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Stability Indicating Method Development and Validation for Simultaneous Estimation of Linagliptin and Metformin HCl in Tablets by HPLC

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ABSTRACT

An accurate, simple, new, precise, rugged and stability indicating method was developed for simultaneous estimation of linagliptin and metformin HCl in tablets. The developed method was rapid and economic. The Chromatographic separation was achieved isocratically on a C-18 column by utilizing di-ammonium hydrogen phosphate buffer: ACN: MeOH (60:20:20 v/v/v) at the flow rate of 1 ml/min with UV detection at 272 nm. The retention times of linagliptin and metformin are 3.180 min and 6.120 min respectively. The method is linear within range 1.25 µg/ml to 10 µg/ml for linagliptin and 250 µg/ml to 2000 µg/ml for Metformin. The correlation coefficient was found to be R^2=0.999 and 1.0 for linagliptin and metformin respectively. Both standard and test solutions proved to be stable for up to 48 h. Forced degradation study showed that the method is stability indicating. The developed method can be used for routine analysis of linagliptin and metformin fixed dose combination.

Keywords: RP-HPLC, Linagliptin, Metformin HCl, Stability indicating, Validation

INTRODUCTION

Linagliptin is an orally-active inhibitor of the dipeptidyl peptidase-4 (DPP-4) enzyme. It is very slightly soluble in water (0.9 mg/ml) (Figure 1). Linagliptin is soluble in methanol (ca. 60 mg/ml), sparingly soluble in ethanol (ca. 10 mg/ml), very slightly soluble in isopropanol (< 1 mg/ml) and very slightly soluble in acetone (ca. 1 mg/ml). Linagliptin is a white to yellowish, slightly hygroscopic but water uptake does not change the crystal modification. The chemical name is 8-[(3*R*)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3- methyl-1-[(4-methylquinazolin-2-yl)methyl]-3,7-dihydro-1*H*-purine-2,6-dione. Molecular formula is $C_{25}H_{28}N_8O_2$, Molecular weight of 472.54 g/mol [1].

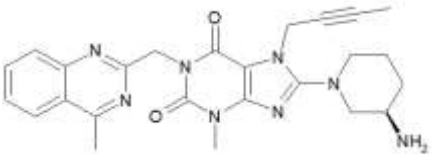

Figure 1: Linagliptin

Metformin hydrochloride (*N*,*N*-dimethylimidodicarbonimidic diamide hydrochloride) is a white to off-white crystalline compound (Figure 2). The pKa of metformin is 12.4. The pH of a 1% aqueous solution of metformin hydrochloride is 6.68. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. Molecular formula=$C_4H_{11}N_5$.HCl, Molecular weight=165.63 [2,3].

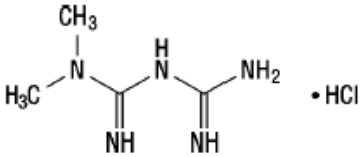

Figure 2: Metformin hydrochloride

MATERIALS AND METHODS

Reagents and chemicals

Linagliptin-MSN Laboratories Limited Hyderabad, metformin HCl, MSN Laboratories Limited Hyderabad. HPLC grade methanol, acetonitrile, water were used. Other chemicals and reagents like diammonium hydrogen phosphate, OPA, HCl, NaOH, $H_2O_2$of AR grade were used.

Instruments used

Analysis was performed by using analytical balance precisa XB220A, HPLC used is Shimadzu LC-2010 with PDA detector. Column used in HPLC is, Waters Spherisorb SCX 10 µm, 250 × 4.6 mm. Other equipments like sonicator, water bath, hot air oven of thermo make were used.

Buffer preparation

Weigh 17 g of diammonium hydrogen phosphate in to 1000 ml of water, sonicate to dissolve and adjust the pH of the solution to 3.0 with ortho phosphoric acid, filter through 0.45 µm membrane filter.

Mobile phase preparation: Mix buffer, acetonitrile and methanol in the ration 60:20:20.

Diluent: Acetonitrile: Water (80:20).

Optimized chromatographic conditions: Waters Spherisorb SCX 10 µm, 250 × 4.6 mm column is used with 272 nm as wavelength, flow rate of 1.0 ml/min, Column Oven Temperature of 40°C, injection volume of 10.0 µl.

Preparation of standard solution

Stock solution of linagliptin standard (500 ppm)

Accurately weighed and transferred 50.0 mg of linagliptin working standard in to a 100 ml volumetric flask. Added 70 ml of diluent and sonicated to dissolve it and made up to the mark with diluent (Figure 3).

Stock solution of metformin HCl standard (4000 ppm)

Accurately weighed and transferred 200.0 mg of metformin HCl working standard in to a 50 ml volumetric flask. Added about 100 ml of diluent and sonicated to dissolve it and made up to the mark with diluent.

Standard preparation of linagliptin (5 ppm) and metformin HCl (1000 ppm)

Transferred 1 ml of stock solution of linagliptin and 25 ml of stock solution of metformin HCl solution in to 100 ml volumetric flask and made up to volume with diluent (Figure 4).

Preparation of placebo

Weighed Placebo equivalent to 12.5 mg of linagliptin and 2500 mg of metformin and transferred into 250 ml volumetric flask. Added about 180 ml of diluent and sonicated for about 30 min. with intermittent shaking. Inserted the magnetic needle and stirred for 15 min. Removed the magnetic needle into a beaker and washed the magnetic needle with diluent. Filtered the sample through 0.45 µm PVDF filter. Further diluted 5 ml to 50 ml with diluent.

Preparation of test solution

Weighed and transferred 5 tablets (equivalent to 12.5 mg of linagliptin and 2500 mg of metformin) in to a 250 ml volumetric flask. Added about 180 ml of diluent and sonicated for about 30 min with intermittent shaking. Inserted the magnetic needle and stirred for 15 min, removed the magnetic needle in to a beaker and washed the magnetic needle with diluent. Transferred the washings in to same flask. Diluted up to the mark with diluent. Filtered the sample through 0.45 µm PVDF filter. Further diluted 5-50 ml with diluent. Injected one blank solution, placebo, standard and test solutions in to the chromatograph.

Method validation

Specificity

Injected blank solution, Placebo, standard and test solutions in to the chromatograph after system suitability. No interference was observed at the RT's of linagliptin and metformin from blank and Placebo solutions (Tables 1-5) [4].

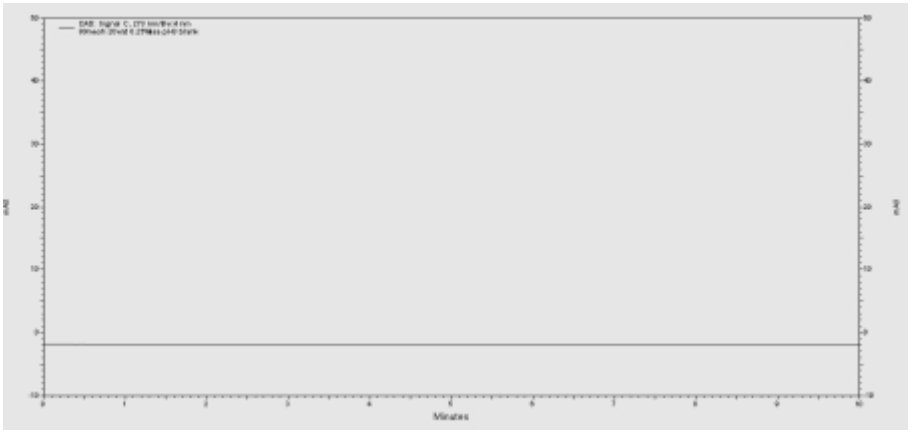

Figure 3: Blank solution

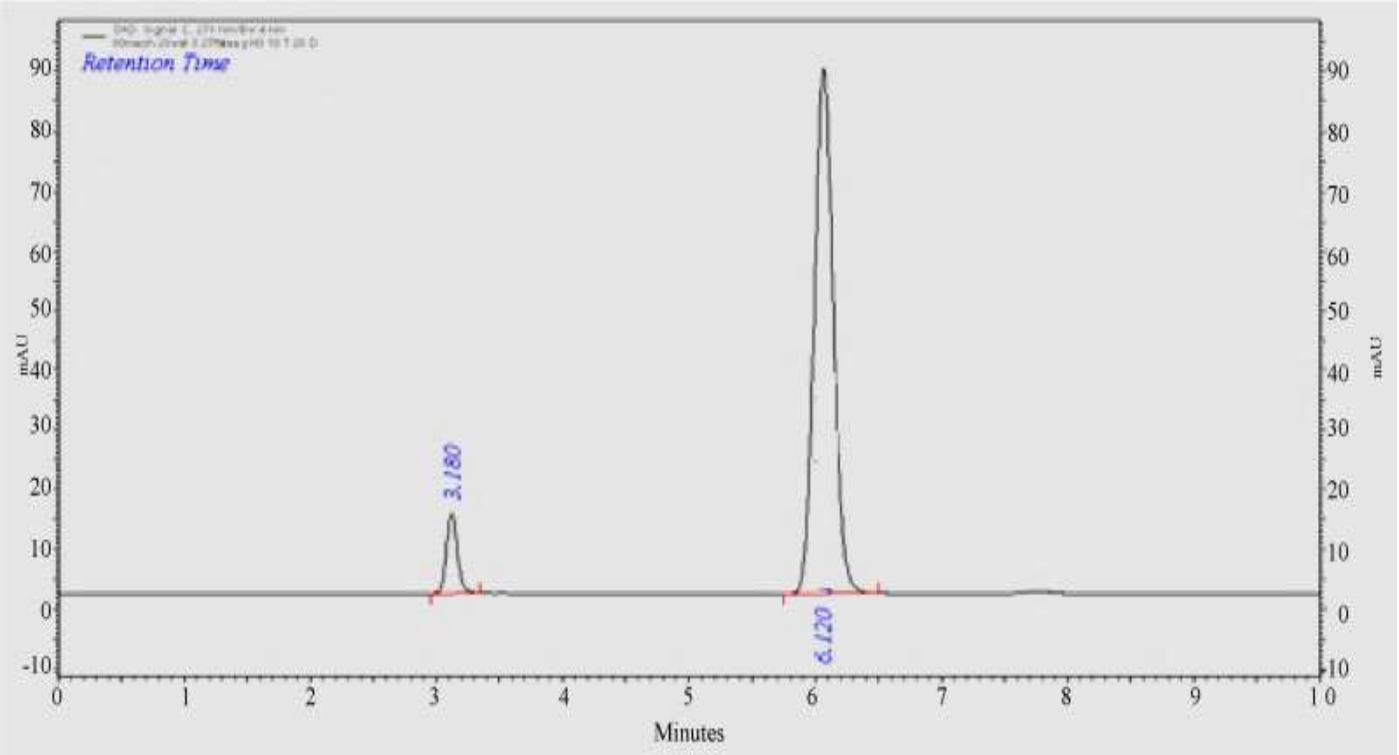

Figure 4: Standard solution

Precision

Determined the precision by preparing and injecting six test preparations. Intermediate precision was performed on a different day, on a different system by using the same lot of samples (Table 1).

Table 1: Method and intermediate precision combined

	Method precision		Intermediate precision		Linagliptin			Metformin		
S. No.	**Assay linagliptin**	**Assay metformin**	**Assay linagliptin**	**Assay metformin**	**Overall Average**	**Overall S.D**	**Overall %RSD**	**Overall average**	**Overall S.D**	**Overall %RSD**
1	99.92	99.55	101.11	100.79						
2	99.66	98.72	100.95	100.79						
3	100.63	99.71	100.44	99.97						
4	101.04	99.38	101.00	99.79						
5	99.79	98.72	100.87	100.57	100.35	0.67	0.67	99.81	0.71	0.71
6	99.44	99.45	99.35	100.24						
Average	100.08	99.26	100.62	100.36						
S.D	0.62	0.43	0.66	0.43						
%RSD	0.62	0.43	0.66	0.43						

Linearity

Determined the linearity from 25%-200% against standard concentration i.e., from 1.25-10 ppm for linagliptin and 25-200 ppm for metformin HCl (Figures 5 and 6).

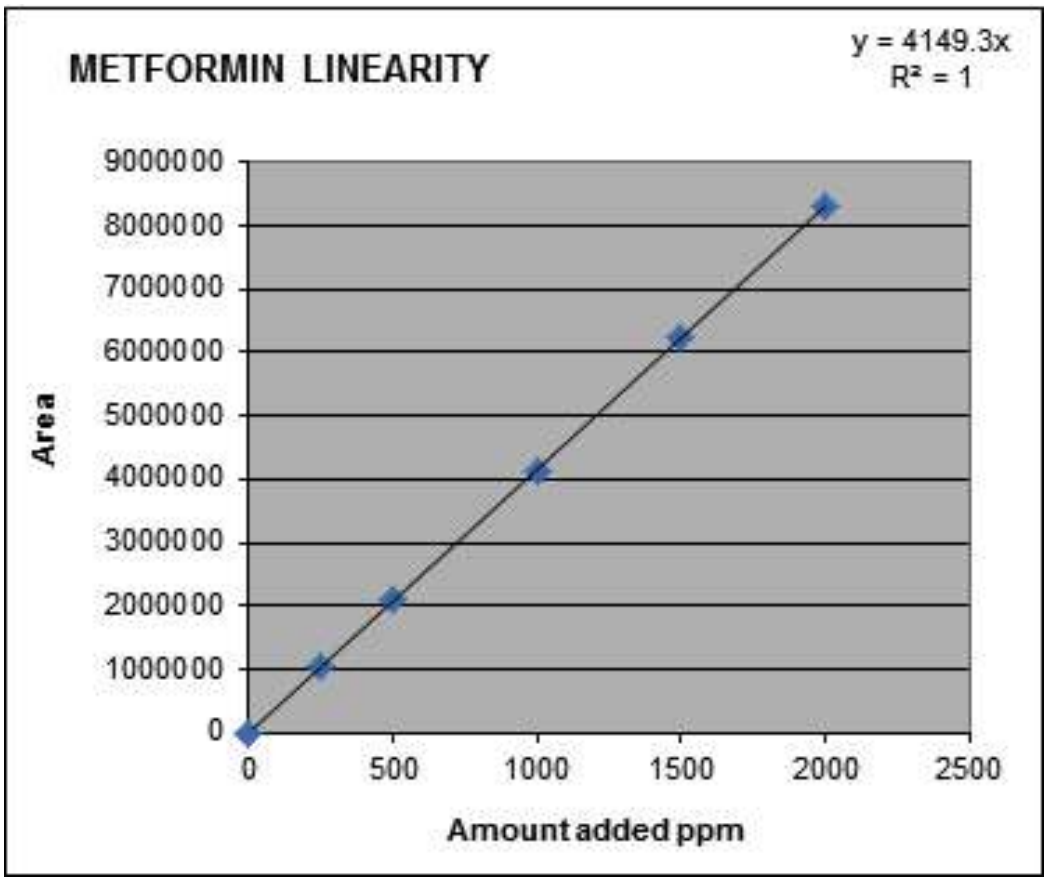

Figure 5: Metformin linearity

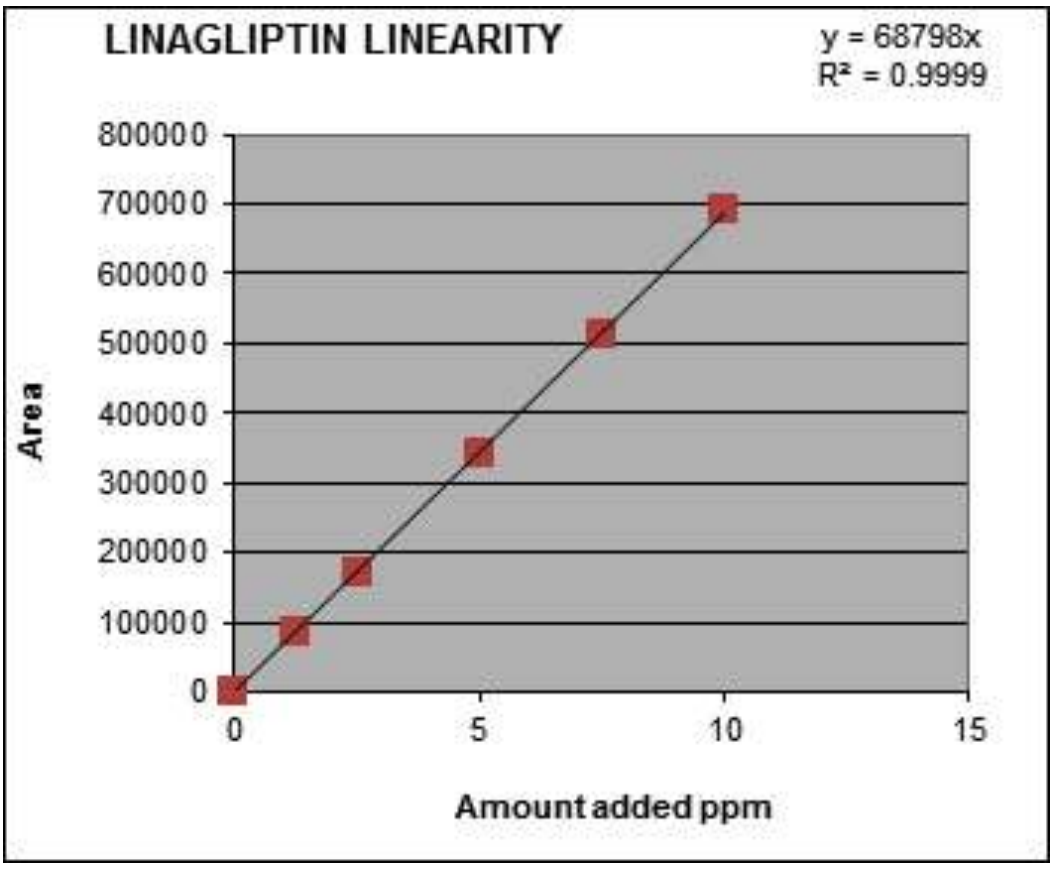

Figure 6: Linagliptin linearity

Accuracy

Performed the accuracy of test method using linagliptin and metformin HCl API and placebo at 80%, 100%, 20% spike levels in triplicate (Tables 2 and 3) (Figures 7-9).

Table 2: Accuracy linagliptin

Linagliptin and metformin accuracy							
Linagliptin accuracy		**Std. Wt. in**	50.03 mg	**Std. Response**	336208	**Potency**	98.87
		Volume (ml)	100	**Sample volume**	250	**M/F**	1
		Dil. Rate	0.01	**Dil. Rate**	10	**Strength**	1
Spike level	**Wt. of sample**	**Sample area**	**mg/ml added**	**mg/ml found**	**% Recovery**	**Average**	**%RSD**
80% ± 01	10.0988	267865	0.04034	0.03981	98.7	99.3	0.60
80% ± 01	10.0234	267654	0.04004	0.03978	99.3		
80% ± 01	10.0123	268765	0.04000	0.03994	99.9		
100% ± 01	12.5781	335505	0.05025	0.04986	99.2	99.2	0.25
100% ± 02	12.5436	335133	0.05011	0.04981	99.4		
100% ± 03	12.5827	334364	0.05027	0.04969	98.9		
120% ± 01	15.0213	402768	0.06001	0.05986	99.7	100.0	0.49
120% ± 02	15.0034	405678	0.05994	0.06029	100.6		
120% ± 03	15.1205	405678	0.06040	0.06029	99.8		

Table 3: Accuracy of metformin

Linagliptin and metformin accuracy							
Metformin accuracy		**Std. Wt.**	200.12 mg	**Std. response**	4062032	**Potency**	99.9
		Volume (ml)	50	**Sample volume**	250	**M/F**	1
		Dil. Rate	0.25	**Dil. rate**	10	**Strength**	1
Spike Level	**Wt. of sample**	**Sample area**	**mg/ml added**	**mg/ml found**	**% Recovery**	**Average**	**%RSD**
80% ± 01	2000.01	3224567	0.07992	0.07935	99.30	99.8	0.63
80% ± 02	2000.34	3234567	0.07993	0.07960	99.60		
80% ± 03	2000.43	3265671	0.07994	0.08036	100.50		
100% ± 01	2500.03	4017909	0.09990	0.09887	99.0	99.4	0.64
100% ± 02	2500.80	4063115	0.09993	0.09999	100.1		
100% ± 03	2500.00	4017909	0.09990	0.09887	99.0		
120% ± 01	3001.09	4821489	0.11992	0.11865	98.9	100.2	1.11
120% ± 02	3000.65	4921345	0.11991	0.12111	101.0		
120% ± 03	3000.32	4902134	0.11989	0.12063	100.6		

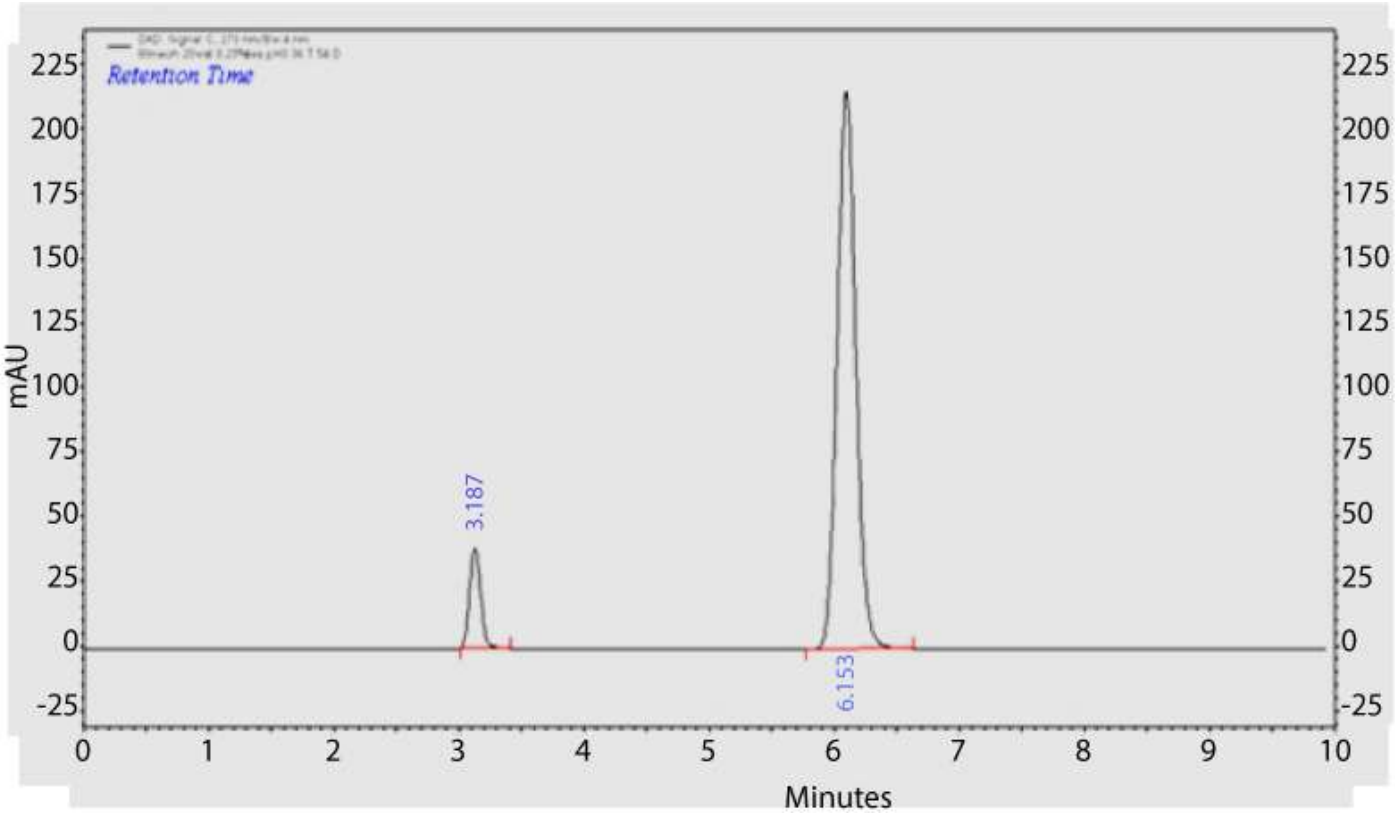

Figure 7: Accuracy (Solution-80%)

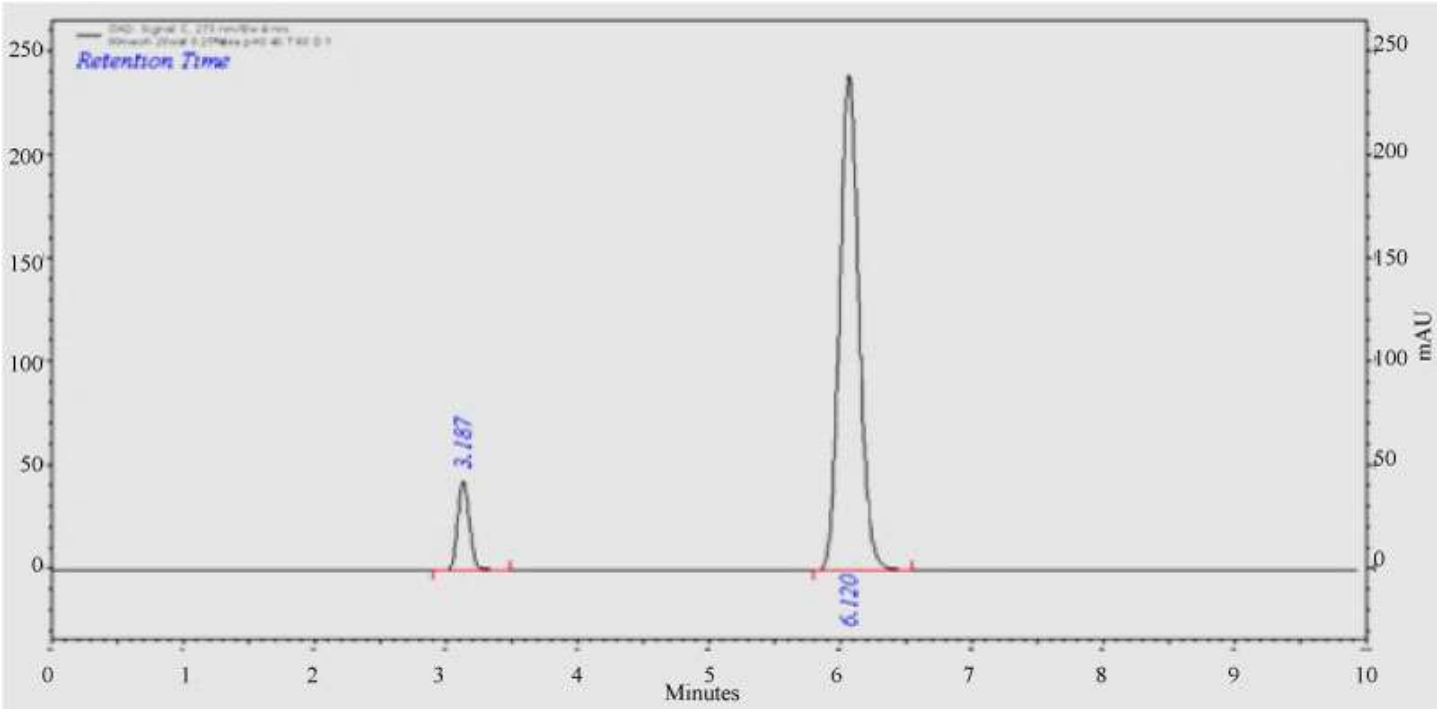

Figure 8: Accuracy (Solution-100%)

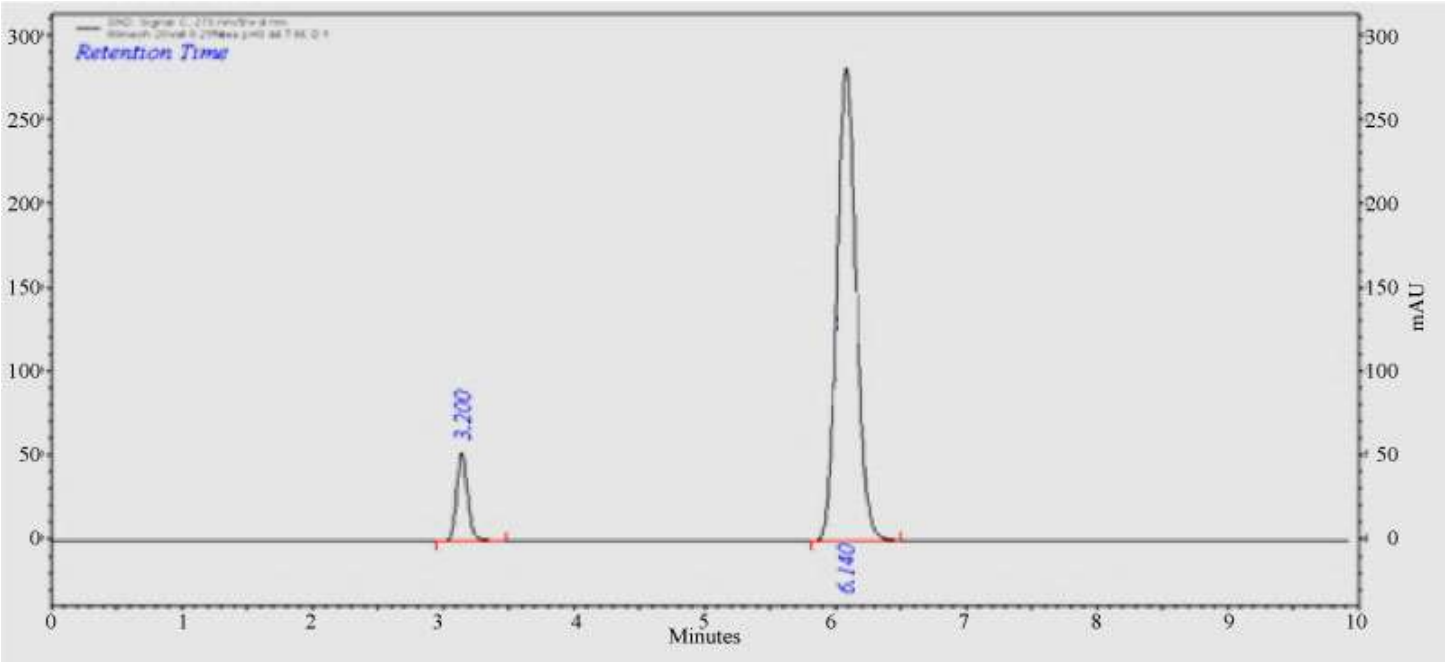

Figure 9: Accuracy (Solution-120%)

Table 4: Solution stability of metformin

Method validation assay test solution stability of metformin							
Weight of tablets		3028.33	**M/F**	1	**Average weight (mg)**		606.8
		Strength (mg)	**Volume (ml)**	250			
		500.00	**Dil. rate**	10			
Standard weight (Initial)	200.25 mg	Volume (ml)	50	Dil. Rate	4	Purity	99.9
Fresh standard weight (24 h)	200.40 mg	Volume (ml)	50	Dil. Rate	4	Purity	99.9
Fresh standard weight (48 h)	200.19 mg	Volume (ml)	50	Dil. Rate	4	Purity	99.9
Sample name	**STD area**	**Sample area**		**Assay (%)**	**Difference**	**Acceptance criteria**	
Test solution (Initial)	4013253	4053875		101.23	NA	Difference in % assay results of initial, 24 h and 48 h shall be NMT 2.0%.	
Test solution (24 h)	4110245	4112348		100.34	0.89		
Test solution (48 h)	4013456	4036742		100.76	0.46		

Table 5: Solution stability of linagliptin

Method validation assay test solution stability of linagliptin							
Weight of tablets		3033.21	**M/F**	1	Average weight (mg)		606.8
		Strength (mg)	**Vol. (ml)**	250			
		2.5	**Dil. Rate**	10			
Standard weight (Initial)	50.05 mg	Volume (ml)	100	Dil. Rate	100	Purity	98.76
Fresh standard weight (24 h)	50.23 mg	Volume (ml)	100	Dil. Rate	100	Purity	98.76
Fresh standard weight (48 h)	50.12 mg	Volume (ml)	100	Dil. Rate	100	Purity	98.76
Sample Name	**STD area**	**Sample area**		**Assay (%)**	**Difference**	**Acceptance criteria**	
Test solution (Initial)	334562	332781		98.53	NA	Difference in % assay results of initial, 24 h and 48 h shall be NMT 2.0%.	
Test solution (24 h)	329107	330268		99.76	-1.23		
Test solution (48 h)	331894	331875		99.19	-0.66		

Forced degradation study

Performed the forced degradation of test method to demonstrate the non-interference of impurities, degradation products in quantification of analyte by various stress conditions (Table 6).

Table 6: Forced degradation study

S. No.	Stress condition	Acceptance criteria	Results
1	Acid degradation	Peak purity shall pass	Passes
2	Base degradation		Passes
3	Peroxide degradation		Passes
4	Thermal degradation		Passes
5	Water degradation		Passes

Robustness

Performed the robustness by altering the flow rate by ± 0.2 ml/min from 1.0 ml/min, column oven temperature by ± 5°C from 40°C and buffer pH by ± 0.2 from 3.0. Prepared the standard solution and checked the system suitability criteria by altering the above mentioned parameters. System Suitability criteria was within the limits for all the altered parameters.

Filter integrity test

Filter integrity test was also performed by using Polyvinylidene Difluoride (PVDF) and Polytetrafluoroethylene (PTFE) filters and checked the difference in assay from centrifuged samples (Table 7).

Table 7: Filter integrity test

Linagliptin				
S. No.	**% Assay**	**% Difference from centrifuged**	**% Assay**	**Difference from centrifuged**
Centrifuged	101.52	NA	101.64	NA
PVDF	100.92	0.6	100.13	1.51
PTFE	100.02	1.5	99.77	1.87
Metformin				
S. No.	**% Assay**	**Difference from centrifuged**	**% Assay**	**Difference from centrifuged**
Centrifuged	101.78	NA	101.07	NA
PVDF	101.16	0.62	101.24	-0.17
PTFE	101.27	0.51	101.33	-0.26

CONCLUSION

A new RP-HPLC method has been developed for simultaneous estimation of linagliptin and metformin HCl in marketed formulation. The method showed good resolution between the two drugs and also with degradants in forced degradation study. The developed method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. It proved to be stability indicating, specific, novel, simple, accurate, precise and cost effective. Hence the proposed RP-HPLC method is suitable for routine assay of linagliptin and metformin in pharmaceutical dosage forms in quality control laboratories.

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