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An Efficient Method For Simultaneous Estimation Of Alogliptin And Metformin In Pharmaceutical Dosage Form By Using Rp-Hplc

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Abstract

Combining alogliptin with metformin is a common way to treat type 2 diabetes patients with high blood sugar. This study's primary goal was to establish an assay that was rapid, accurate, and precise as well as a rapid reversed-phase high performance liquid chromatographic (RP-HPLC) approach that could simultaneously estimate both metformin and alogliptin from a combination drug product. The suggested procedure involves separating the two drugs in reversed-phase mode using a Water C18 250 4.6 mm, 5 column that is kept at room temperature. Acetonitrile: Ammonium Phosphate buffer pH 3.5 (70:30 v/v) was the ideal mobile phase mixture, with a flow rate of 1.0 mL/min, and VWD detection at 214 nm. ICH guidelines were followed in the method's validation. With correlation values of 0.998 and 0.999, respectively, Alogliptin and Metformin showed linearity in the concentration ranges of 6.25-18.75 g/mL and 250-750 g/mL. With an RSD of less than 2.0%, the mean percent recovery of triplicate samples at each level for both medicines was determined to be between 98 and 100 percent. For metformin and alogliptin, the standard calibration curve was established in the concentration range of 500 g/mL and 12.5 g/mL, respectively. Metformin's percent of label claim was determined to be 100.48 and alogliptin's to be 100.11. The established HPLC method's validation is linear, accurate, precise, particular, and robust. It is a time- and cost-saving technique with several uses in quantification, routine analysis, forced degradation research, stability investigations, and other fields.

Keywords: Alogliptin, Metformin, RP-HPLC, Validation, ICH, Quality Control Analysis

INTRODUCTION

In addition to a healthy diet and exercise routine, metformin and alogliptin are used as a combination medicine to treat type 2 diabetes. The most popular biguanide antidiabetic drug is metformin, which has the chemical formula N,N-Dimethylimidodicarbonimidic diamide. These medications were created using galegine, a guanidine derivative found in Galega officinalis (Fig 1). Analytical techniques for metformin in various combinations have been developed. 1 Alogliptin is a very effective, noncovalent DPP-4 inhibitor (Fig. 2). Analytical techniques for alogliptin in mixtures have been established in various ways[2].

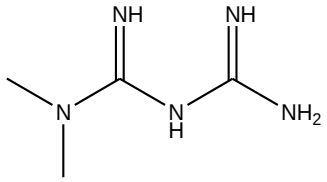

Fig 1 Chemical Structure of Metformin

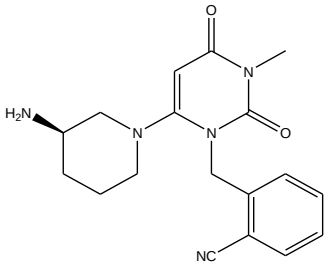

Fig 2 Chemical Structure of Alogliptin

It included a comparison of UPLC and UPLC-MS/MS techniques for locating Metformin and Alogliptin in drug combos.[3] In order to simultaneously determine the amounts of metformin hydrochloride, alogliptin benzoate, and repaglinide in tablets, Mahrouse, M. A., et al. developed an experimental approach for optimization and robustness determination in ion pair RP-HPLC method development.[4] For the simultaneous measurement of metformin and alogliptin in human plasma, Ashutosh, K. S. et al. established a novel verified stability indicating RP-HPLC approach.[5] The method for the simultaneous determination of alogliptin and metformin hydrochloride tablet dosage form by RP-HPLC was developed and validated by Kumar, A. P. et al. A number of expensive reagents and chemicals, including triethylamine, ortho-phosphoric acid, etc., were used to develop the method.[6] To determine the precise quantification of each drug component in the dosage form and to validate the developed method in accordance with ICH guidelines on various parameters such as Specificity, Accuracy, Precision, Linearity, Range, and Robustness, the current research work presents a quick, accurate, sensitive, cost-effective method for simultaneous estimation of Metformin and alogliptin from the tablet formulation.

MATERIALS AND METHODS

Chemicals and Reagents

All of the chemicals used in this procedure were HPLC and analytical grade. Alembic Pharmaceutics Limited, Vadodara, kindly provided the alogliptin and metformin API. For the assessment of a commercial formulation, Takeda Pharms USA's Kazano [alogliptin (12.5mg) and metformin (500mg)] was purchased from the market.

Instrument Parameters and Chromatographicconditions

HPLC chromatography was carried out utilising an Amkette Analytics LC-100 liquid chromatographic system, an ultraviolet (UV) detector, and a fixed injector fitted 20 l loop. A Water's C18 250 4.6 mm, 5

Kromasil column kept at room temperature was utilised for the chromatographic separation. Acetonitrile: Ammonium Phosphate buffer pH 3.5 (70:30 v/v) was the ideal mobile phase, and VWD detection was carried out at 214 nm with a flow rate of 1.0 mL/min..

Preparation of Alogliptin (125 µg/ml)and Metformin(5000 µg/ml) standard stock solution:

Weighing 12.5 mg of Alogliptin, it was then transferred to a volumetric flask with a 100 ml capacity and made up with the mobile phase. Using a volumetric flask, 500 mg of metformin was weighed, transferred, and made up with the mobile phase.

Preparation of standard solution of binary mixtures of Alogliptin(12.5 µg/ml) and Metformin (500 µg/ml)

1ml from the Alogliptin stock solution and 1ml from Metformin stock solution was transferred to 10ml volumetric flask and make up with the mobile phase.

Analysis of Pharmaceutical dosage form-Tablet by developed method

Preparation of Sample solution:

A 100 ml volumetric flask was filled with tablet powder that was weighed to equal 500 mg of metformin and 12.5 mg of alogliptin. 60 ml of mobile phase was then added to make up the volume. Pipette 1 ml of the aforementioned solution into a volumetric flask with a 10 ml capacity, then add the mobile phase. Inject above solution 20 µl for Analysis.

HPLC method validation

The optimized chromatographic method was validated by evaluating specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness. The validation of the method was performed as per ICH Guidelines.

RESULT AND DISCUSSION

HPLC method development

Selection of wavelength

Alogliptin and Metformin were showing significant absorption at 214 nm. Thus, 214 nm was selected as wavelength for analysis (Fig. 3).

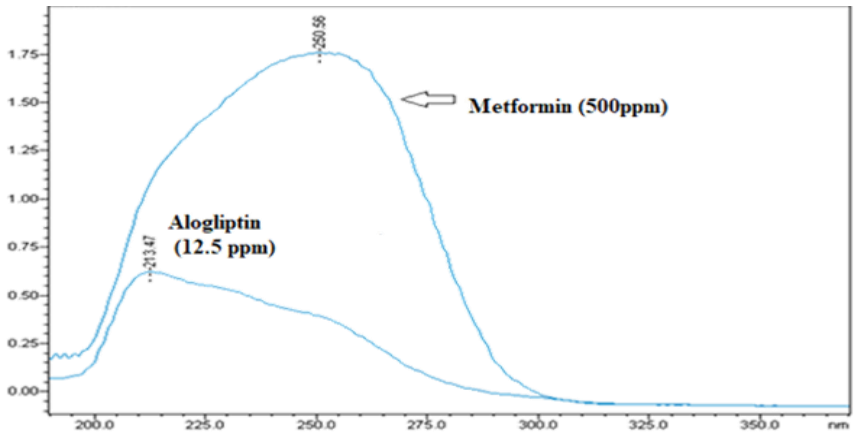

Fig 3: UV Spectra of Alogliptin (12.5 ppm) and Metformin (500 ppm) in Acetonitrile

Development of RP-HPLC Method

Selection of wavelength

The UV detection wavelength utilised in an HPLC method determines the sensitivity of the method. The ideal wavelength is the one that results in the best response when drugs are being detected. The specified wavelength is used. AGP and MET had significant absorption at 214 nm. As a consequence, 214 nm was selected as the analysis's wavelength. After that, overlay spectra were obtained by scanning these drug solutions in the UV between 200 and 400 nm.

Selection of Mobile Phase

The trial included multiple mobile phases that included Methanol, Water, and Acetonitrile in varied proportions and volumes at varying flow rates. The mixture of Buffer(Acetate) at pH 5.5:Acetonitrile (30:70) at 1.0 mL/min flow rate was shown to be superior to the other mixture in terms of peak shape, theoretical plate, and asymmetry in several experiments..

Optimization of flow rate

1.0 ml/min flow rate, proved to be better than the other in terms of resolution, peak shape and shorter retention time.

Trials are summarizing in following table:

Table No. 1 List of Mobile Phase trials

Sr No	Mobile Phase	Remark
1	Water: Methanol (40:60)	One peak observed
2	Water: Methanol (40:60)	Peak of MET Confirmed
3	Water: Methanol (40:60)	Peak of AGP did not merge with the peak of MET
4	Water: Methanol (60:40)	Retention time reduced

5	Water: Methanol (20:80)	Retention time reduced but second peak did not find
6	Methanol (100)	Still second peak did not find
7	Water: Acetonitrile (50:50)	No second peak observed
8	Water: Acetonitrile (30:70)	Still second peak did not observe
9	Water: Methanol: Acetic acid (30:70:0.1)	Second peak observed even by using an Acetic acid
10	Water: Acetonitrile: Acetic acid (30:70:0.1)	Second peak observed even by using an Acetic acid with Acetonitrile
11	Water (pH 4.0): Methanol (50:50)	Second peak observed by reducing the pH of Water
12	Water (pH 4.0): Methanol (50:50)	Peak of AGP Confirmed
13	Water (pH 4.0): Methanol: TEA (50:50:0.1)	Peak shape of AGP did not become sharp by using TEA
14	Buffer (pH 4.0): Methanol (50:50)	Peak shape became good but peak of MET Found at solvent peak time
15	Buffer (pH 4.5): Methanol (50:50)	Run time Increased but Both peaks follow SST Parameters
16	Buffer (pH 4.5): Methanol (30:70)	Run time Increased
17	Buffer (Acetate) at pH 5.5: Acetonitrile (30:70)	Retention time reduced and Both peaks resolved

Trial 1:

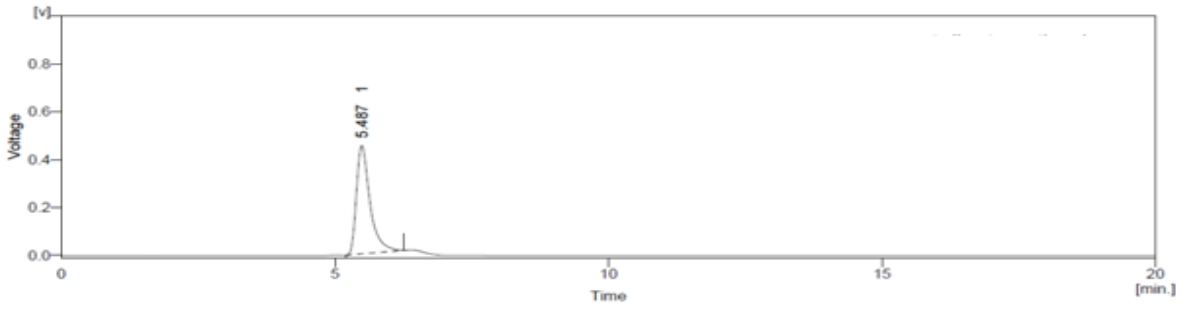

Figure 4 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Water: Methanol (40:60)

Trial 2:

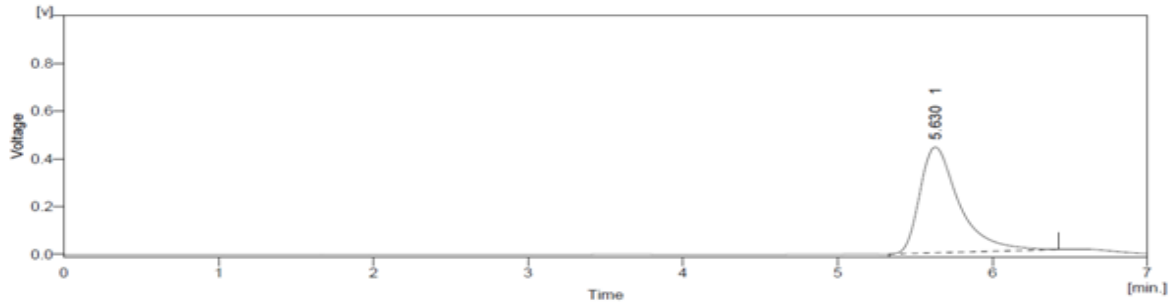

Figure: 5 HPLC Chromatogram of MET 500ppm in Water: Methanol (40:60)

Trial 3:

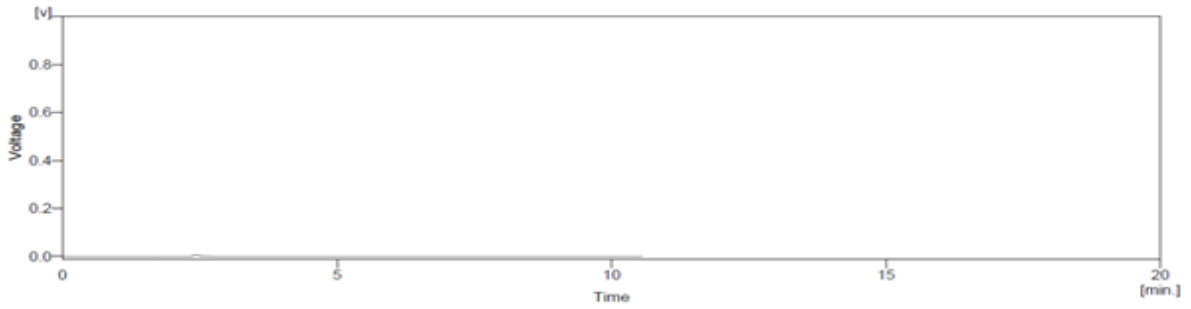

Figure: 6 HPLC Chromatogram of AGP 12.5ppm in Water: Methanol (40:60)

Trial 4:

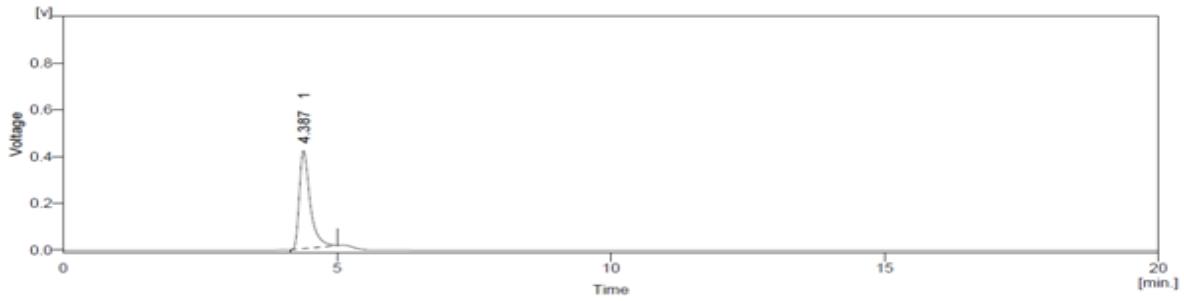

Figure 7 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Water: Methanol (60:40)

Trial 5:

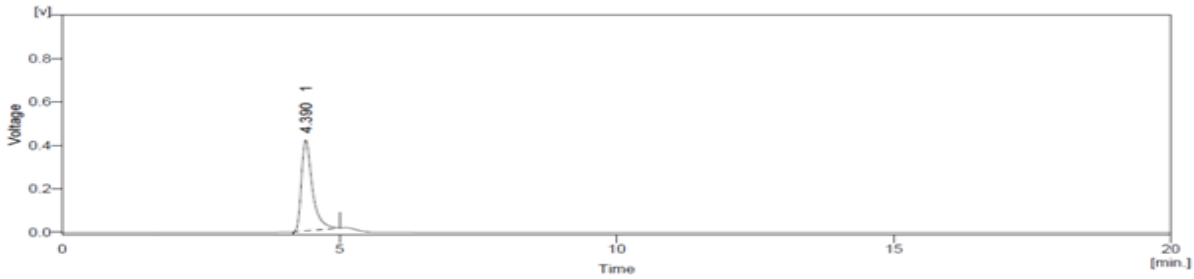

Figure: 8 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Water: Methanol (20:80)

Trial 6:

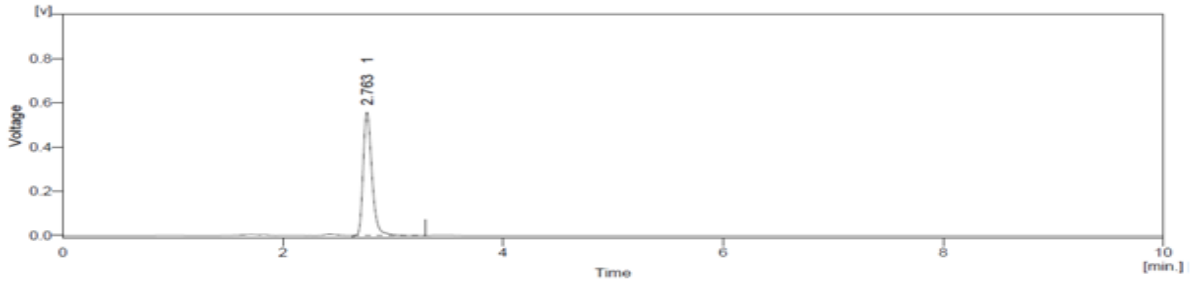

Figure: 9 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Methanol (100)

Trial 7:

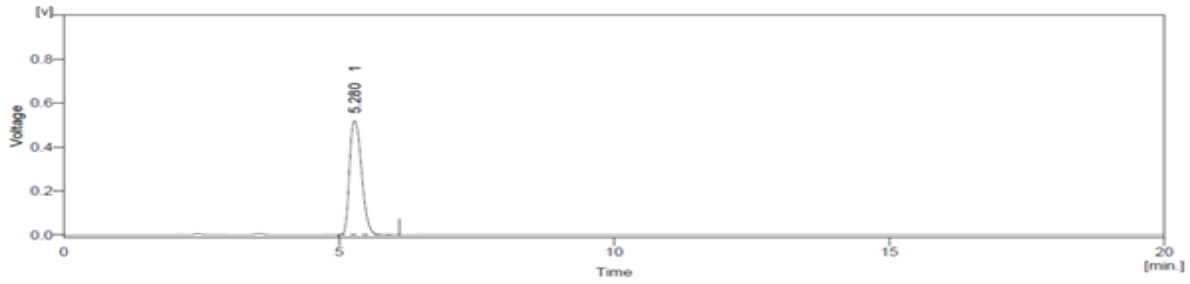

Figure: 10 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Water: Acetonitrile (50:50)

Trial 8:

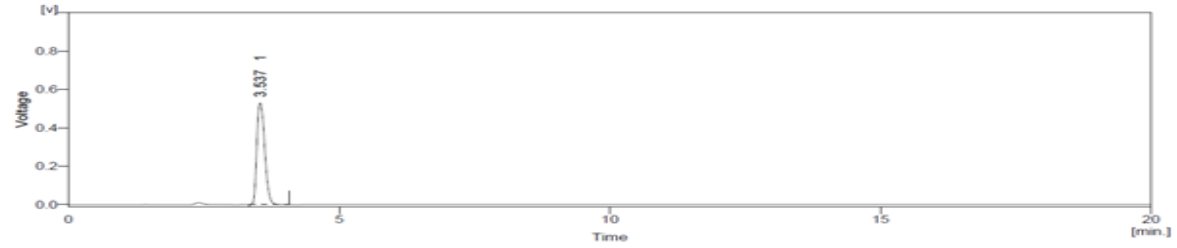

Figure: 11 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Water: Acetonitrile (30:70)

Trial 9:

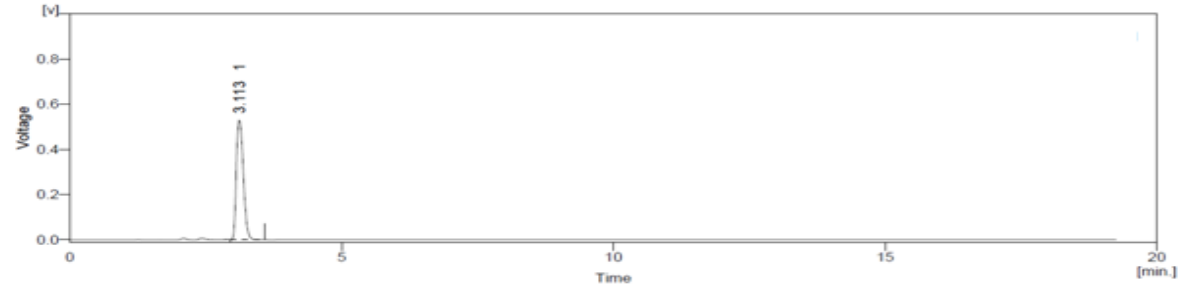

Figure: 12 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Water: Methanol: Acetic acid (30:70:0.1)

Trial 10:

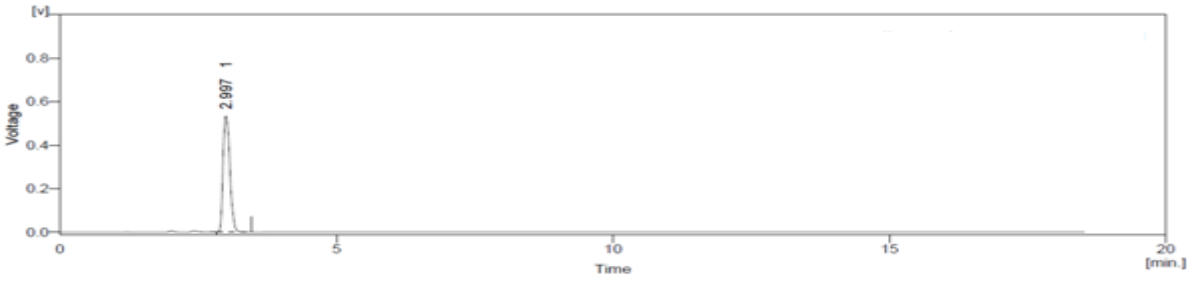

Figure: 13 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Water: Acetonitrile: Acetic acid (30:70:0.1)

Trial 11:

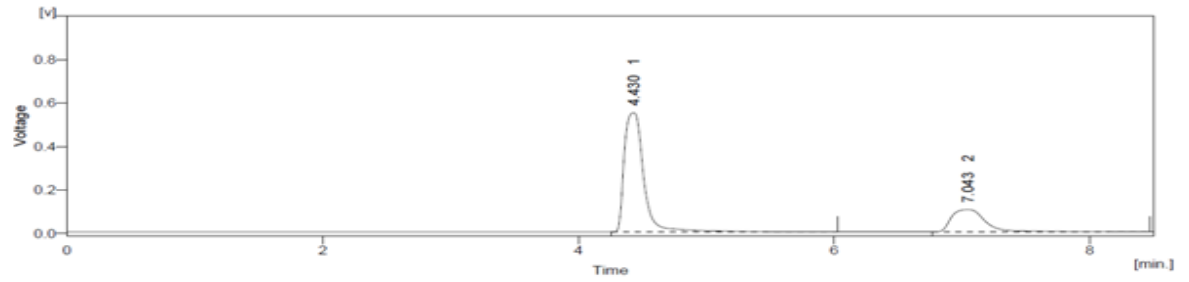

Figure: 14 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Water (pH 4.0): Methanol (50:50)

Trial 12:

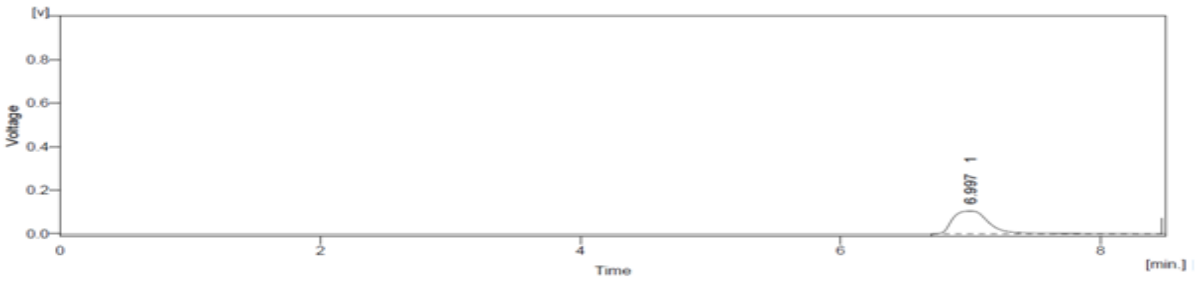

Figure: 15 HPLC Chromatogram of AGP 12.5ppm in Water (pH 4.0): Methanol (50:50)

Trial 13:

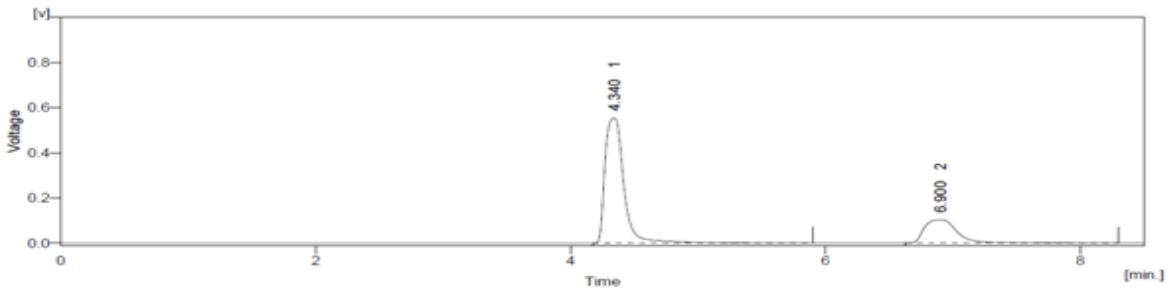

Figure: 16 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Water (pH 4.0): Methanol: TEA (50:50:0.1)

Trial 14:

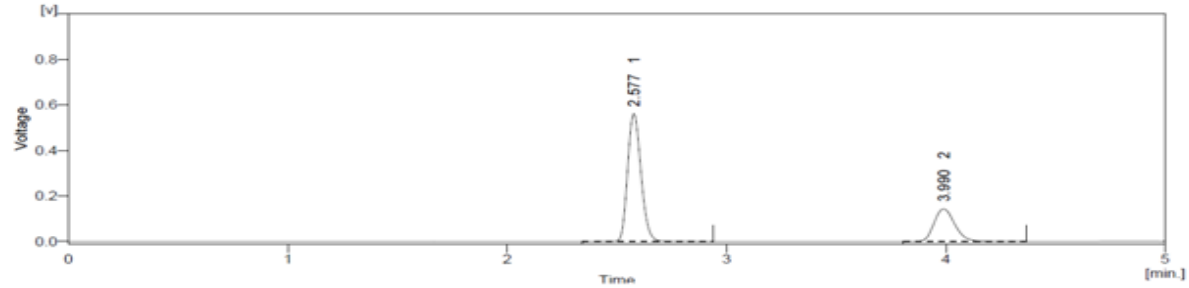

Figure: 17 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Buffer (pH 4.0): Methanol (50:50)

. Trial 15:

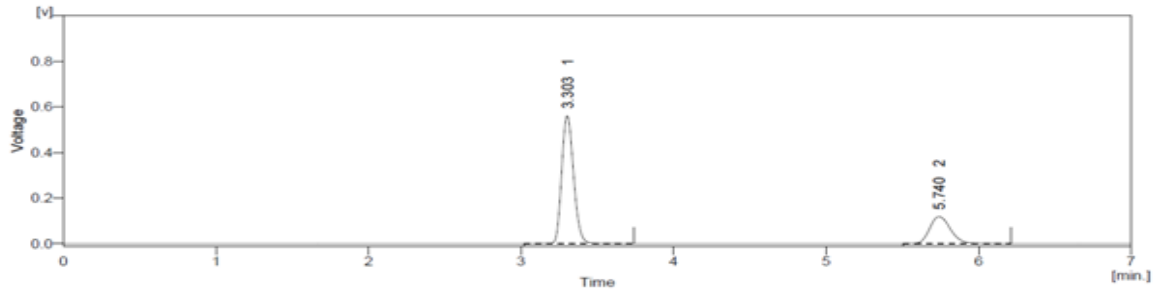

Figure: 18 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Buffer (pH 4.5): Methanol (50:50) (Final)

Trial 16:

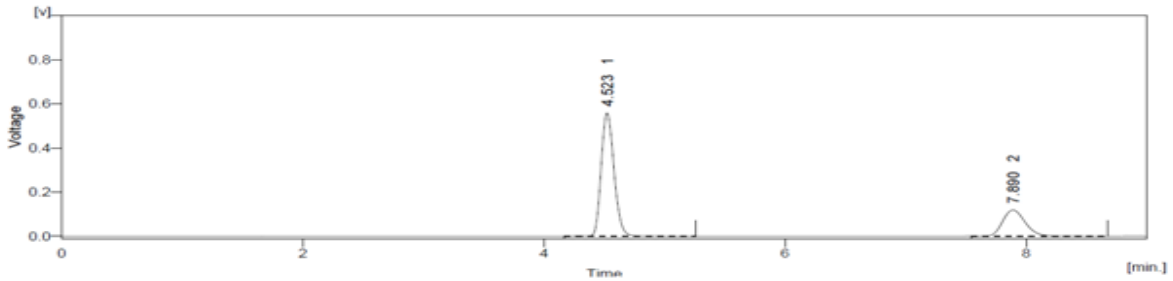

Figure: 19 HPLC Chromatogram of AGP 12.5ppm and MET 500ppm in Buffer (pH 4.5): Methanol (30:70)

Optimized chromatographic conditions

The optimized mobile phase consists of Buffer (Ammonium Phosphate, pH 3.5): Acetonitrile in a ratio of 30:70 v/v at a flow rate of 1 ml/min. The retention time of metformin and alogliptin was 3.3 min and 5.7 min, respectively (Fig. 4).

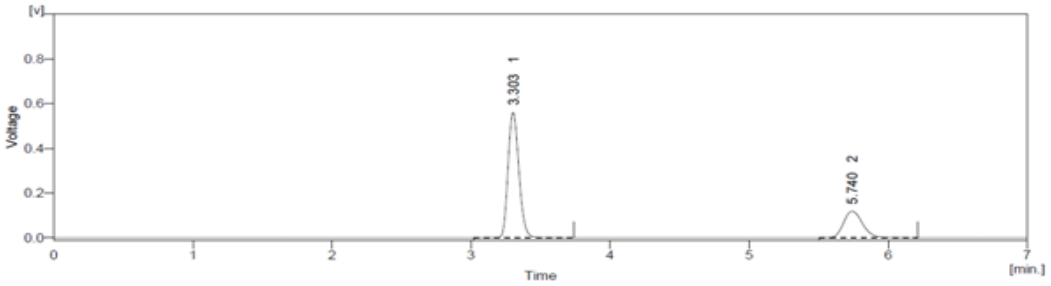

Fig 20 HPLC Chromatogram of Alogliptin 12.5ppm and Metformin 500 ppm in Buffer (pH 3.5): Acetonitrile (30:70)

HPLC method validation

Specificity

The chromatograms of Metformin and Alogliptin show no interference with the chromatogram of Metformin and Alogliptin blank, so the developed method is found to be Specific (Fig. 5, 6, 7).

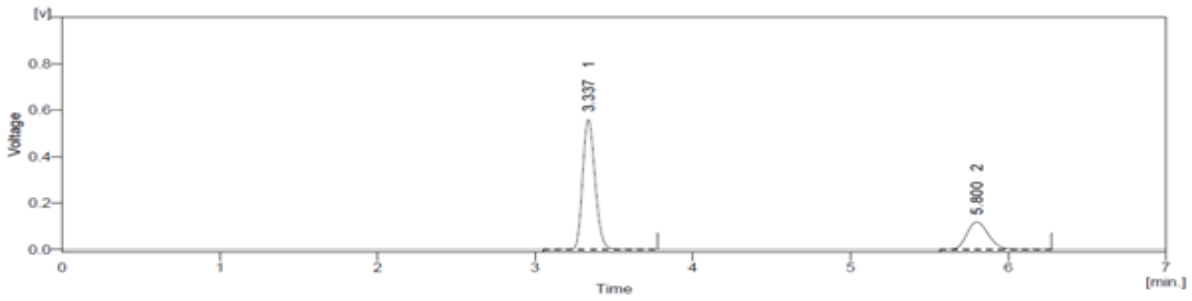

Fig. 21 Chromatogram of Metformin and Alogliptin standard

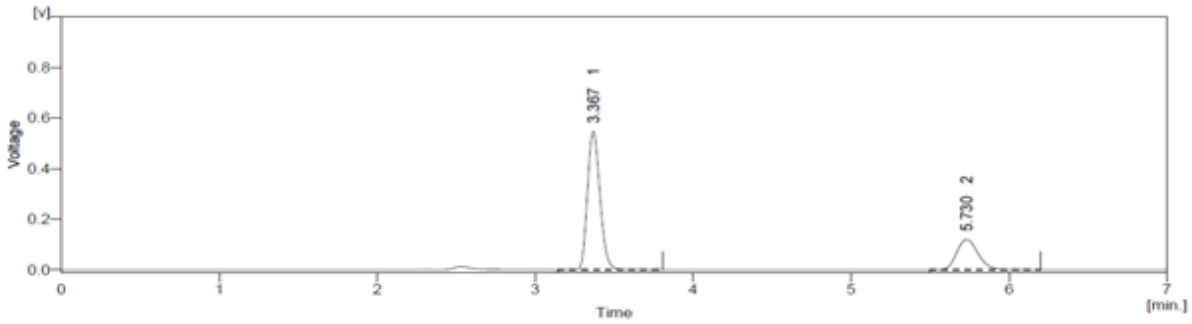

Fig. 22 Chromatogram of Metformin and Alogliptin sample

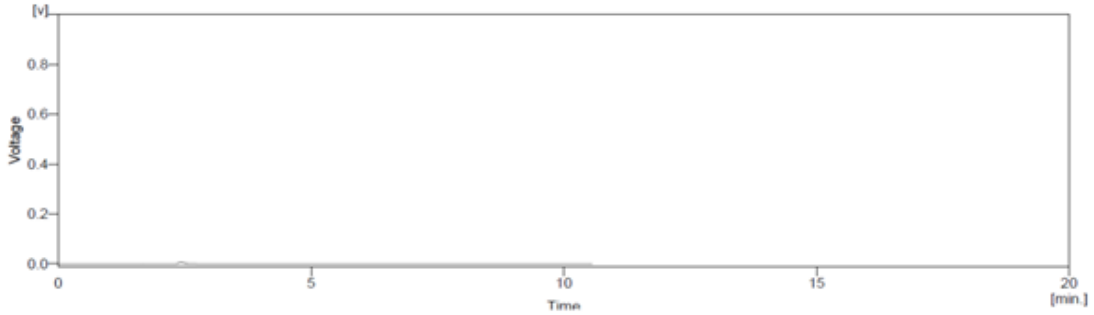

Fig. 23 Chromatogram of Blank

Linearity

Alogliptin and Metformin Hydrochloride Standard Stock Solution was transferred in the proper volume to a volumetric flask with a 10 ml capacity. A solution comprising 6.25-18.75 g/ml of Alogliptin and 250-750 g/ml of metformin hydrochloride was produced by adjusting the volume with mobile phase. Alogliptin and metformin were able to attain correlation coefficients of 0.998 and 0.999, respectively.

Table 2 Linearity data for Metformin

Concentration(µg/ml)	Area
250	1047.84

375	2031.29
500	2994.2
625	3845.59
750	4759.24

Table 3 Linearity data for Alogliptin

Concentration(µg/ml)	Area
6.25	563.184
9.375	815.254
12.5	1094.28
15.625	1354.12
18.75	1571.05

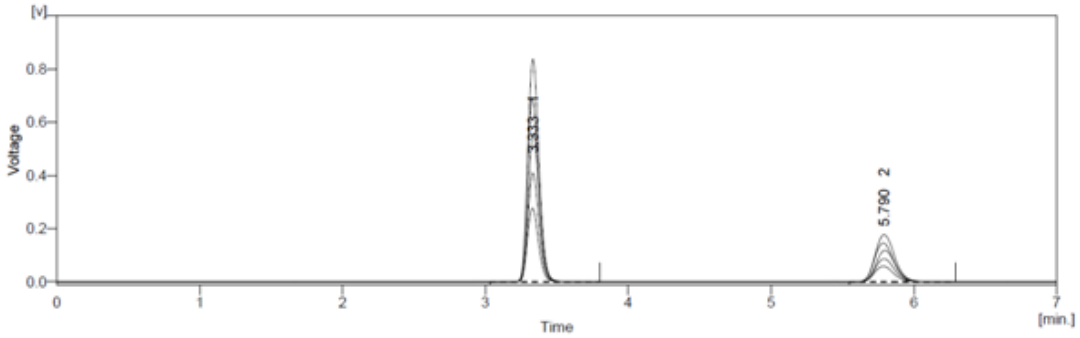

Fig. 24 Overlay chromatogram of Alogliptin and Metformin

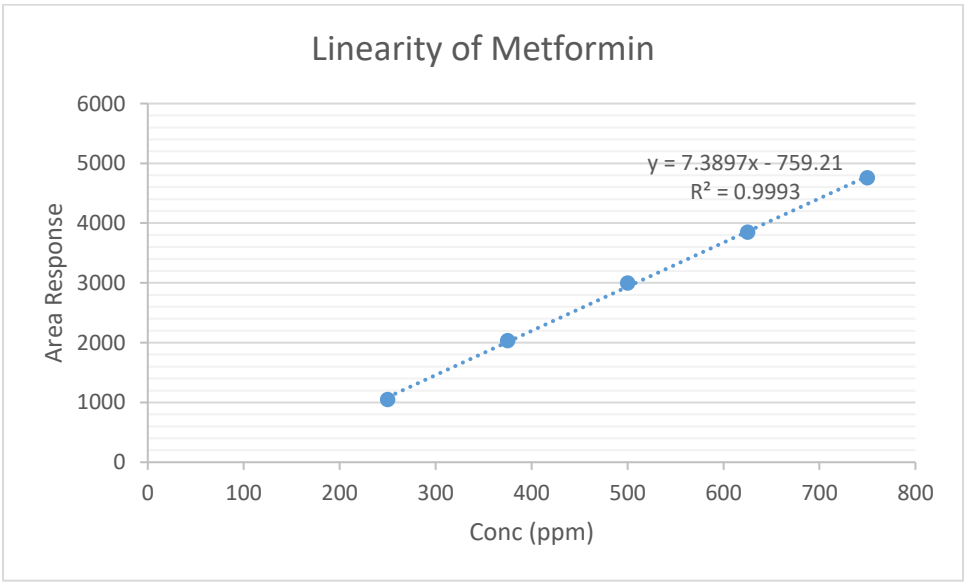

Fig. 25 Calibration Curve of Metformin (250-750 µg/ml).

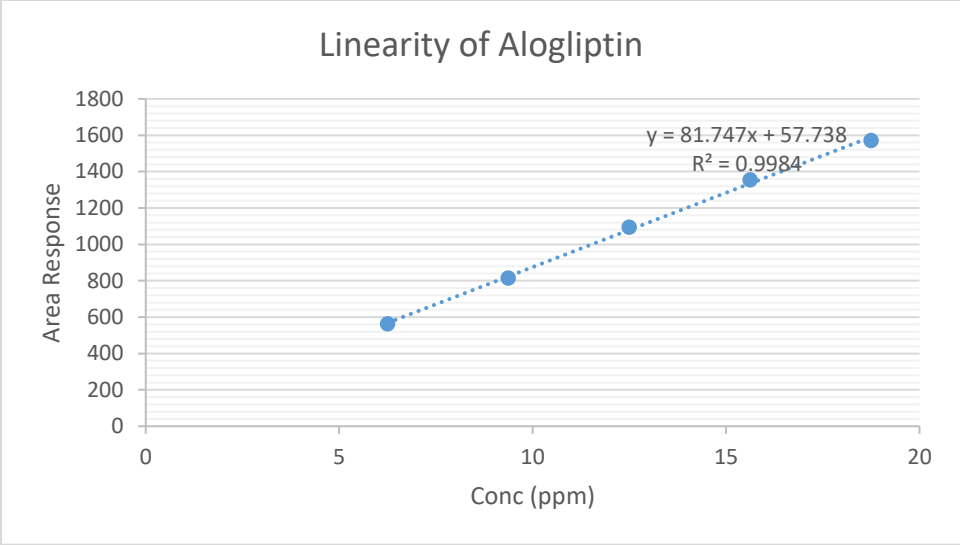

Fig. 26 Calibration Curve of Alogliptin (6.25-18.75 µg/ml)

Precision

Method Precision

The data for Method Precision of peak area measurement for Alogliptin (12.5 µg/ml) and Metformin (500 µg/ml) based on six measurements of the same solution of Alogliptin (12.5 µg/ml) and Metformin (500 µg/ml).The % RSD for Alogliptin and Metformin was found to be 0.661 and 0.216 respectively.

Table 4 Method Precision data for Metformin

Conc. (µg/ml)	Area	Mean	% R.S.D
500	2932.991	3041.133	0.21
	2939.122		
	2945.179		
	2951.236		
	2936.104		
	2942.167		

Table 5 Method Precision data for Alogliptin

Conc. (µg/ml)	Area	Mean	% R.S.D
12.5	1011.843	1111.932	0.66
	1014.118		
	1016.314		
	1018.600		

	1012.957
	997.758

Intraday and Interday precision

Intraday precision was determined through standard solution containing (250,500,750 µg/ml) of Metformin and (6.25, 12.5, 18.75 µg/ml) of Alogliptin were analyzed three times on the same day and % R.S.D was calculated. Interday precision was determined through standard solution containing (250,500,750 µg/ml) of Metformin and (6.25, 12.5, 18.75 µg/ml) of Alogliptin were analyzed three times on different day and % R.S.D was calculated.

Table 6 Intraday precision data for estimation of Metformin

	Metformin	
Conc. (µg/ml)	**Mean Area**	**% R.S.D**
250	1399.381	0.10
500	2931.032	0.25
750	4441.086	0.25

Table 7 Intraday precision data for estimation of Alogliptin

	Alogliptin	
Conc. (µg/ml)	**Mean Area**	**% R.S.D**
6.25	450.266	0.25
12.5	1004.907	1.03
18.75	1564.914	0.25

Table 8 Interday precision data for estimation of Metformin

Conc. (µg/ml)	**Mean Area**	**% R.S.D**
250	1392.580	0.59
500	2918.676	0.30
750	4423.957	0.35

Table 9 Interday precision data for estimation of Alogliptin

Conc. (µg/ml)	Mean Area	% R.S.D
6.25	442.656	1.32
12.5	999.855	0.96
18.75	1553.479	0.54

Accuracy

Sample solution was taken in three different flask label A, B and C with different concentration at 80%, 100%, and 120% of standard solution spiked in it and diluted up to 10ml. The area of each solution peak was measured at 214nm. The amount of Metformin and Alogliptin was calculated at each level and % recoveries were computed.

Table 10 Recovery data for Metformin

Conc. Level (%)	Sample amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery
80 %	250	200	200.341	100.363	100.230
	250	200	199.156	99.804	
	250	200	201.164	100.524	
100 %	250	250	251.456	100.700	99.917
	250	250	250.468	100.114	
	250	250	247.349	98.938	
120 %	250	300	301.957	100.539	99.758
	250	300	297.9278	99.276	
	250	300	298.648	99.458	

Table 11 Recovery data for Alogliptin

Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery
80 %	6.25	5	5.098	100.518	100.436

	6.25	5	4.988	99.928	
	6.25	5	5.047	100.862	
100 %	6.25	6.25	6.207	100.857	99.478
	6.25	6.25	6.154	98.729	
	6.25	6.25	6.214	99.848	
120 %	6.25	7.5	7.552	100.687	99.532
	6.25	7.5	7.421	99.448	
	6.25	7.5	7.451	99.463	

LOD and LOQ

LOD and LOQ were evaluated by injecting the dilution of standard solution and the standard deviation (SD) of the intercepts was calculated.

Table 12 LOD data for Metformin and Alogliptin

Metformin	Alogliptin
LOD = 22.648µg/ml	LOD = 1.456µg/ml

Table 13 LOQ data for Metformin and Alogliptin

Metformin	Alogliptin
LOQ = 64.486 µg/ml	LOQ = 3.264 µg/ml

Robustness

Robustness was examined by changing one by one and their effect was evaluated on standard preparation.

1. pH of Mobile phase was changed (± 0.2) 4.7 and 4.3.
2. Ratio of Mobile phase was changed (±2) Buffer: Acetonitrile (28:72) and Buffer: Acetonitrile (32:68)

Table 14 Robustness data for Metformin.

SR NO.	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	2909.046	3001.549	2902.809	3031.082
2	2927.293	3013.897	2921.026	3068.915
3	2945.681	3026.184	2942.531	3090.220
% R.S.D	0.586	0.423	0.637	1.011

Table 15 Robustness data for Aloglipin.

SR NO.	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	1019.828	963.749	1024.548	981.788
2	1046.503	968.276	1044.180	988.430
3	1053.248	972.817	1052.094	996.207
% R.S.D	1.550	0.424	1.244	0.663

Analysis of Marketed Formulation by developed method

For the treatment of Type 2 Diabetes mellitus, combination pharmaceutical dose forms of Metformin and Alogliptin are available. Prior to now, a number of HPLC analytical techniques for Metformin and Alogliptin were published, either alone or in combination with other medications. An HPLC investigation on the combination of metformin and alogliptin has been published. But for the simultaneous estimate of Metformin and Alogliptin for combination pharmaceutical dosage form, it is necessary to design and validate a straightforward and accurate HPLC analytical approach. The findings of the estimate were comparable to the labelled value of each medication in the combination dose form. These findings demonstrate the effectiveness of the devised technique for routine quality control of the dosage form in enterprises. It is accurate, precise, quick, and convenient.

rapid

Table 16 Analysis on marketed formulation

Tablet	Kazano	
Label claim	**Metformin (500 mg)**	**Alogliptin (12.5 mg)**
Assay	100.487	100.154

CONCLUSION

According to ICH recommendations for Simultaneous Estimate of Metformin and Alogliptin in Their Combined Dosage Form, a straightforward, speedy, accurate, sensitive, and cost-effective approach for simultaneous estimation and precise RP-HPLC method has been designed and validated. The new RP-HPLC technique is found to be linear, accurate, exact, specific, and robust by validation. It can be effectively obtained for regular quality control examination of combined dose forms of metformin and alogliptin. This technique may now be used to the normal laboratory testing of the combination dose forms of the drugs metformin and alogliptin.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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