
Stdev.	619.794	22394.004
%RSD	0.3	0.3
Tailing Factor	1.177	1.380
Theoretical Plate count	8958	3668

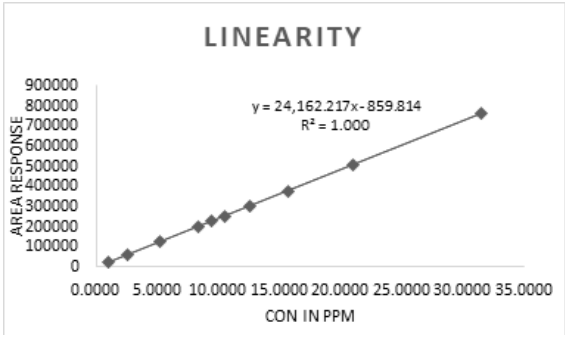


Figure 2: Linearity graph of sitagliptin

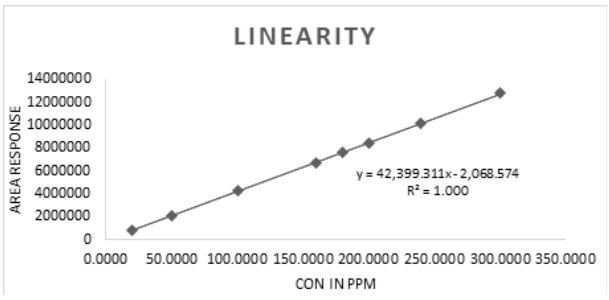


Figure 3: Linearity graph of sitagliptin

Table 2: Forced degradation studies of Sitagliptin Phosphate and Metformin HCl

Sitagliptin Phosphate forced degradation mass balance sheet

Name of the sample	*RRT*	*As such sample*	*Thermal stressed at 80°C for 72 hours*	*Humidity stressed at 72 hours*	*Photolytic stressed sample*	*As such sample*	*5N HCl Stressed at 80°C for 4 hours*	*1N NaOH Stressed at 80°C for 4 hours*	*10%H_2O_2 Stressed at 80°C for 4 hours*	*Neutral Stressed at 80°C for 5 hours*
Single Point Threshold	---	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Peak Purity Index	---	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Total Impurities	---	0.08	0.01	0.09	0.11	0.06	0.16	9.61	9.35	11.38
Assay	---	102.70	100.50	101.20	101.10	104.70	102.80	97.70	98.40	97.10
RS + Assay	---	102.78	100.51	101.29	101.21	104.76	102.96	107.31	107.75	108.48
Mass Balance	---	---	97.8	98.6	98.5	---	98.3	102.4	102.9	103.6

Metformin HCl forced degradation mass balance sheet

Name of the sample	*RRT*	*As such sample*	*Thermal stressed at 80°C for 72 hours.*	*Humidity stressed at for 72 hours.*	*Photolytic stressed control sample*	*As such sample*	*5N HCl Stressed at 80°C for 4 hours.*	*1N NaOH stressed at 80°C for 4 hours.*	*10%H_2O_2 Stressed at 80°C for 4 hours.*	*Neutral stressed at 80°C for 5 hours.*
Single Point Threshold	---	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Peak Purity Index	---	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Assay	---	102.00	100.40	100.40	99.80	97.80	97.70	99.20	99.90	99.60
RS + Assay	---	102.07	100.45	100.47	99.86	97.87	99.73	99.78	99.95	99.65
Mass Balance	---	---	98.4	98.4	97.8	---	101.9	102.0	102.1	101.8

Table 3: Comparison between method precision and intermediate precision

Sample set No.		*50/1000 mg*	
		Sitagliptin	*Metformin*
Method precision	1	98.5	100.8
	2	98.3	100.8
	3	98.1	100.6
	4	98.3	100.8
	5	96.9	99.7
	6	98.2	100.4
Intermediate precision	7	99	100.4
	8	99.2	100.7
	9	99.3	101
	10	99.5	101.1
	11	98.7	100.7
	12	98.6	100.1
Average		98.55	100.59
Stdev		0.69	0.39
%RSD 0.70		0.39	

Table 4: Statistical results of linearity levels of sitagliptin

S. No.	*Conc in ppm*	*Area response*
1	1.0519	25111
2	2.6297	62806
3	5.2593	126987
4	8.4149	201627
5	9.4668	227550
6	10.5186	254128
7	12.6224	303092
8	15.7780	379434
9	21.0373	507692
10	31.5559	762206
	Slope	24162.217
	Intercept	-859.814
	%Intercept	-0.34
	Cor. Coeff.	1.000
	Reg.Coeff	1.000

Specificity is the ability to prove that no interference from diluent, Std, placebo and impurities with the main analyte and method found specific with respective to diluent, placebo and impurities of sitagliptin and metformin. Different stress degradation conditions are established for sitagliptin and metformin tablets along with placebo to verify the method capability throughout the product's life cycle. The study is established by using PDA detector with lab solution software. From the data method, stability indicate in nature. Since in all the degradation conditions method found specific.[13,14]

Table 5: Statistical results of linearity levels of metformin

S. No.	*Conc in ppm*	*Area response*
1	20.0080	862594
2	50.0200	2111395
3	100.0399	4310042
4	160.0639	6718145
5	180.0719	7619458
6	200.0799	8468331
7	240.0959	10100370
8	300.1198	12813425
	Slope	42399.311
	Intercept	-2068.574
	%Intercept	-0.02
	Cor. Coeff.	1.000
	Reg.Coeff	1.000

Table 2 shows forced degradation studies of sitagliptin phosphate and metformin HCl.

Evaluated method precision and intermediate precision by studying the variations in assay of a homogeneous sample analyzed by several analysts various instruments on separate days. Prepared blank, standard solution and sample solutions as per described in the test procedure. Injected blank solution and six replicates sample solution. Six different model evaluations of the product calculate. Table 3 shows a comparison between method precision and intermediate precision.

The linearity of ten levels has been established from 10 to 300% of working concentration of sitagliptin (1–30 ppm) and 10 to 150% of metformin (20–300 ppm) from standard stock solutions are prepared from 0.25 mg/mL sitagliptin and 1-mg/mL metformin respectively. The linearity levels are prepared against concentration vs area response and statistical results are established for sitagliptin and metformin.[15]

Table 6: Accuracy values for sitagliptin

Set	*%Levels*	*Mean area response*	*mg added*	*mg added (Actual)*	*mg recovered*	*%recovery*	*Mean %recovery*	*%RSD*
1	25	16.11	16.11	15.5139	15.4680	99.7		
2	25	16.15	16.15	15.5525	15.5856	100.2	100.1	0.4
3	25	16.13	16.13	15.5332	15.5912	100.4		
1	50	32.17	32.17	30.9797	30.7350	99.2		
2	50	32.04	32.04	30.8545	30.8273	99.9	99.6	0.4
3	50	32.10	32.10	30.9123	30.8166	99.7		
1	100	64.47	64.47	62.0846	61.3731	98.9		
2	100	64.46	64.46	62.0750	61.6372	99.3	98.9	0.4
3	100	64.44	64.44	62.0557	61.1387	98.5		
1	150	96.47	96.47	92.9006	91.9174	98.9		
2	150	96.70	96.70	93.1221	91.8697	98.7	98.8	0.1
3	150	96.54	96.54	92.9680	91.8966	98.8		
Average of all levels					99.4			
%RSD for all levels (4x3 levels)					0.6			

Table 7: Accuracy values for metformin HCl

Set	*%Levels*	*Mean area response*	*mg added*	*mg added (Actual)*	*mg recovered*	*%recovery*	*Mean %recovery*	*%RSD*
1	25	2024829	254.26	252.4039	252.6475	100.1		
2	25	2028573	254.07	252.2153	253.1146	100.4	100.3	0.2
3	25	2031708	254.19	252.3344	253.5058	100.5		
1	50	4040650	500.86	497.2037	504.1710	101.4		
2	50	4041143	500.46	496.8066	504.2325	101.5	101.4	0.2
3	50	4028506	500.22	496.5684	502.6557	101.2		
1	100	8129087	1008.01	1000.6515	1014.3046	101.4		
2	100	8136918	1008.88	1001.5152	1015.2817	101.4	101.1	0.5
3	100	8068309	1009.49	1002.1207	1006.7210	100.5		
1	150	12711482	1575.17	1563.6713	1586.0716	101.4		
2	150	12725791	1577.17	1565.6567	1587.8570	101.4	101.4	0.0
3	150	12709040	1575.90	1564.3959	1585.7669	101.4		
Average of all levels					101.1			
%RSD for all levels (4x3 levels)					0.5			

Table 8: Robustness values for sitagliptin and metformin

Validation parameter	*Acceptance criteria*		*Results for Sitagliptin*			*Results for Metformin*		
	The System suitability parameters should pass for all the conditions		*Tailing factor NMT 2.0*	*Theoretical Plates NLT 2000*	*%RSD NMT 2.0*	*Tailing factor NMT 2.0*	*Theoretical Plates NLT 1000*	*%RSD NMT 2.0*
Robustness	Original Condition		1.150	8521	0.1	1.44	3620	0.1
	Change in mobile phase flow rate 0.8 ± 0.1 mL/min	0.7 mL/min	1.180	10144	0.1	1.42	3584	0.1
		0.9 mL/min	1.160	8754	0.1	1.45	3070	0.1
	Change in column oven temperature 30 ± 5°C (25°C and 35°C)	25°C	1.180	9013	0.1	1.43	3117	0.1
		35°C	1.170	9836	0.2	1.44	3448	0.2
	Change in mobile phase proportion ± 2% (organic ratio change)	-2%	1.20	8829	0.1	1.460	3581	0.1
		+2%	1.180	9407	0.1	1.450	3514	0.0
	Change in pH (± 0.2) in pH (± 0.2)	- 0.2	1.20	8331	0.0	1.470	3544	0.1
		+0.2	1.20	9718	0.1	1.450	3510	0.1

Tables 4 and 5shows statistical results of linearity levels of sitagliptin and metformin

The accuracy levels are prepared by adding API to the placebo of sitagliptin and metformin in the 25 to 150% of working concentrations. Figures 2 and 3 shows the linearity graph of sitagliptin. They are analyzed in triplicate preparations. The method was found accurate for sitagliptin and metformin. Table 6 shows accuracy values for sitagliptin.

Table 7 shows accuracy values for metformin HCl. In order to explain in normal use in method parameters very small and deliberate analysis under variations method again delivers buildable results. Robustness is studied for changes in flow (± 0.1), Change in temperature (± 5°C), change in pH (± 0.2) and change in mobile phase (± 2%). Table 8 shows robustness values for sitagliptin and metformin.

Evaluated the stability in different time constant in interval Solution and model and solution by infusion analytical solution (59 and 60 hours) at room temperature (25°C)

CONCLUSION

A new stability indicative evaluation method for sitagliptin created and verified metformin tablets in a single chromatographic condition with run time 10 minutes. Concentrations of the solutions are finalized as Sitagliptin and respectively for metformin 0.01 and 0.2 mg/mL. A linearity curve achieved with 0.999 correlation coefficient and recovery achieved with acceptable limits ± 2% for both the components and precision values are obtained with RSD <2. The method found specific with degradation impurities, blank and placebo solutions. Forced degradation studies are evaluated with Lab solution software and homogeneity of the method is achieved by peak purity criteria i.e., peak purity index ≥ Single point Threshold. Hence, method can be used for the regular analysis.

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