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Original Article

RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION AND STABILITY INDICATING STUDY OF METFORMIN AND LINAGLIPTIN IN PURE AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: The objective of this study was to develop a simple, efficient, specific, precise and accurate Reverse phase High Performance liquid chromatography method for the simultaneous estimation of Metformin and Linagliptin Pharmaceutical Dosage form.

Methods: The separation method was carried out using reverse phase C18 column, Inertsil ODS – 3V (250 mm x 4.6 mm x 5µm). The mobile phase used was a mixture of Phosphate buffer (1.625 g of Potassium Di Hydrogen Ortho Phosphate and 0.3 g of Di Potassium Hydrogen Ortho Phosphate in 550 ml water) pH 4.5 and Acetonitrile in the ratio of 60:40 (v/v) at isocratic mode. The flow rate was 1.0 mL/min, column temperature was 30°C and eluents were monitored at 280 nm using waters 2695 alliance HPLC instrument equipped with the Waters 2998 PDA detector and Empower 2 software.

Results: With the optimized method, the retention times of Metformin and Linagliptinwere found to be 3.048 and 4.457 respectively, with theoretical plate count and asymmetry as per the ICH limits. The method has shown a good linearity in the concentration range of 500-3000µg/ml from Metformin and 2.5-15µg/mL for Linagliptin with Regression coefficient (R2) of 0.99 and 0.99. The percentage assays were found to be 99.28% and 99.54% respectively for Metformin and Linagliptin. The method was found to be accurate (with percentage mean recoveries 100% for Metformin HCl and 100% for Linagliptin), precise, robust, stable and Degradation studies are conducted under various conditions.

Conclusion: The proposed method was validated in accordance with ICH guidelines and hence, can be successfully applied to the simultaneous estimation of Metformin and Linagliptin tablet formulations.

Keywords: Metformin and Linagliptin, Simultaneous estimation, Reverse phase HPLC, Validation, Degradation studies.

INTRODUCTION

Metformin HCl is an oral hypoglycemicdiabetic drug which comes under the class Biguanides. It is chemically 1, 1-Dimethyl biguanide monohydrochloride. It is the first line drug for treating Type-2 Diabetes mellitus. Metformin acts by suppressing hepatic gluconeogenesis and glucose output from the liver. It is official in USP-2010, BP-2012, and IP-2007 [1-3]. It is the first line drug of choice for the treatment of type 2 diabetes, particularly in overweight or obese people and those with normal kidney function. Metformin activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance and metabolism of glucose and fats. Metformin is an anti-diabetic agent [4, 5]. Activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells. The chemical structure of Metformin is shown in fig. 1.

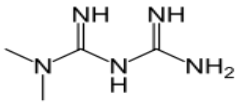

Fig. 1: Structure of Metformin

Linagliptin is described chemically as 1H-Purine- 2,6-Dione, 8-[(3R) -3-amino-1-piperidinyl] -7-(2-butyn-1- yl) -3,7-dihydro-3-methyl-1-[(4-methyl-2-quinazolinyl) methyl] -The empirical formula is $C_{25}H_{28}N_8O_2$. The structural formula is shown in fig (2). Linagliptin is a white to yellowish or only slightly hygroscopic solid substance. It is very slightly soluble in water (0.9 mg mL-1). Linagliptin is soluble in methanol (CA. 60 mg mL-1), sparingly soluble in ethanol (CA. 10 mg mL-1), very slightly soluble in isopropanol (<1 mg mL-1), and very slightly soluble in acetone (CA. 1 mg mL-1). Linagliptin is an oral drug that reduces blood sugar (glucose) levels in patients with type 2 diabetes. Linagliptin is a member of a class of drugs that inhibit the enzyme, DI peptidyl peptidase-4 (DPP-4). Following a meal, insertion hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP) are released from the intestine, and their levels increase in the blood. GLP-1 and GIP reduce blood glucose by increasing the production and release of insulin from the pancreas. GLP-1 also reduces blood glucose by reducing the secretion by the pancreas of the hormone, glucagon, a hormone that increases the production of glucose by the liver and raises the blood level of glucose. The net effect of increased release of GLP-1 and GIP is to reduce blood glucose levels. Linagliptin inhibits the enzyme, DPP-4, that destroys GLP-1 and GIP and thereby increases the levels and activity of both hormones. As a result, levels of GLP-1 and GIP in the blood remain higher, and blood glucose levels fall. Linagliptin reduces blood glucose levels by inhibiting DPP-4 and increasing the levels of GLP-1 and GIP [6-8]. The chemical structure of Linagliptin is shown in fig. 2.

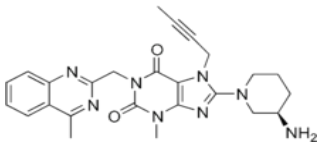

Fig. 2: Structure of Linagliptin

For the simultaneous estimation of drugs present in multicomponent dosage forms, HPLC method is considered to be most suitable for this is a powerful and rugged method. Many Methods have been reported in the literature for the estimation of Metformin Hydrochloride [9-19] and Linagliptin [7, 8, 20-23]

individually and in combination. However, there is no simple method with shorter run times has been reported for the simultaneous estimation of Metformin Hydrochloride with Linagliptin. The present investigation was aimed at developing a fully validated RP-HPLC method for the simultaneous estimation of Metformin and Linagliptin in pure and pharmaceutical dosage forms that is more economical, simple and accurate than the previous methods.

MATRIALS AND METHODS

Instruments used

The chromatographic determination was performed on waters 2695 alliance HPLC instrument equipped with the waters 2998 PDA detector and Empower 2 software. The different columns were used during method trials such as Inertsil ODS-3V C18column (250 mm×4.6 mm, 5µ particle size), Boston C18 (150 mmX4.6 mm, 5µ), Zodiac C18 (250 mm×4.6 mm, 5µ), etc. Other equipment used were Schimadzu electronic balance AY220, Global Digital pH meter DPH 500, ultrasonic cleaner (Frontline FS 4, Mumbai, India).

Chemicals and reagents

Standard gift samples of Metformin Hydrochloride and Linagliptinwere obtained from Lara drugs Pvt. Ltd., Hyderabad, India. Marketed formulation of combination was purchased from local markets. Acetonitrile, Methanol and water were purchased from HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Potassium Di Hydrogen Phosphate and Di PotassiumHydrogen Phosphate were purchased from E. Merck, Mumbai, India. All the solvents and reagents were of HPLC grade.

Preparation of standard solution

Accurately weighed standards of Metformin HCl (2000 mg) andLinagliptin (10 mg) were weighed accurately 2000mg of Metformin and 10 mg of Linagliptinwas transferred into 50 ml of volumetric flask dissolved and diluted to volume with the mobile phase and sonicated for 15 min. Pipette out 5 ml of this solution into 25 ml volumetric flasks and make up the volume with the mobile phase.

Preparation of sample solution

Twenty tablets were weighed (average weight 2000 mg) and powdered using mortar and pestle. The quantity of powder equivalent to 500 mg of Metformin HCl and Linagliptini. e., 2000 mg was transferred to a 50 ml volumetric flask. The content was dissolved in the mobile phase, sonicated for15 minutes to dissolve the drug as completely as possible. The solution was then filtered through 0.45µNylon disposable Syringe filter. The volume was then made to mark with the mobile phase. This is the standard stock solution. From the standard stock solution, an aliquot of 5 ml solution was transferred to a 25 ml volumetric flask and diluted to mark with the mobile phase

RESULTS AND DISCUSSION

Method development

Initially, many method trials were performed using different mobile phases, different columns, and varying chromatographic conditions in an attempt to obtain the best separation and resolution between Metformin HCl and Linagliptinas shown in fig. 3. The finalized method involved the use of a mixture of Phosphate buffer (1.625 g. of Potassium Di Hydrogen Ortho Phosphate and 0.3 g of Di Potassium Hydrogen Ortho Phosphate in 550 ml water); pH 4.5 and Acetonitrile in the ratio of 60:40 (v/v) as the mobile phase at isocratic mode and eluents were monitored at 280 nm using UV-Visible spectrophotometer as the detector allowing the adequate separation of both the compounds using the column Inertsil ODS - 3V C18 (250 mm x 4.6 mm x 5µm particle size) at a flow rate of 1.0 ml/min and column temperature 30°C. Sample injection volume was 1.0 ml/minas shown in fig. 4.

Assay procedure

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected five times and the chromatograms were recorded. This procedure was repeated for the sample solution too. The averages of peak areas were determined for standard and sample solutions. The concentration of the drug was calculated using the following formula

Amount of drug in each tablet

$$= \frac{\text{Peak area of test}}{\text{Peak area of std}} \times \frac{\text{Std dilution factor}}{\text{Sample dilution factor}} \times \frac{\text{average weight of tablets}}{\text{label claim}} \times \text{potency}$$

The results are described in table 1.

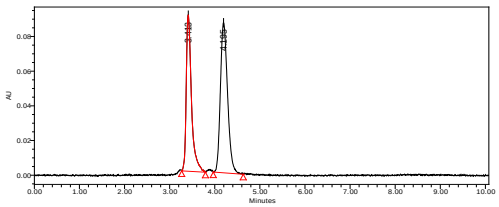

Fig. 3: Typical chromatogram for the trial

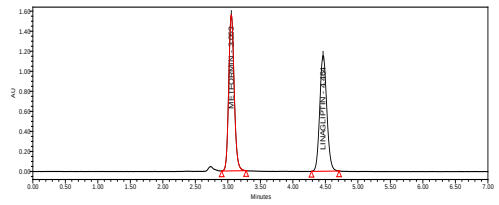

Fig. 4: Typical chromatogram for the standard

Table 1: Assay results of Metformin and Linagliptin

Drug	% Assay
Metformin	99.28
Linagliptin	99.54

Method validation [20-23]

System suitability

The suitability of the chromatography system was tested before each stage of validation. Six replicates of working standard solution are injected and the chromatograms are recorded. The % Relative Standard Deviation (%RSD) of retention times, asymmetry, theoretical plate count and of peak areas (should not be more than 2%) was determined as shown in table 2 and fig. 5.

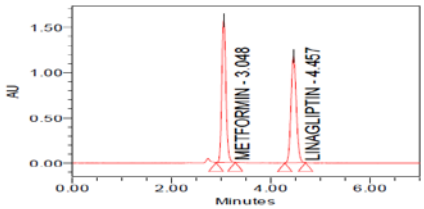

Fig. 5: Typical chromatogram for the standard

Table 2: System suitability parameters

Parameters	Metformin	Linagliptin
Retention time	3.048	4.457
Resolution	-	7.970
Theoretical plates	6782	8231
Tailing	1.147	1.096

Accuracy

To the pre-analyzed sample solution, a known amount of standard solution (usually 5-20%) was spiked at three different levels (50%, 100%, and 150%). These solutions were injected in three replicates and Percentage Mean Recoveries are determined for Metformin and Linagliptin which should lie between 98-102%. The results are described in table 3, 4 and fig. 6, 7 and 8.

Table 3: Accuracy for Metformin

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	% recovery	Mean
50%	2045.00	4520363	3958.065	3958.42	100	100
50%	2045.00	4525808	3958.065	3958.42	100	
50%	2045.00	4524672	3958.065	3958.42	100	
100%	4090.88	9055238	7917.832	7930.00	100	100
100%	4090.88	9056005	7917.832	7930.00	100	
100%	4090.88	9053824	7917.832	7930.00	100	
150%	6135.0	13518335	11874.194	11838.50	100	100
150%	6135.00	13516919	11874.194	11838.50	99	
150%	6135.00	13568589	11874.194	11838.50	100	

Table 4: Accuracy for Linagliptin

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	% recovery	Mean
50%	2045.00	4358197	19.990	19.96	100	100
50%	2045.00	4356218	19.990	19.95	100	
50%	2045.00	4352141	19.990	19.93	100	
100%	4090.88	8707432	39.989	39.88	100	100
100%	4090.88	8700880	39.989	39.85	100	
100%	4090.88	8706209	39.989	39.87	100	
150%	6135.0	13056602	59.971	59.80	100	100
150%	6135.00	13022412	59.971	59.64	99	
150%	6135.00	13048584	59.971	59.76	100	

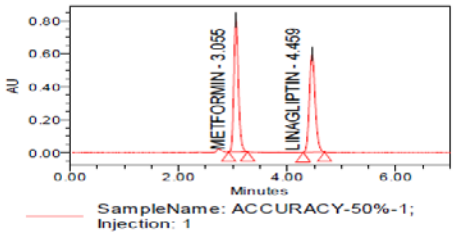

Fig. 6: chromatogram of 50% accuracy level

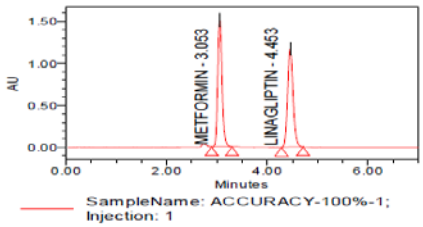

Fig. 7: chromatogram of 100% accuracy level

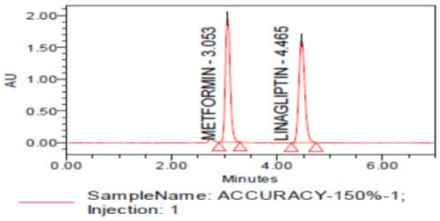

Fig. 8: chromatogram of 150% accuracy level

Precision

The precision of the method (Intra-day variation) was determined by repeatedly injecting the sample solution (8mg/ml of Metformin HCl and 0.5mg/ml Linagliptin) six times. The retention times and peak areas of six replicates are recorded. The precision is expressed as the % RSD of Peak areas and it should not be more than 2%. The results are described in table 5 and 6.

Table 5: Intraday precision

S. No.	Sample weight	Metformin	Linagliptin	% Assay (metformin)	% Assay (linagliptin)
1	4090.88	9058615	8707008	99	100
2	4090.88	9055892	8706154	99	100
3	4090.88	9058196	8705139	99	100
4	4091.88	9056969	8701982	99	100
5	4090.88	9053353	8700572	99	100
6	4090.88	9054070	8703774	99	100
Average Assay:				99	100
SD				0.02	0.03
%RSD				0.02	0.03

Table 6: Interday Precision

S. No.	Sample weight	Metformin	Linagliptin	% Assay (metformin)	% Assay (linagliptin)
1	4091	9058245	8702651	100	100
2	4091	9057486	8703148	100	100
3	4091	9058954	8705246	100	100
4	4091	9056847	8701431	100	100
5	4091	9055078	8706054	100	100
6	4091	9059834	8704297	100	100
Average Assay:		9057740.7	8704297	100	100
SD		1677	2234	0.02	0.03
%RSD				0.02	0.03

Linearity

The calibration curve was constructed by plotting peak area against concentration of solutions. Metformin and Linagliptin were found to be linear in the concentration range of 500-3000µg/mL (25 % to 150%) and 2.5-15µg/mL (25% to 150%) respectively. The results show that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The results of linearity were represented in tables 7. And the results for calibration curves are given in fig. 9 & 10.

Table 7: Linearity of Metformin and Linagliptin

Linagliptin and metformin Con. c%	Metformin area	Metformin µg/ml	Linagliptin area	Linagliptin µg/ml
25	2336359	500	2148043	2.5
50	4524579	1000	4354630	5
75	6788434	1500	6536100	7.5
100	9057249	2000	8704856	10
125	11379261	2500	10866198	12.5
150	13583129	3000	13086472	15.00

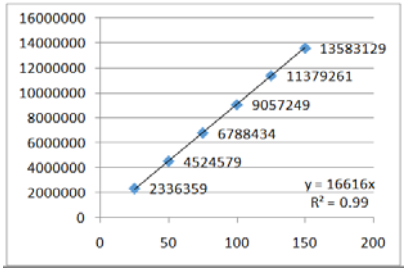

Fig. 9: Linearity Curve for Metformin

Robustness

The robustness of the method is determined under normal operating conditions different conditions such as change in flow rate and detection wave length. 10 µl of standard and sample solutions are injected by varying wavelength (282, 284, 286 nm) and the flow rate (0.8 ml/min, 1.0 ml/min, 1.2 ml/min) and the chromatograms are recorded and changes in parameters are observed. The results are shown in table 8 and 9

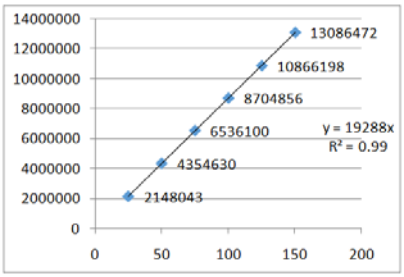

Fig. 10: Linearity curve for Linagliptin

Table 8: Robustness for Metformin

S. No.	Sample name	Change	RT	Area	Tailing	Plate count
1	Flow1	0.8 ml	3.810	11279449	1.211	7134
2	Flow 1	0.8 ml	3.805	11359450	1.210	6800
3	Flow 1	0.8 ml	3.804	11325956	1.230	6935
4	Flow 2	1.2 ml	2.546	7368090	1.158	5916
5	Flow 2	1.2 ml	2.544	7343308	1.179	5989
6	Flow 2	1.2 ml	2.542	7329988	1.191	6128
7	Temp 1	+5°C	3.048	8967960	1.194	6494
8	Temp 1	+5°C	3.052	8962415	1.173	6423
9	Temp 1	+5°C	3.047	8971075	1.256	6581
10	Temp 2	-5°C	3.041	8956081	1.158	6602
11	Temp 2	-5°C	3.034	8922707	1.198	6732
12	Temp 2	-5°C	3.045	9003965	1.155	6679

Forced degradation studies

Degradation studied is performed under different conditions like acid, base, peroxide, photo and Thermal. In each degradation study for both Metformin and Linagliptin it was observed that purity angle is less than the threshold value, it indicated the no interference of degradants with the drug peaks so the peak was said to be pure. Degradation studies reveal that the developed method was stability indicating hence, this method can easily and conveniently adopt for routine quality control analysis of Metformin and Linagliptin in pure and its pharmaceutical dosage forms. It was observed that there was marked degradation in the chromatograms, and the data given in Tables 10&11. Purity plots for Metformin (Fig.11a-11e) and Linagliptin (Fig.12a-12e) were shown.

Table 9: Robustness for Linagliptin

S. No.	Sample name	Change	RT	Area	Tailing	Plate count
1	Flow 1	0.8 ml	5.519	11117649	1.169	9036
2	Flow 1	0.8 ml	5.504	11133699	1.186	9236
3	Flow 1	0.8 ml	5.501	11149431	1.208	8958
4	Flow 2	1.2 ml	3.694	7214335	1.134	7607
5	Flow 2	1.2 ml	3.682	7215805	1.144	7584
6	Flow 2	1.2 ml	3.678	7191727	1.154	7446
7	Temp 1	+5°C	4.403	8828575	1.143	8352
8	Temp 1	+5°C	4.419	8802088	1.148	8290
9	Temp 1	+5°C	4.417	8821620	1.182	8012
10	Temp 2	-5°C	4.363	8832817	1.185	8753
11	Temp 2	-5°C	4.343	8813283	1.177	8447
12	Temp 2	-5°C	4.369	8872802	1.146	8560

Table 10: degradation studies for metformin

Mode of degradation	Conditions	Sample weight	Area	% Assay	Purity Angle	% Deg.	Purity Threshold
Control	No treatment	-	-	-	-	-	-
Acid degradation (5N HCl)	40°C/5 minutes	4091	7287975	80	-19	0.746	0.891
Alkali degradation (1N NaOH)	80°C/1 hours	4091	7681508	84	-15	0.818	1.062
Peroxide (30%W/V H_2O_2)	80°C/10 minutes	4091	7790730	85	-14	0.702	0.994
Light (200watts hrs/min)	105°C/72 hours	4091	8593479	94	-5	0.961	1.085
Heat (105°C/72hr)	25°C/72 hours	4091	8544169	94	-5	0.743	0.910

Table 11: Degradation studies for Linagliptin

Mode of degradation	Conditions	Sample weight	Area	% Assay	% Deg.	Purity Angle	Purity Threshold
Control	No treatment	-	-	-	-	-	-
Acid degradation (5N HCl)	40°C/5 minutes	4091	6973352	80	-20	9.048	14.724
Alkali degradation (1N NaOH)	80°C/1hour	4091	7367552	84	-16	9.563	16.016
Peroxide (30%W/V H_2O_2)	80°C/10 minutes	4091	7666328	88	-12	9.328	13.864
Light (200watts hrs/min)	105°C/72 hours	4091	8068628	92	-8	9.109	13.038
Heat (105°C/72hr)	25°C/72 hours	4091	8031372	92	-8	8.275	15.559

Purity Plots for Metformin

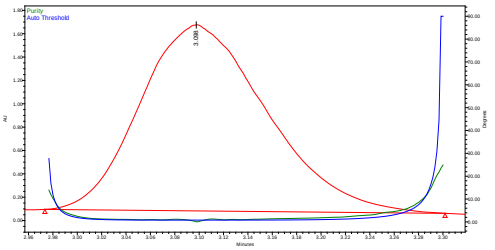

Fig. 11a: Acid degradation

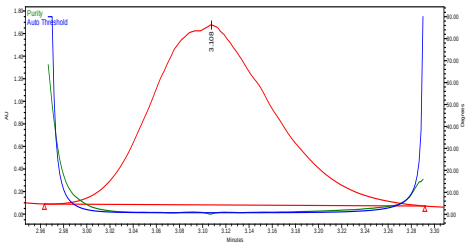

Fig.11 b: Alkali degradation

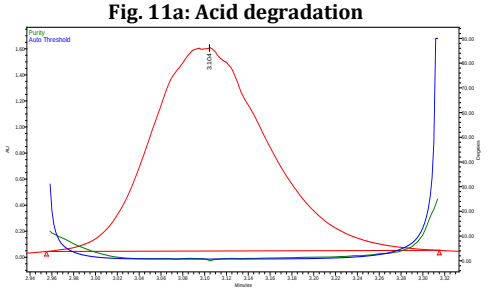

Fig. 11c: thermal degradation

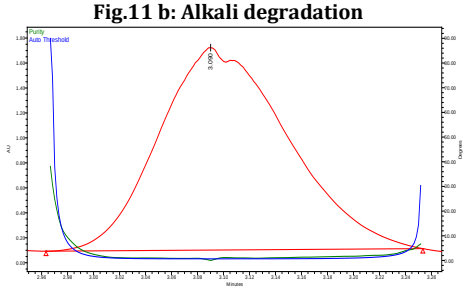

Fig. 11d: Peroxide degradation

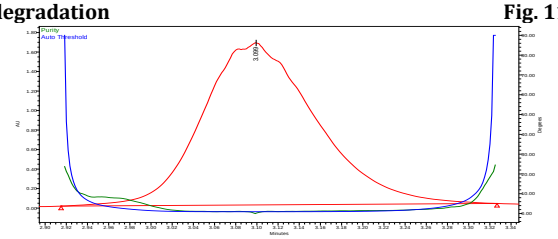

Fig. 11e: Photolytic degradation

Purity Plots for Linagliptin

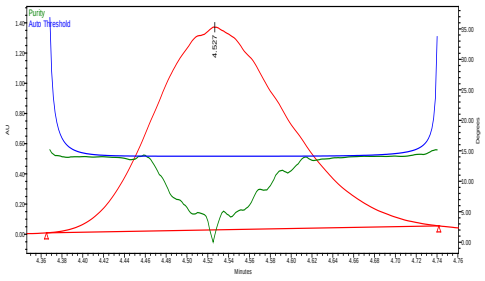

Fig. 12a: Acid degradation

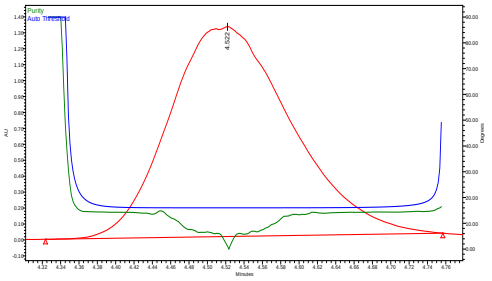

Fig. 12b: Alkali degradation

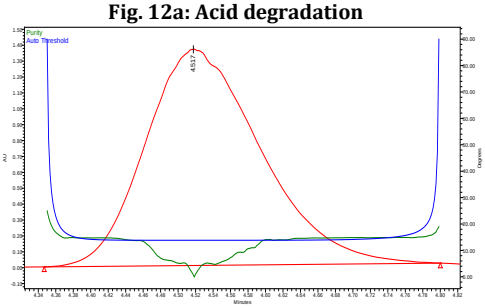

Fig. 12c: photolytic degradation

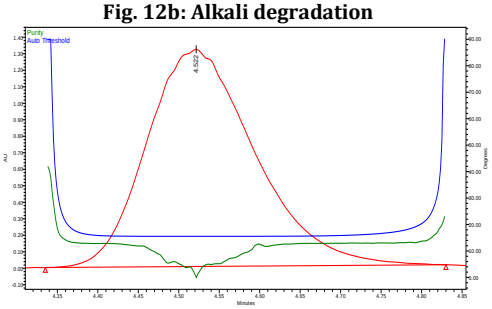

Fig. 12d: Thermal degradation

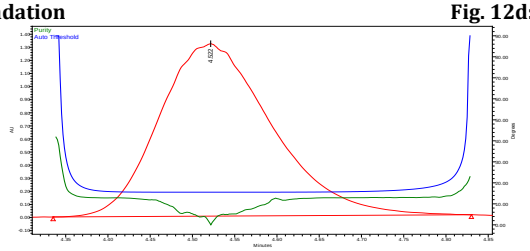

Fig. 12e: Peroxide degradation

CONCLUSION

The developed RP-HPLC method was developed and validated as per ICH guidelines in terms of specificity, accuracy, precision, linearity, robustness, limit of detection and limit of quantitation for the simultaneous quantitative estimation of Metformin and Linagliptin. The correlation coefficients were greater than 0.99 for both the drugs. The precision results were good enough to say that the method developed is precise and reproducible.

Accuracy studies revealed that mean recoveries after spiking experiments were between 99 and 101%, indicative of accurate method. Degradation studies reveal that the developed method was stability indicating hence, this method can easily and conveniently adopt for routine quality control analysis of Metformin and Linagliptin in pure and its pharmaceutical dosage forms.

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CONFLICT OF INTEREST

Declared None

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