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STABILITY INDICATING CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HCl & ANAGLIPTIN IN ITS SYNTHETIC MIXTURE BY HPLC

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ABSTRACT

A simple, rapid and accurate stability indicating RP-HPLC method was developed for the simultaneous estimation of Metformin HCl & Anagliptin in its synthetic mixture. The method showed a linear response for concentration of range of 5-15µg/ml using Potassium Phosphate Buffer(PH 7): Acetonitrile solution in the ratio of (35:65) as the mobile phase with detection at 240 nm and flow rate 1ml/min and the retention time for Metformin HCl and Anagliptin was found to be 4.227 and 5.893 respectively. The method was validated for the Specificity, Precision, Robustness, Accuracy, LOD, LOQ, Assay. The drug undergoes degradation under Acidic, Basic, Oxidation, Photolytic and Thermal degradation. All the peaks of degraded product were resolved from active ingredient with significantly different retention time. This method can be employed as a stability indicating one.

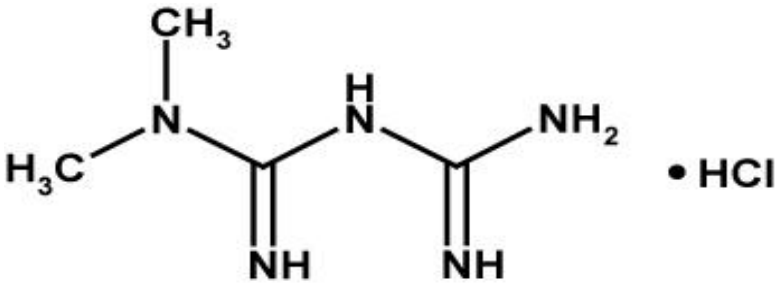
KEYWORDS: Metformin HCl, Anagliptin, RP-HPLC, Degradation Studies.

INTRODUCTION

Metformin HCl

Metformin hydrochloride acts as anti diabetic drug belongs to the class of Biguanide. The drug is effectively used in the treatment of type 2 diabetes. Molecular formula for Metformin HCl is C4H11N5.HCl and its molecular weight is 165.63gm/mol. The mechanism of action of metformin hydrochloride is suppressing glucose production from liver and to lesser extent increase tissue sensitivity to insulin, increase glucose uptake, preventing lipid biosynthesis,

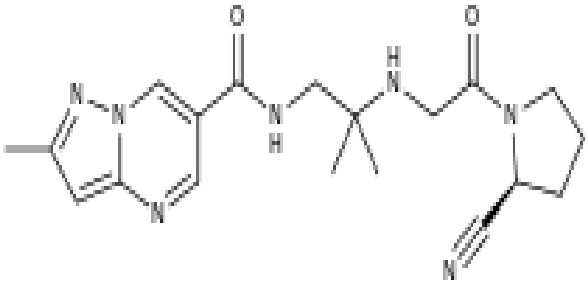
and promoting fatty acid oxidation. The common side effect of intacking drugs are muscle pain or weakness, trouble breathing, headache, swelling or rapid weight gain.



Metformin Hydrochloride

Anagliptin

Anagliptin belongs to a class of DPP – IV inhibitor which also called as a gliptin which prevent degradation of incretin. Molecular formula for Anagliptin is C19H25N7O2. Mechanism of anagliptin is that significantly inhibition of the plasma DPP-IV activity and increase the plasma active GLP-1 levels. Anagliptin competitively inhibit the DPP-IV. This enzyme breakdown the incretins GLP-1, gastrointestinal hormones released in response to a meal. By preventing GLP-1 inactivation, they are able to to increase secretion of insulin and suppress the release of glucagon by the alpha cells of pancreas. This leads to blood glucose level to normal.



Anagliptin

EXPERIMENTAL

MATERIAL AND METHODS

Metformin HCl and Anagliptin were received from oasis laboratory. Methanol, Potassium dihydrogen phosphate and HPLC grade water received from Merck Specialities Pvt. Ltd. Mumbai.

INSTRUMENTATION

The analysis of the drug was carried out on a Shimadzu SPD – 20 A detector. The HPLC column C18(25cm*0.46cm) Hypersil BDS were used for separation purpose. Systronic 119 UV Visible Spectrophotometer is used.

Chromatographic conditions

Mobile phase consists mixture of Potassium Phosphate Buffer(ph7) - Acetonitrile in the ratio of (35:65)%v/v. The mobile phase was pumped from the solvent reservoir to the column at a flowrate 1ml/min. Column temperature was maintained at a ambient. UV detection performed at 240 nm. Injection volume is 20µL and run time is 8 minutes.

Selection of detection wavelength

UV detector was selected, as it is reliable and easy to set at constant wavelength. A fix concentration of analyte were analysed at different wavelength. As per the response of analyte 240 nm wavelength was selected.

Preparation of standard solutions

(A) Metformin HCl standard stock solution: (100 µg/mL).

A 10 mg of Metformin HCl was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with mobile phase.

(B) Anagliptin standard stock solution: (100 µg/mL).

A 10 mg of Anagliptin was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with mobile phase.

(C) Preparation of standard solution of binary mixtures of Metformin HCl (10 µg/mL) and Anagliptin(10 µg/mL).

Take 1 mL from the Metformin HCl stock solution and 1mL from Anagliptin stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

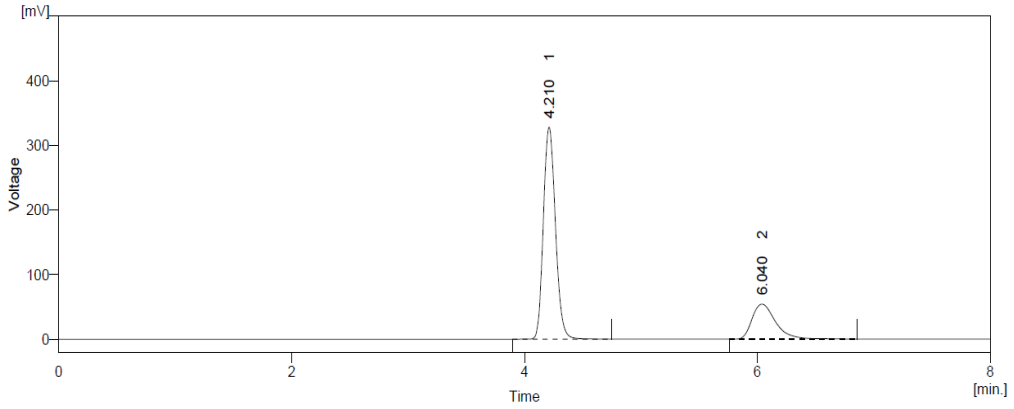


Figure 1: Simple chromatogram of standard Metformin HCl and Anagliptin.

Preparation of calibration graph

The linearity response for metformin hydrochloride and anagliptin assay method were determined by preparing and injecting solutions with concentration of 5,7.5,10,12.5 and 15µg/ml of metformin hydrochloride and anagliptin both. Linearity curves are shown in figure 2 and figure 3.

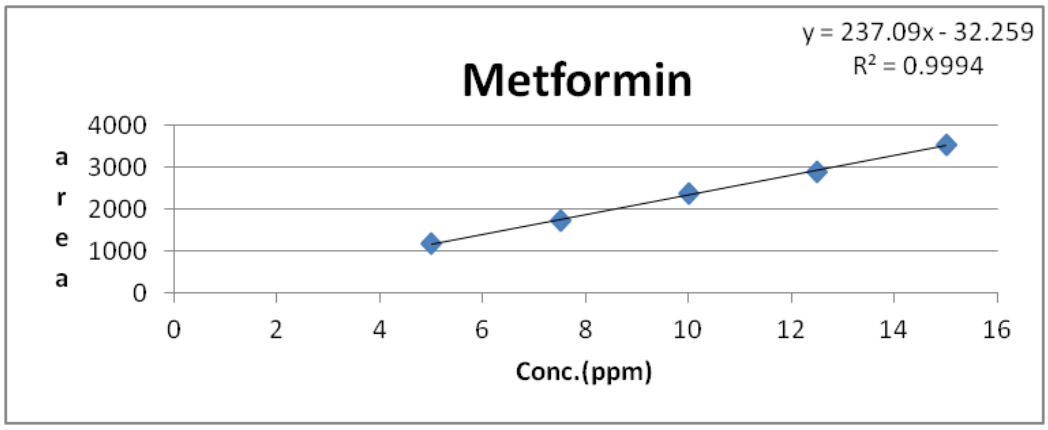


Figure 2 Linearity curve for Metformin HCl.

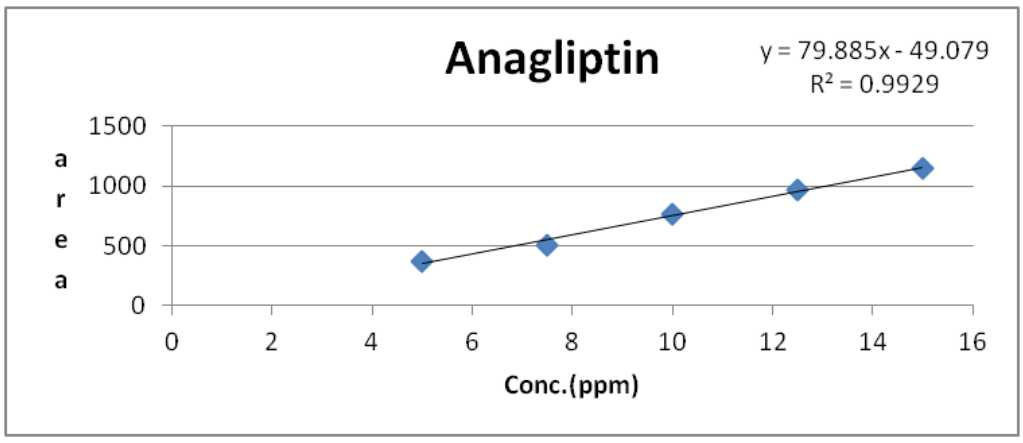


Figure 3 Linearity curve for Anagliptin.

Sample solution preparation

Take tablet powder equivalent to 10 mg of Metformin HCl and 10 mg of Anagliptin was transferred to a 100 ml volumetric flask, add 60 ml of mobile phase and shake for 15 minutes and make up the mobile volume with mobile phase . The solution filtered through whatman filter paper no. 42.

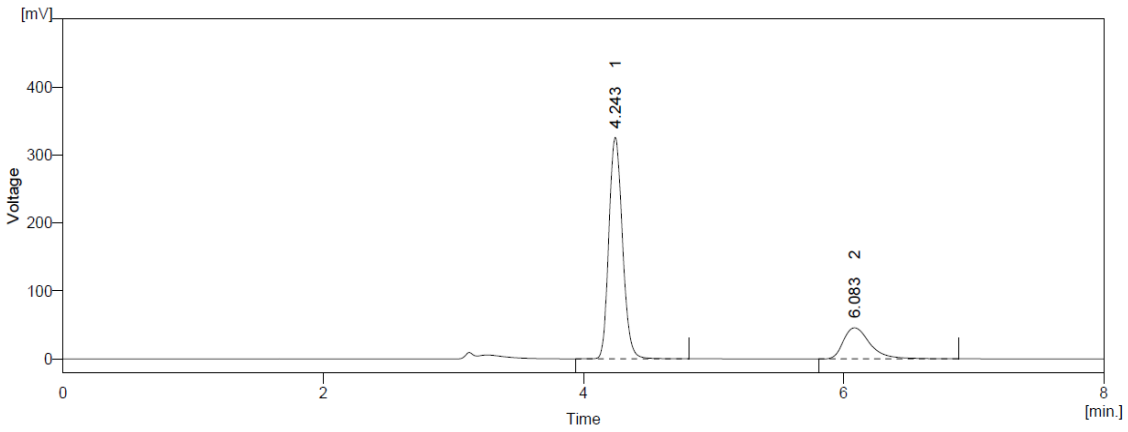


Figure 4: Simple chromatogram of sample Metformin HCl and Anagliptin.

FORCED DEGRADATION STUDIES

To evaluate the stability indicating preparation of the developed HPLC method, forced degradation studies were carried out in accordance to the ICH guidelines, to produce the possible relevant degradant and test its chromatographic behavior. Intentional degradation was attempted to stress conditions of photolytic degradation Acid hydrolysis(0.1 N HCl), Base hydrolysis (using 0.1 N NaOH), Oxidative degradation (using 3% H2O2) and Thermal degradation to evaluate the ability of the proposed to method to separate Metformin HCl and Anagliptin from its degradation product. Metformin HCl and Anagiptin at a concentration of 10µg/ml was used in all degradation studies. After completion of degradation processes the solution were neutralized.

1. Acid Degradation

Acid decomposition studies were performed by Transferring 1 ml of stock solution in to 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and kept for 5 hrs at RT. Then the volume was adjusted with diluent to get 10 µg/ml for Anagliptin and 10 µg/ml for Metformin HCl. Then degraded product neutralized by adding 2 ml of 0.1N NaOH.

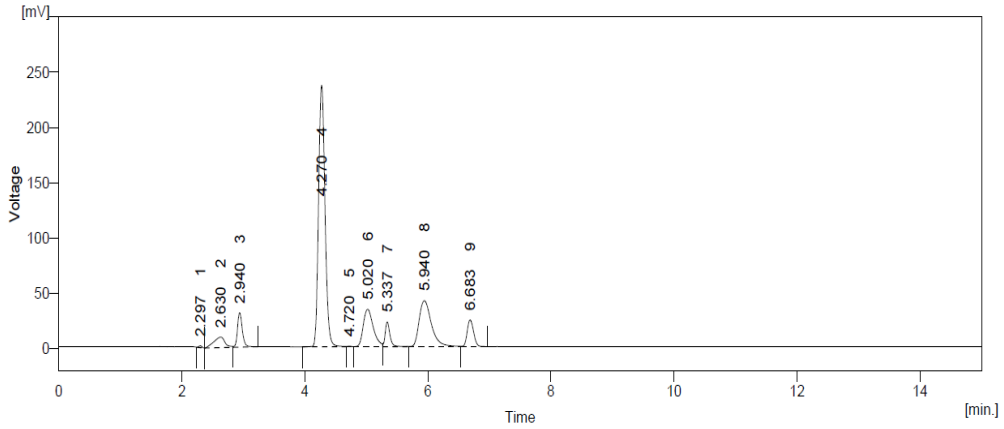


Figure 5: Chromatogram of Anagliptin and Metformin HCl degradation Sample.

2. Base degradation

Basic decomposition studies were performed by Transferring 1 ml of stock solution in to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and kept for 4 hrs. Then the volume was adjusted with diluent to get 10 µg/ml for Anagliptin and 10 µg/ml for Metformin HCl .Then degraded product neutralized by adding 2 ml of 0.1 N HCl.

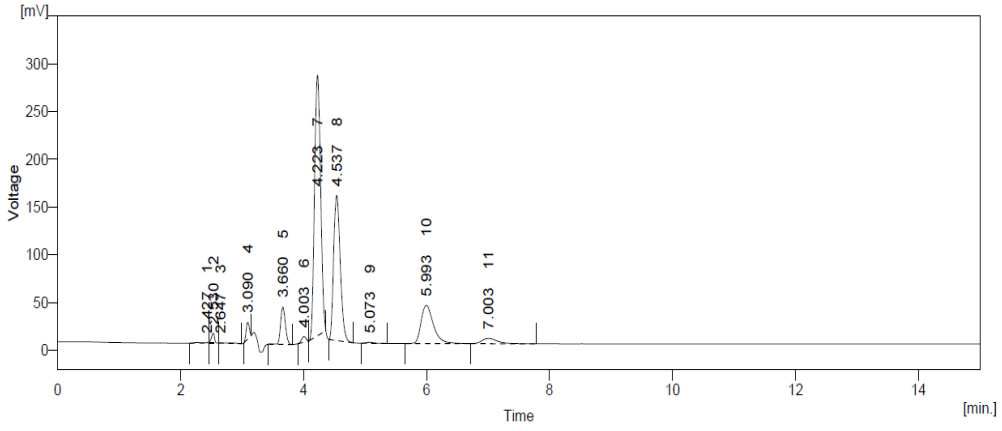


Figure 6: Chromatogram of Anagliptin and Metformin HCl Base Degradation Sample.

3. Oxidative degradation

Oxidative decomposition studies were performed by Transferring 1ml of stock solution in to 10 ml of volumetric flask. Two ml of 3% H_2O_2 solutions was added and mixed well and kept for 3 hrs. Then the volume was adjusted with diluent to get 10 µg/ml for Anagliptin and 10 µg/ml for Metformin HCl.

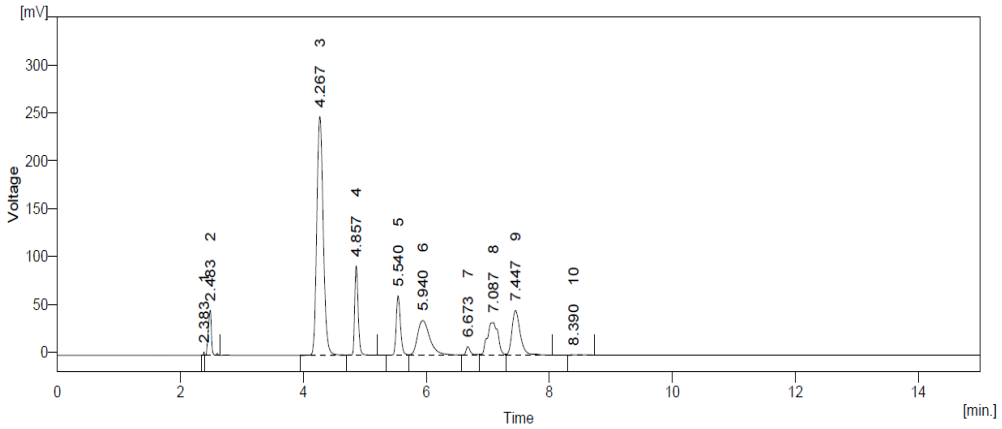


Figure 7: Chromatogram of Anagliptin and Metformin HCl Oxidation Degradation Sample.

4. Photo degradation

Photo Degradation studies were performed by Transferring One ml of stock solution in to 10 ml of volumetric flask. The volumetric flask was keep in UV Chamber for 36 hrs. Then the volume was adjusted with diluent to get 10 µg/ml for Anagliptin and 10 µg/ml for Metformin HCl.

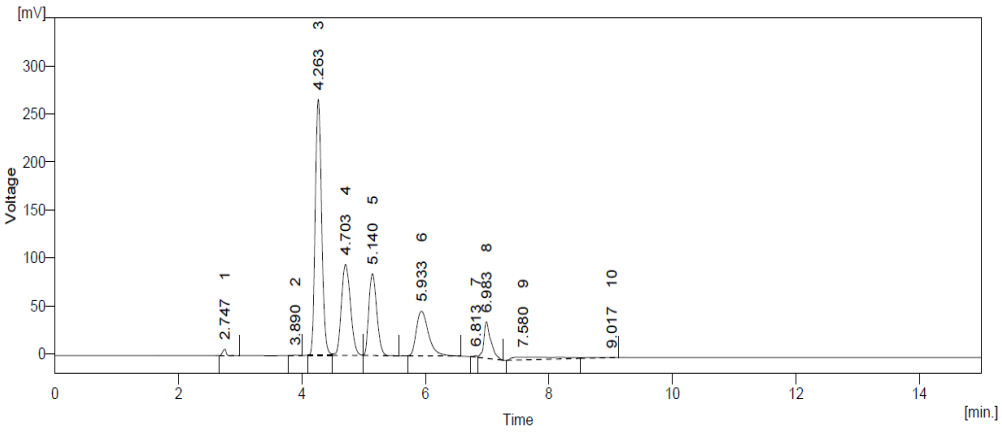


Figure 8: Chromatogram of Anagliptin and Metformin HCl Photo Degradation sample.

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5. Thermal degradation

Thermal Degradation studies were performed by weigh powder and exposed to dry heat in an oven at 70°C for 4 hrs. The powder was removed from the oven for proper dilution and chromatograms were taken.

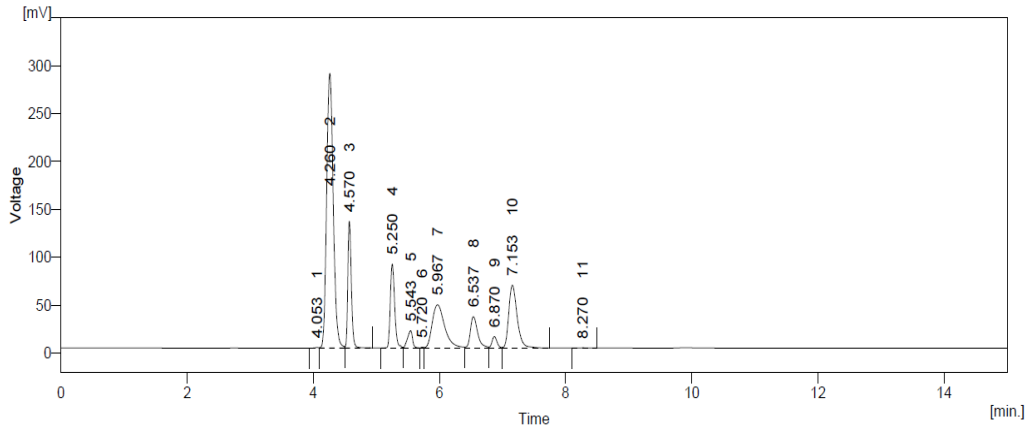

Figure 9: Chromatogram of Anagliptin and Metformin HCl Thermal Degradation Sample.

Table 1: Metformin HCl % Degradation.

Metformin HCl				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	1753.085	27.578	1732.481	28.429
Base	1958.531	19.091	1870.012	22.748
Thermal	2089.416	13.684	2088.755	13.711
Oxidation	1834.725	24.205	1824.279	24.637
Photo	1963.769	18.874	1966.037	18.781

Table 2: Anagliptin % Degradation.

Anagliptin				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	581.702	23.031	576.313	23.745
Base	532.762	29.507	559.785	25.931
Thermal	602.378	20.296	619.157	18.076
Oxidation	509.431	32.594	502.086	33.566
Photo	666.640	11.793	633.191	16.219

Table 3: Peak Purity Data.

Graph	Peak Purity of Metformin	Peak Purity of Anagliptin
Metformin Std	0.996	-
Anagliptin Std	-	0.992
Standard Combination	0.995	0.993
Sample	0.996	0.993

Validation of the Method

The analytical method was validated with respect to parameter such as linearity, precision, accuracy, specificity, LOD, LOQ and robustness.

1. Specificity

The Chromatograms of Metformin HCl and Anagliptin standards and Metformin HCl and Anagliptin sample show no interference with the Chromatogram of Metformin HCl and Anagliptin Blank, so the Developed method is Specific.

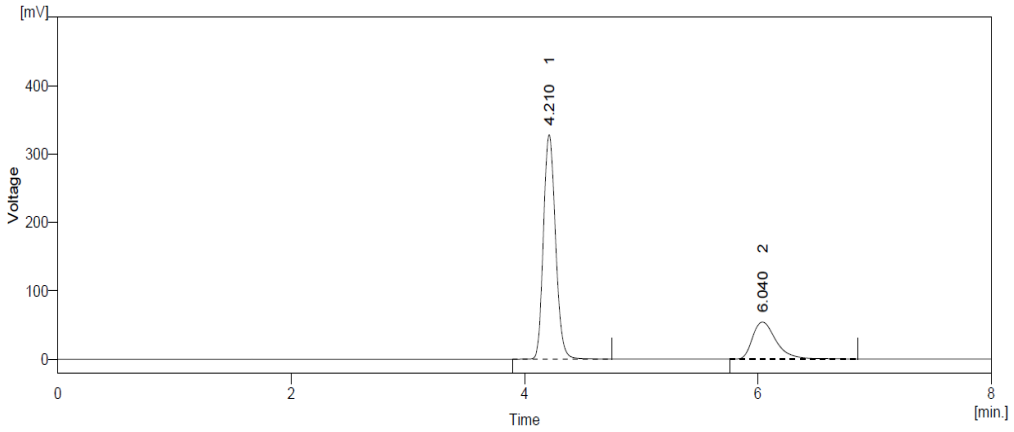

Figure 10: Chromatogram of Metformin HCl and Anagliptin std.

2. Linearity

The linearity for Anagliptin and Metformin HCl were assessed by analysis of combined standard solution in range of 5-15 µg/ml and 5-15 µg/ml respectively, 5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Anagliptin (100 µg/ml) andMetformin HCl (100 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 5,7.5,10,12.5 and 15 µg/ml, and 5,7.5,10,12.5 and 15 µg/ml for Anagliptin and Metformin HCl respectively.

In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Correlation co-efficient for calibration curve Anagliptin and Metformin HClwas found to be 0.999 and 0.993 respectively.

The regression line equation for Anagliptin and Metformin HCl are as following.

For Anagliptin**y = 79.885x - 49.079** and For Metformin HCl: **y = 237.09x - 32.259**

Table 4: Linearity data of Anagliptin.

Sr.No	Concentration(µg/ml)	Area
1	5	374.295
2	7.5	506.446
3	10	759.086
4	12.5	965.878
5	15	1143.139

Table 5: Linearity data of Metformin HCl.

Sr.No	Concentration(µg/ml)	Area
1	5	1167.171
2	7.5	1723.787
3	10	2357.679
4	12.5	2903.972
5	15	3540.739

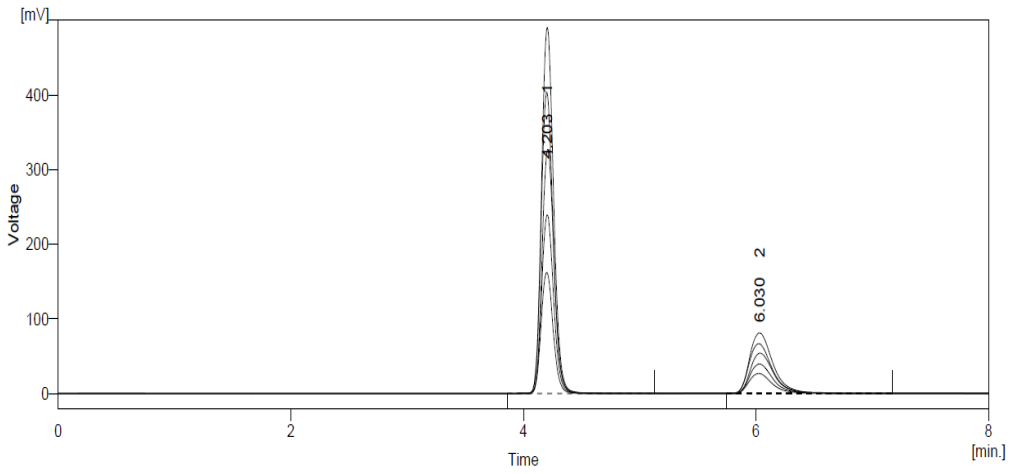

Figure 11: Overlay chromatogram of different concentrations of mixtures of Anagliptin and Metformin HCl.

3. Precision

1. Repeatability

The data for repeatability of peak area measurement for Anagliptin (10 µg/ml) and Metformin HCl (10 µg/ml) based on six measurements of same solution of Anagliptin (10 µg/ml) and Metformin HCl (1 µg/ml).The % RSD for Anagliptin and Metformin HCl was calculated.

Table 6: Repeatability data of Anagliptin.

Anagliptin				
Sr No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	10	756.069	754.781±7.822	1.036
		757.636		
		739.145		
		760.659		
		756.785		
		758.394		

Table 7: Repeatability data for Metformin HCl.

Metformin HCl				
Sr No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	10	2348.176	2347.811 ±17.898	0.762
		2312.763		
		2357.650		
		2362.391		
		2350.579		
		2355.305		

2. Intraday precision

Standard solution containing (5,10,15 µg/ml) of Metformin HCl and (5,10,15 µg/ml) of Anagliptin were analyzed three times on the same day and % R.S.D was calculated.

Table 8: Intraday precision data for Anagliptin.

	Anagliptin		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	370.682 ± 3.504	0.945
2	10	753.292 ± 4.565	0.606
3	15	1873.734± 34.114	1.820

Table 9: Intraday precision data for estimation of Metformin HCl.

	Metformin HCl		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	1155.942 ± 12.538	1.084
2	10	2337.599 ± 20.868	0.893
3	15	5821.644 ± 79.529	1.366

3. Interday precision

Standard solution containing (5,10,15 µg/ml) of Metformin HCl and (5,10,15 µg/ml) of Anagliptin were analyzed three times on the different day and % R.S.D was calculated.

Table 10: Interday precision for Anagliptin.

	Anagliptin		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	370.171 ± 2.645	0.715
2	10	753.030± 4.015	0.533
3	15	1874.963± 25.414	1.355

Table 11: Interday precision data for estimation of Anagliptin.

	Metformin HCl		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	1155.288 ± 12.518	1.083
2	10	2332.068± 19.778	0.848
3	15	5824.127± 34.961	0.600

4. Accuracy

For Metformin HCl

5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 240 nm. The amount of Metformin HCl was calculated at each level and % recoveries were computed.

Table 12: Recovery data for Metformin HCl.

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	5	4	3.951	98.775	99.877 ± 1.074
2		5	4	4.037	100.920	
3		5	4	3.997	99.935	
4	100 %	5	5	4.959	99.190	99.676 ± 0.440
5		5	5	5.002	100.047	
6		5	5	4.990	99.791	
7	120 %	5	6	5.995	99.917	99.694 ± 0.323
8		5	6	5.959	99.323	
9		5	6	5.991	99.842	

For Anagliptin

5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 240 nm. The amount of Anagliptin was calculated at each level and % recoveries were computed.

Table 13: Recovery data for Anagliptin.

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	5	4	3.984	99.608	100.836 ± 1.135
2		5	4	4.074	101.848	
3		5	4	4.042	101.051	
4	100 %	5	5	5.001	100.020	100.651 ± 0.655
`		5	5	5.066	101.328	
6		5	5	5.030	100.604	
7	120 %	5	6	6.067	101.113	100.628 ± 0.493
8		5	6	6.008	100.127	
9		5	6	6.039	100.646	

5. LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercept was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 240 nm. The amount of Anagliptin was calculated at each level and % recoveries were computed.

Limit of Detection

Table 14: Limit of Detection data for Metformin HCl and Anagliptin.

Anagliptin	Metformin HCl
LOD = 3.3 x (SD / Slope) = 3.3 x (30.918/79.885) = 1.277 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (26.300/237.09) = 0.366 µg/ml

Limit of Quantfication

Table 15: Limit of Quantification for Metformin HCl and Anagliptin.

Anagliptin	Metformin HCl
LOQ = 10 x (SD / Slope) = 10 x (30.918/79.885) = 3.870 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (26.300/237.09) = 1.109 µg/ml

6. Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.

2. pH of Mobile phase was changed (± 0.2) 6.8 and 7.2.

3.Ratio of Mobile phase was changed(±2) Buffer: Acetonitrile (33:67) and Buffer: Acetonitrile (37:63)

Table 16: Robust data of Anaglitin.

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	784.044	737.168	776.477	722.078	775.788	735.688
2	779.889	726.899	761.361	706.461	753.525	728.551
3	793.777	744.728	782.478	731.072	781.787	745.552
% R.S.D	0.907	1.215	1.407	1.730	1.933	1.159

Table 17: Robust data of Metformin HCl.

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	2407.049	2266.889	2388.608	2213.107	2388.072	2262.340
2	2444.830	2303.912	2421.301	2256.792	2413.936	2299.297
3	2458.841	2313.412	2430.597	2270.785	2428.156	2315.732
% R.S.D	1.099	1.071	0.914	1.339	0.843	1.193

7. Analysis of Synthetic Mixture by developed method

Table 18 Assay of Synthetic Mixture

Tablet	Synthetic Mixture	
Label claim	Metformin HCl (10mg)	Anagliptin (10mg)
Assay (% of label claim*) Mean ± S. D.	99.695±0.857	99.882±1.563

The assay results were comparable to labelled value of each drug in combined dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

RESULT AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Metformin HCl and Anagliptin preferably analyzed by reverse phase columns and accordingly C18 column was selected so the elution of the compound from the column was influenced by polar mobile phase. The concentration of the Potassium phosphate buffer and Acetonitrile were optimized to give symmetric peaks with short run time based on asymmetric factor and peak are obtained. Different mobile phase tried but Potassium Phosphate Buffer(ph7): Acetonitrile (35:65) v/v gives well resolved and good symmetrical peaks. The retention time for Metformin HCl and Anagliptin were found to be 4.227 and 5.893 minute respectively. The RSD value for accuracy and precision is less then 2% which indicate that developed method is accurate and precise. The degree of reproducibility of the result obtained as a result of small and deliberate variations in the method parameter has proven that the method is robust. The results of assay indicate that the method is selective for the analysis of synthetic mixture of Metformin HCl and Anagliptin.

CONCLUSION

The above developed stability indicating RP – HPLC method was successfully validated in terms of accuracy, precision, linearity, robustness and reproducibility. This method was found to be simple, rapid, accurate, robust, precise and reproducible. The above developed stability indicating RP – HPLC method can be applied for routine quantitative analysis of synthetic mixture of Metformin HCl and Anagliptin.

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