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Research article

Validation of stability indicating high performance liquid chromatographic method for simultaneous determination of assay of Linagliptin and Metformin drugs in the pharmaceuticals tablet formulations using bupropion as a common internal standard

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Key words: Linagliptin, Metformin Hydrochloride, Bupropion Hydrochloride, High Performance Liquid Chromatographic, Force degradation studies, Assay.

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Abstract

Linagliptin is a DPP-4 inhibitor developed by Boehringer Ingelheim and used for the treatment of type II diabetes. Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM).Validation of stability indicating Simple, Specific, Precise, Accurate, Linear, Rugged, Robust High Performance Liquid Chromatographic method of analysis for simultaneous determination of assay of Linagliptin and Metformin drugs in the pharmaceuticals Tablet formulations using Bupropion as a common internal standard was performed. The assay was accomplished using a mixture of Ammonium phosphate buffer (pH 3.00), and methanol in the volume ratio of 40:60 v/v as mobile phase on an Inertsil ODS2, 150 mm x 4.6mm, 5µ as chromatographic column at a flow rate 0.800 mLmin-1and with a uv detector at a wavelength 233nm. The temperature of auto injector and column oven was 10^0C and 30^0Creceptively. The Injection volume of HPLC system kept as 30µL.linearity of the analytical method was evoluted at concentration range of 0.5123 µg/ml to 11.2299 µg/ml for Linagliptin and 159.6713 µg/ml to 3500.0690 µg/ml for Metformin respectively with Correlation coefficient (r) value more than 0.999.The LOD and LOQ was 0.1036µg/mL and 0.3140 µg/mL for Linagliptin and 46.4871µg/mL and 140.8700µg/mL for Metformin respectively. The retention time found to be 3.35 min for Linagliptin, 7.80min for Metformin and 3.00 min for Internal standard. Specificity, Method Precision, System Precision, Ruggedness, Robustness, Recovery, Stability of analytical solution, Filter paper selection study, Stress testing (Force Degradation) at various conditions were performed as per the ICH (Q2) recommendations. All the results were found within acceptance criteria.

Introduction

Linagliptin is a DPP-4 inhibitor developed by Boehringer Ingelheim and used for the treatment of type II diabetes. Linagliptin is a more potent inhibitor of DPP-4 than other drugs that belong to the same class Linagliptin is a competitive and reversible dipeptidyl peptidase (DPP)-4 enzyme inhibitor that slows the breakdown of insulinotropic hormone glucagon-like peptide (GLP)-1 for better glycemic control in diabetes patients. GLP and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones that increase the production and release of insulin from pancreatic beta cells and decrease the release of glucagon from pancreatic alpha cells. This results in a overall decrease in glucose production in the liver and increase an of insulin in a glucose-dependent manner. The chemical name is (R)-8-(3-Aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methylquinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione. The molecular formula for Linagliptin is C25H28N8O2. The molecular weight of Linagliptin is 472.55. Linagliptin is light yellowish-white to yellow-white and slightly hygroscopic solid substance. 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. The pKa of Linagliptin is 8.6 [1-7].

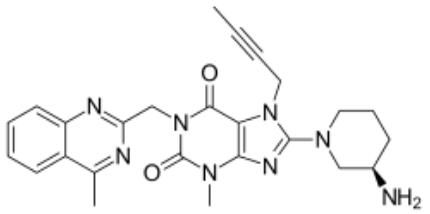

Figure 1. Chemical structure of Linagliptin.

Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin may induce weight loss and is the drug of choice for obese NIDDM patients. Use of metformin is associated with modest weight loss. When used alone, metformin does not cause hypoglycemia; however, it may potentiate the hypoglycemic effects of sulfonylureas and insulin. Its main side effects are dyspepsia, nausea and diarrhea. Dose titration and/or use of smaller divided doses may decrease side effects. Metformin should be avoided in those with severely compromised renal function (creatinine clearance < 30 ml/min), acute/decompensated heart failure, severe liver disease and for 48 hours after the use of iodinated contrast dyes due to the risk of lactic acidosis. Lower doses should be used in the elderly and those with decreased renal function. Metformin decreases fasting plasma glucose, postprandial blood glucose and glycosolated hemoglobin (HbA1c) levels, which are reflective of the last 8-10 weeks of glucose control. Metformin may also have a positive effect on lipid levels. In 2012, a combination tablet of linagliptin plus metformin hydrochloride was marketed under the name Jentadueto for use in patients when treatment with both linagliptin and metformin is appropriate.

The chemical name is 3-(diaminomethylidene)-1,1-dimethylguanidine hydrochloride. The molecular formula is $C_4H_{11}N_5.HCl$. The molecular weight of Metformin Hydrochloride is 165.63.The molecular weight of Metformin is 129.17. Metformin Hydrochloride is white to off-white powder and soluble in organic solvents such as methanol and is practically insoluble in acetone, ether and chloroform. The pKa of Metformin is 12.4 [1-7].

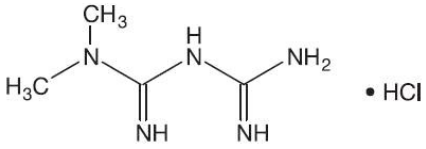

Figure 2. Chemical structure of Metformin Hydrochloride.

Bupropion Hydrochloride an antidepressant of the aminoketone class, is chemically unrelated to tricyclic, tetracyclic, selective serotonin re-uptake inhibitor, or other known antidepressant agents. The chemical name is (±)-1-(3-chlorophenyl)-2-[(1,1-dimethylethyl)amino]-1-propanone hydrochloride. The molecular formula is $C_{13}H_{18}ClNO.HCl$. The molecular weight of Bupropion Hydrochloride is 276.20 and that of free Bupropion is 239.74.Bupropion is white to powder and has a pKa of 8.22. Bupropion is very slightly soluble in water and soluble in organic solvents such as methanol [1-7].

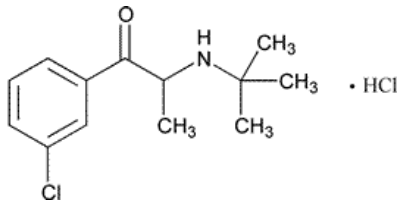

Figure 3. Chemical structure of Bupropion Hydrochloride.

While Reviewing Literature for analytical method of analysis it was observed that many methods have been reported for determination of linagliptin and metformin in combination and individually [5-23] but none of the reported HPLC methods have not been validated using internal standard to compensate any processing related and method related variability. Most of the published method is not performed stability-indicating studies (Acid, Alkali, Peroxide, Thermal, Photolytic, Humidity degradation) which is mandatory as per the ICH (Q2) recommendations.

The main objective of the work is to develop and validate stability indicating HPLC method of analysis which is Simple, Specific, Precise, Accurate, Linear, Rugged, Robust etc. for simultaneous determination of assay of Linagliptin and Metformin drugs in the pharmaceutical Tablet formulations using Bupropion as an common internal standard.

Experimental

Material and methods

Instrumentation

Shimadzu Prominence HPLC system equipped with dual pump, SIL-HTc auto-sampler with cooler, column oven, variable wavelength UV detector and a data acquisition system (Lab Solution Software) were used for the simultaneous determination of assay of Linagliptin and Metformin drugs in the pharmaceutical Tablet formulations using Bupropion as a common internal standard.

Reagents and Materials

The reagents used during analysis include Methanol [HPLC Grade], Water [Milli-Q /HPLC Grade], Ammonium phosphate (AR Grade), Phosphoric acid (GR Grade), Linagliptin (Batch No.LNG/A-499/025, Valid up to: May 2017), Metformin Hydrochloride (Batch No. MD00690113, Valid up to: 09 July 2017) and Bupropion Hydrochloride (Control No. 2-ED-0920812, Valid up to: 27 July 2017) were used obtained as a gift samples from Wockhardt and Lupin Pharmaceutical limited. Fixed dose combination tablets containing 2.5 mg Linagliptin and 1000 mg Metformin Hydrochloride of Lupin Ltd.(Batch

No.606917, Mfg. Date: Sept 2016, Exp. Date: Feb 2018) was purchased from Local medical, Aurangabad (Maharashtra).Ammonium phosphate Buffer, Rinsing solution, Mobile Phase was prepared by dissolving required volume and quantity of reagents and chemicals.

Analytical solutions

Stock solutions having concentrations approximately, 102.1829 µg/mL of Linagliptin in methanol, 7692.4594 µg/mL of Metformin in methanol and 1666.7305 µg/mL of Bupropion in methanol were prepared and solutions were filtered through 0.45µm nylon membrane filterwith discarding first 2 mL of the filtrate before use. The solution of bupropion was used as internal standard dilution solution during various experiments performed in an analytical method validation and assay calculations of pharmaceutical formulation.

Standard solutions having concentrations approximately, 3.75 µg/ml of Linagliptin, 1500.00 µg/mL of Metformin and 50.000µg/mL of Bupropion were prepared in mobile phase and use as a reference solution for related activities and system suitability. Filter the solution through 0.45µm nylon membrane filter with discarding first 2 mL of the filtrate before use.

Sample solution having concentrations 3.75 µg/ml of Linagliptin, 1500.00 µg/mL of Metformin and 50.000 µg/mL of Bupropion was prepared in mobile phase by dissolving a quantity of powder equivalent to Strength of 2.5 mg of Linagliptin and 1000 mg Metformin and use as a sample solution for related activities. Filter the solution through 0.45µm nylon membrane filter with discarding first 2 mL of the filtrate before use.

Results and Discussion

Method development

Primarily, numerous trials for optimization of method was performed using different mobile phases composition, different ratios of organic to buffer ,different organic solvents , different buffer with different pH, different stationary phases, different internal standards and variable chromatographic settings in an effort to achieve the finest peak resolution and separation between Linagliptin, Metformin and internal standard as depicted in Figure 4.

A summarized chromatographic condition was as follows:

Mobile phase: Methanol and Ammonium phosphate in water (60:40v/v),

Rinsing Solution: Methanol: Mill-Q water (60:60v/v)

Chromatographic Column: Inertsil ODS2, 150 mm x 4.6mm, 5µ

Wavelength:	233 nm
Column Oven Temperature:	30 ^{0}C
Sample cooler Temperature:	10 ^{0}C
Flow rate:	0.800 ml per minute
Injection Volume:	30 µl
Run Time:	10 minute
Retention Time (minute):	Linagliptin-5.35 Metformin-7.80 Bupropion-3.00

Analytical method validation

The Analytical method was optimized and validated in accordance with the current ICH guidelines and recommendations by means of a vision to accomplish Simple, Specific, Precise, Accurate, Linear, Rugged, Robust method [8-30].

Specificity

For the evaluation of specificity; Blank solution, placebo solutions, sample solution, standard solution in triplicate were injected into HPLC system No. interference was observed from blank solution and placebo at the retention time of chromatographic peak of Linagliptin, Metformin and internal standard. Peak purity was passes (purity angle was less than purity threshold) for Linagliptin and Metformin and % assay difference with respect to method precision was found 0.10% for Linagliptin and 0.30% for Metformin.

The typical chromatograms of various samples under optimized HPLC conditions was depictedin Figure 4.

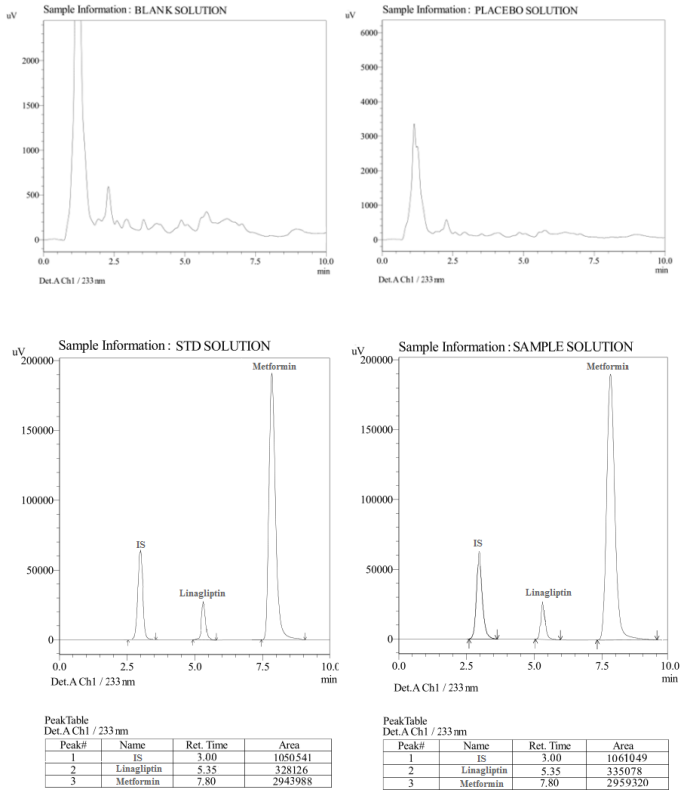

Figure 4. Typical chromatograms of Blank solution, Placebo solution, Sample solution & standard Solution

System Precision

Six replicates injections of standard solution was injected in to the HPLC system and the chromatograms and area ratio of Linagliptin to the Bupropion and Metformin to the Bupropion are recorded. % RSD for area ratio of Linagliptin to the Bupropion and Metformin to the Bupropion of six replicate injections of standard solution was found 0.79% and 0.15% respectively implies that system is precise as tabulated in table no.1.

Table 1. . Result of system precision

Injection No.	Area ratio (Linagliptinto Bupropion)	Area ratio (Metformin to Bupropion)
1	0.3113	2.8039
2	0.3111	2.8027
3	0.3107	2.7939
4	0.3123	2.8024
5	0.3120	2.8013
6	0.3126	2.8059
Mean	**0.31167**	**2.80168**
Standard Deviation	**0.000745**	**0.004122**
% R.S.D.	**0.24**	**0.15**

Method Precision

For the evaluation of Method precision of the analytical method, six samples from homogenous mixture of single batch were prepared as per the test procedure of methodology and analyzed on HPLC system .%RSD for % assay of Linagliptin and Metformin of six samples found was 0.16% and 0.15% as tabulated in Table no.2

Table 2. Result of method precision

Sample No.	% Assay of Linagliptin	% Assay of Metformin
1	99.1	99.7
2	99.3	99.4
3	99.5	99.5
4	99.1	99.6
5	99.2	99.8
6	99.4	99.7
Mean	**99.3**	**99.6**
Standard Deviation	**0.16**	**0.15**
% R.S.D.	**0.16**	**0.15**

Method Ruggedness

The ruggedness was evaluated through analysis of six samples from a homogenous mixture of single batch by different analyst by using different column, different system and different day. % RSD for % assay of ruggedness found was 0.10 % for Linagliptin and 0.32 % for Metformin and Overall % RSD found was 0.14 % for Linagliptin and 0.29 % for Metformin as tabulated in Table no. 3

Accuracy (Recovery)

Accuracy of the analytical method was evaluated at a known concentration of Linagliptin and Metformin at about 50%, 100% and 150% of test concentration of sample solution and 50% (1X Blend) and 150% (3x Blend) was calculated. % accuracy at individual level and overall average of % Recovery at all level for both Linagliptin and Metformin was found 99% to 100% as tabulated in table no. 4

Table 3. Result of ruggedness

Sr. No.	Linagliptin		Metformin	
	% Assay of Linagliptin Method precision	% Assay of Linagliptin Ruggedness	% Assay of Metformin Method precision	% Assay of Metformin Ruggedness
1	99.1	99.0	99.7	99.6
2	99.3	99.2	99.4	98.7
3	99.5	99.1	99.5	99.4
4	99.1	99.2	99.6	99.2
5	99.2	99.3	99.8	99.5
6	99.4	99.2	99.7	99.4
Mean	**99.3**	**99.2**	**99.6**	**99.3**
Standard Deviation	**0.16**	**0.10**	**0.15**	**0.32**
% R.S.D.	**0.16**	**0.10**	**0.15**	**0.32**
Overall Mean	**99.4**		**99.5**	
Overall S.D.	**0.14**		**0.29**	
Overall R.S.D.	**0.14**		**0.29**	

Table 4. Result of recovery

Spike level in %	Linagliptin				Metformin			
	% Recovery	Mean	SD	% RSD	% Recovery	Mean	SD	% RSD
50% (Assay)	99.2 99.5 99.4	**99.4**	**0.15**	**0.15**	99.7 99.6 99.5	**99.6**	**0.10**	**0.10**
100% (Assay)	99.3 99.1 99.4	**99.3**	**0.15**	**0.15**	99.8 99.7 99.6	**99.7**	**0.10**	**0.10**
150% (Assay)	99.5 99.7 99.4	**99.5**	**0.15**	**0.15**	99.9 99.8 99.8	**99.8**	**0.06**	**0.06**
50% (1X Blend)	**99.7** 99.3 99.7	**99.6**	**0.23**	**0.23**	**99.4** 99.2 99.3	**99.3**	**0.10**	**0.10**
150% (3X Blend)	99.9 99.8 99.9	**99.9**	**0.06**	**0.06**	99.9 100.0 99.9	**99.9**	**0.06**	**0.06**
Overall Mean	**99.5**				**99.7**			
Overall SD	**0.25**				**0.24**			
Overall % RSD	**0.25**				**0.24**			

Linearity

For the evolution of the linearity of the analytical method, a mixture of standard solution of Linagliptin and Metformin in a concentration range of 0.5123 µg/ml to 11.2299 µg/ml for Linagliptin and 159.6713 µg/ml to 3500.0690 µg/ml for Metformin respectively were prepared as per the test procedure of methodology and analyzed on the HPLC system.

Correlation coefficient (r) value for Linagliptin and Metformin using a regression equation with a 1/ (concentration^2) of weighting factor was calculated.

Correlation coefficient (r) valuewas found 0.99942 for Linagliptin and 0.99990 for Metformin. Lower limit of Detection (LOD) and Lower limit of Quantification (LOQ) was calculated using following formulas.

Limit of detection (LOD) =3.3 X S.D. of Y intercept / Slope of the calibration curve.

Limit of Quantification (LOQ) =10 X S.D. of Y intercept / Slope of the calibration curve.

The LOD and LOQ for Linagliptin were0.1036 µg/ml and 0.3140 µg/ml.

The LOD and LOQ for metformin were 46.4871 µg/ml and 140.8700 µg/ml.

The linearity plot was depicted in Figure 5 for Linagliptin and Metformin.

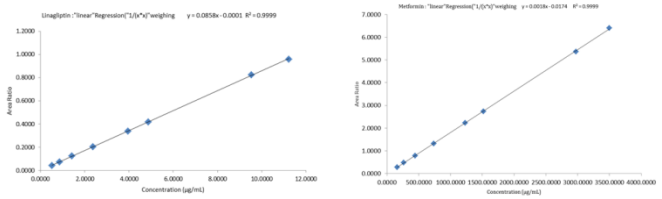

Figure 5. Linearity plot for Linagliptin and Metformin.

Results was tabulated in table no.5.

Table 5. Result of linearity

Sample no.	Linagliptin		Metformin	
	Conc. in µg/mL	Area Ratio	Conc. in µg/mL	Area Ratio
1	0.5123	0.0435	159.6713	0.2837
2	0.8539	0.0733	266.1188	0.4742
3	1.4231	0.1234	443.5313	0.7879
4	2.3718	0.2027	739.2188	1.3207
5	3.9530	0.3371	1232.0310	2.2353
6	4.8682	0.4181	1517.2800	2.7414
7	9.5454	0.8239	2975.0590	5.3607
8	11.2299	0.9601	3500.0690	6.4014
Slope	**0.0858**		**0.0018**	
Intercept	**0.00010**		**-0.00174**	
CC (r)	**0.9999**		**0.99990**	

The results of the linearity confirmed that an excellent correlation was exists between area ratio and concentration of both drugs within the concentration range.

Stability in analytical solution

For the evolution of stability in analytical solution; standard solution and sample solution was prepared freshly injected on the HPLC system at initially and different time intervals up to 50 hours and 54 hours respectively and the results of standard solution and sample solution were recorded. Absolute % difference and similarity factor were calculated.

For sample solution; absolute % difference between the initial result and results obtained at different time

intervals was found 0.30 % for Linagliptin and 0.60% for Metformin.
For standard solution; similarity factor between the initial result and results obtained at different time intervals was found 99.6 for Linagliptin and 99.5 for Metformin.
The sample solution and standard solution are stable up to 50 hours and 54hours respectively on bench top at room temperature.

Filter paper study

Filter paper study was performed to measure the analysis impact of filter paper used during various experiments of analytical method validation. For the evolution of the filter paper study of the analytical method, standard solution was prepared as per test procedure of methodology and distributed the standard solution in two different portions. One portion centrifuged at 4000 rpm for 5 minutes and second portion was filter through 0.45-µm nylon membrane filter with discarding first 2mL of the filtrate and all the samples were analyzed on HPLC system.
Similarity factor between as such standard solution and filtered standard solution was found 100.9 for Linagliptin and 100.9 for Metformin.
% absolute difference between average % assay of centrifuged sample solution and filtered sample solution was found 0.20% for Linagliptin and 0.20% for Metformin.
Form the results it was concluded that the 0.45-µm nylon membrane filter with discarding first 2mL of the filtrate is suitable for the determination of the Assay Linagliptin and Metformin in tablet formulation.

Forced degradation study

Forced degradation study was performed by treating sample tablet of 2.5MG/1000MG strength containing 1000 mg Metformin Hydrochloride and 2.5 mg Linagliptin under acidic, basic, peroxide, thermal, photolytic and humidity conditions but somewhat degradation of the Linagliptin observed under peroxide stress condition and slightly acidic degradation detected for Metformin as tabulated in table no. 6.

Table 6. Results of force degradation

Degradation Condition	% Degradation	
	Linagliptin	Metformin
AcidTreated	1.1	4.4
AlkaliTreated	1.3	3.3
PeroxideTreated	5.8	1.1
Thermal Treated	0.9	0.3
Photolytic Treated	0.7	0.4
Humidity Treated	0.3	0.5

Method robustness

Robustness of the analytical method was evaluated by accomplishment of analysis under marginally changed in the chromatographic method of analysis such as change in detection wavelength, change in flow rate, change in composition of the mobile phase and change in column oven temperature, and the assay results were compared with the assay result of method precision i.e. with finalized chromatographic conditions. The analytical method used is robust for change in flow rate, change in column oven temperature, and change in wavelength and change organic component of mobile phase.
The result was tabulated in table no. 7 and in table no. 8.

Table 7. Result of robustness for linagliptin

Sr. No	Method precision	Minus Flow	Plus Flow	Minus Temp	Plus Temp	Minus Organic comp. (MeOH)	Plus Organic comp. (MeOH)	Minus Wavelength	Plus Wavelength
1	99.1	99.0	99.3	98.9	99.2	99.0	98.9	99.1	99.0
2	99.3	98.9	99.0	99.1	98.9	98.8	99.2	99.3	98.8
3	99.5	99.1	99.1	99.4	99.5	98.9	98.8	99.4	99.3
4	99.1								
5	99.2	Not applicable							
6	99.4								
Overall mean		**99.2**	**99.2**	**99.2**	**99.2**	**99.1**	**99.2**	**99.3**	**99.2**
Overall SD		**0.19**	**0.16**	**0.19**	**0.20**	**0.23**	**0.22**	**0.15**	**0.21**
Overall %RSD		**0.19**	**0.16**	**0.19**	**0.20**	**0.23**	**0.22**	**0.15**	**0.21**

Table 8. Result of robustness for metformin

Sr. No	Method precision	Minus Flow	Plus Flow	Minus Temp	Plus Temp	Minus Organic comp. (MeOH)	Plus Organic comp. (MeOH)	Minus Wavelength	Plus Wavelength
1	99.7	99.3	99.1	99.2	99.7	99.4	99.4	99.6	99.5
2	99.4	99.2	99.3	99.7	99.4	99.6	99.6	99.1	99.3
3	99.5	99.5	99.4	99.3	99.5	99.5	99.2	99.3	99.0
4	99.6								
5	99.8	Not applicable							
6	99.7								
Overall mean		**99.5**	**99.5**	**99.5**	**99.6**	**99.6**	**99.5**	**99.5**	**99.5**
Overall SD		**0.20**	**0.22**	**0.21**	**0.15**	**0.14**	**0.19**	**0.22**	**0.24**
Overall % RSD		**0.20**	**0.22**	**0.21**	**0.15**	**0.14**	**0.19**	**0.22**	**0.24**

Range

From the analytical procedure data of precision, accuracy and linearity, the range of the analytical method used for simultaneous determination of assay of Linagliptin and Metformin drugs in the pharmaceutical Tablet formulations using Bupropion as a common internal standard was tabulated in table no. 9.

Table 9. Result of range

Name of Analyte (s)	Concentration (µg/mL)
Linagliptin	0.5123 µg/ml to 11.2299 µg/ml
Metformin	159.6713 µg/ml to 3500.0690 µg/ml

Analysis of Marketed Products

The potency test of marketed tablet products were performed after the complete validation of the method for simultaneous determination of assay of Linagliptin and Metformin drugs in the pharmaceutical Tablet formulations using Bupropion as a common internal standard were performed by the proposed validated method.

The potency of tested brands was found to be within the limit of 98.00-102.00%. The results are tabulated in table no. 10.

Table 10. Result of potency of marketed products

Sr. No.	Brandname code	Linagliptin Label Claimed (mg)	Linagliptin Amount found (mg)	Linagliptin Potency (%)	Metformin Label Claimed (mg)	Metformin Amount found (mg)	Metformin Potency (%)
1	Lina and Met **A**	2.5	2.52	100.6	1000	1008.5	100.9
2	Lina and Met **B**	2.5	2.51	100.1	850	853.5	100.4
3	Lina and Met **C**	2.5	2.53	100.9	500	503.0	100.5

Conclusions

This is the first reported High Performance Liquid Chromatographic method developed used for simultaneous determination of assay of Linagliptin and Metformin drugs in the pharmaceuticals Tablet formulations using Bupropion as a common internal standard was stability indicating as recommended by ICH guidelines and validated for Specificity, System precision, Method precision, Ruggedness, Robustness, Accuracy etc. The present analytical method has a widespread linear concentration range augmenting its applicability to different strength of Linagliptin and Metformin tablet formulations. The chromatographic method may also be applied for simultaneous estimation of analytes in plasma, serum, urine after using appropriate sample extraction technique. Thus the method is Simpler, Accurate and Economical as compare to the previous methods.

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