Formulation Analytical Method Development And Validation Of Anti- Diabetic Drugs

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Abstract- Rapid and accurate HPLC method has been developed for the simultaneous estimation of Linagliptin and Lobeglitazone in tablet dosage form. Chromatographic separation of both anti-diabetic drugs was achieved on a Hypersil BDS C18 column (250 mm × 4.6 mm, 5 µm) using a mobile phase consisting of 760 mL methanol and 240 mL of buffer (0.2% FTA in water), at a flow rate of 1.0 mL/min. Detection was carried out at 275 nm. The separation was achieved in less than 10 minutes. The method was validated as per ICH guidelines for parameters including linearity, accuracy, precision, system suitability, specificity, robustness, limit of detection (LOD), limit of quantitation (LOQ), and range. Linearity, accuracy, and precision were found to be within acceptable limits over the range of 0.5–3.0 µg/mL for Lobeglitazone and 5–30 µg/mL for Linagliptin. The method proved to be sensitive, specific, and suitable for routine quality control of these drugs in pharmaceutical formulations.

Keywords: *High-Performance Liquid Chromatography, Linagliptin and Lobeglitazone, Anti-diabetic drugs, Pharmaceutical formulations, Method validation.*

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from defects in insulin secretion, insulin action, or both. In recent years, combination therapy using oral hypoglycaemic agents has gained attention for better glycaemic control and reduced side effects. Diabetes not only affects glucose metabolism but also leads to long-term complications involving the heart, kidneys, eyes, and nerves. According to the World Health Organization, diabetes is a major cause of blindness, kidney failure, heart attacks, stroke, and lower limb amputation. The chronic nature of the disease and its complications place a substantial burden on healthcare systems worldwide (World Health Organization). Type 2 diabetes is strongly associated with lifestyle factors such as physical inactivity, unhealthy diet, and obesity. Early diagnosis and effective treatment are essential to prevent or delay the onset of complications. Oral hypoglycaemic agents (OHAs) are widely used in type 2 diabetes management, and their combination therapies have shown enhanced therapeutic benefits (DeFronzo et al). Recent pharmacological advancements have led to the development of novel drug classes like DPP-4 inhibitors (e.g., Linagliptin) and thiazolidinediones (e.g., Lobeglitazone). These agents not only reduce blood glucose levels but also exhibit protective effects on beta-cell function and insulin sensitivity, making them suitable for long-term management (Nauck and Meier).

Linagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor used in the management of type 2 diabetes mellitus. It works by inhibiting the DPP-4 enzyme, which degrades incretin hormones such as GLP-1 (glucagon-like peptide-1). These incretins help stimulate insulin release and suppress glucagon secretion in a glucose-dependent manner. Linagliptin has a unique pharmacokinetic profile as it is primarily excreted via bile and faces, requiring no dose adjustment in patients with renal impairment.

Lobeglitazone is a novel thiazolidinedione (TZD) that acts as a selective agonist of peroxisome proliferator-activated receptor gamma (PPAR-γ). It improves insulin sensitivity in adipose tissue, muscle, and the liver. Unlike older TZDs, Lobeglitazone is effective at a lower dose (0.5 mg) with a favourable safety profile. It also exhibits potential anti-inflammatory and anti-atherogenic effects, making it a promising candidate for combination therapy in diabetes.

When used together, Linagliptin and Lobeglitazone provide complementary mechanisms—enhancing insulin secretion and improving insulin sensitivity—resulting in improved glycemic control with a reduced risk of hypoglycaemias.

MATERIALS AND METHODS

Chemicals And Reagent

Linagliptin and Lobeglitazone were obtained as complimentary samples from Yarrow Chem Products, Mumbai, India. High-performance liquid chromatography (HPLC) grade solvents and reagents, including water, methanol, acetonitrile, and acetic acid, were used throughout the study. Tri-fluor acetic acid (TFA) was employed as a modifier in the mobile phase preparation. All chemicals and solvents used were of analytical or HPLC grade and required no further purification before use.

Instrumentation

The High-Performance Liquid Chromatography (HPLC) system used for the analysis was a Younglin HPLC system, integrated with a UV detector (Model: 730D). Chromatographic separation was achieved using a Hypersil BDS C18 analytical column having dimensions of 250 mm × 4.6 mm with a 5 µm particle size. The entire system operation and data processing were controlled using Autochrom 3000 software. Manual injection mode was used for sample loading. A pH meter (M Lab) was employed for buffer and mobile phase pH adjustments. The weighing of samples and standards was carried out using a Shimadzu analytical balance (Model: ATX224). UV-Visible spectroscopic analysis was performed using a Shimadzu UV-1800 spectrophotometer (Japan Corporation).

An ultra sonicator (RC-System MU-1700, Serve Well Instruments) was used to ensure proper dissolution and degassing of the mobile phase and samples.

Preparation of Mobile Phase

Prepared Homogeneous mixture of 760ml HPLC grade Methanol and 240 ml of Buffer Shake well, filter this solution through 0.2 um membrane filter paper. And sonicate this mobile phase for about 5 minutes.

Preparation of Stock Solution:

Weigh accurately 50 mg of Linagliptin standard and 5 mg of Lobeglitazone standard. Transfer in 100 ml volumetric flask, Add about 90 ml of diluent to the volumetric flask, Shake well and sonicate for 5 min to get completely dissolve shake well and sonicate for 5 min make-up the volume to 100 ml. Pipet-out 2 ml from stock solution and transferred to 20ml flask to volumetric flask.

Standard solution:

Pipette out 2ml from the stock solution and Dilute it into 20ml with the help of Diluent and shake well. Then Sonicate it for 2min and filter it through Used 0.2 um syringe filter before used.

Method Development

After finishing four experimental trials with variations in run time, column and mobile phase the drug observed to be in good peak shape at fourth trial. The % RSD, Tailing Factor and Theoretical plate shows that the drug is within the acceptance criteria. The approach was found to be satisfactory Optimized Chromatographic Condition.

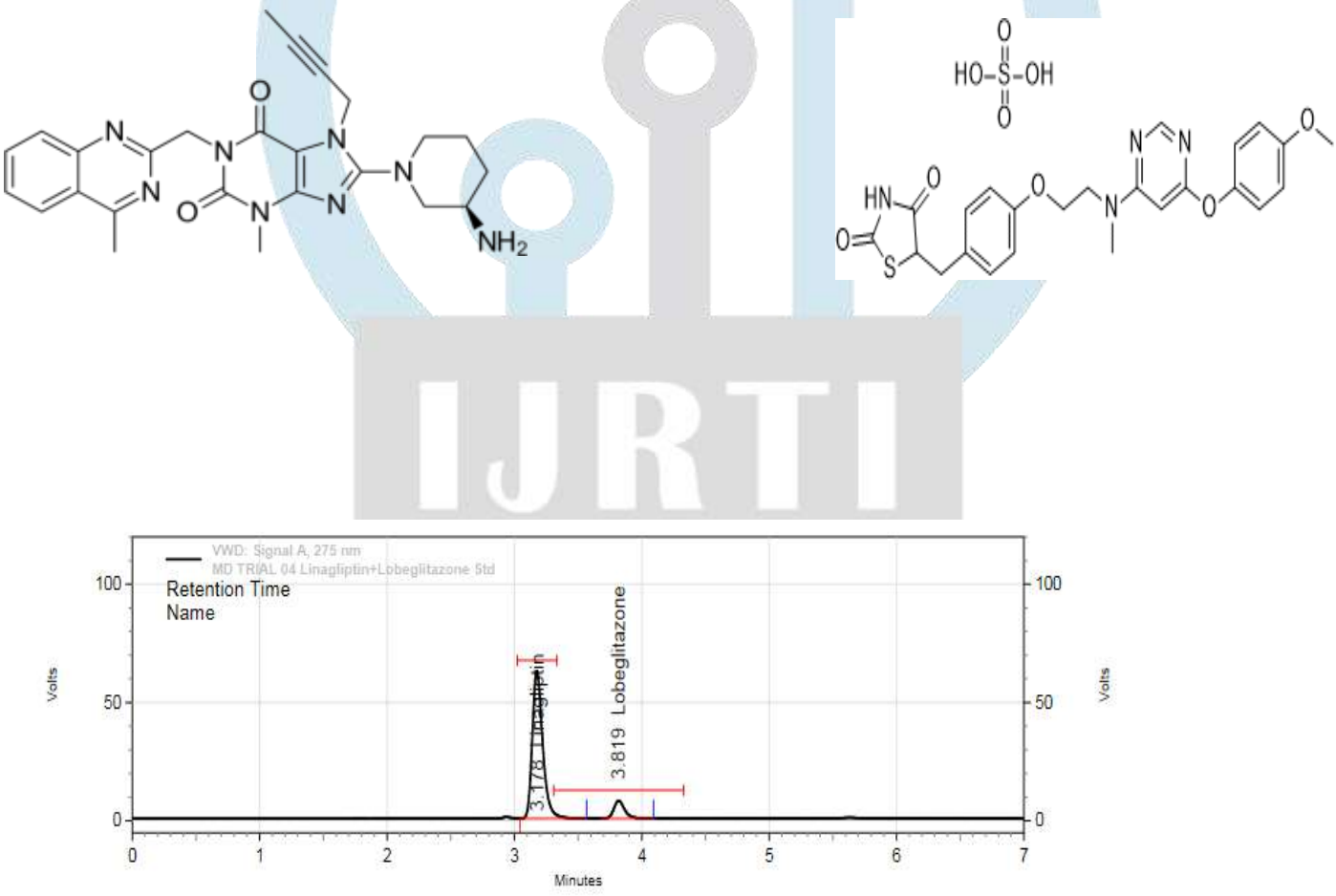


Fig 3: Chromatogram of optimized method for Linagliptin and Lobeglitazone

Result and Discussion

Parameters	Result
Mobile Phase	MeOH :Buffer ; 76:24
Column	Hypersil, C18 (250mm x 4.6 ID, Particle size :5 um)
Flow rate	1.00ml/min
Injection volume	20ul
Temperature	Ambient
Wavelength	275
Run time	7 min
Elution Mode	Isocratic
Diluent	Mobile Phase

Specificity

Specificity was evaluated by chromatograms of mobile phase blank, placebo solution, standard solution of Linagliptin and lobeglitazone and its sample solution.

Sr. No	Solution	
1	Blank	0.00
2	Plasma Blank	0.00
3	Linagliptin Standard Sample	3.178
4	Linagliptin Test Sample	3.173
5	Lobeglitazone Standard Sample	3.805
6	Linagliptin Test Solution	3.810

Table 2: Specificity of Linagliptin and Lobeglitazone

There is no interference from the blank and placebo at the retention time of Linagliptin chromatographic peak. Retention time for Linagliptin in standard solution, Test solution, individual solution are matching with each other. So this system is specific for the analysis of Linagliptin, hence specificity is justified.

System Suitability

These parameters were shown to be within specified limits. Column efficiency (theoretical plates), resolution factor and peak asymmetry factor, tailing factor, LOD, LOQ are the system suitability parameters. These parameters of the optimized methods were found satisfactory.

Standard Solution in order to conduct the test.

Name	Area	RT (min)	TP(NLP2000)	TF(NMT2)	Resolution (NLT-2)
Standard_Inj_01	5986354	3.173	7714	1.43	4.26
Standard_Inj_02	5987268	3.168	7735	1.38	4.27
Standard_Inj_03	5991506	3.173	7745	1.42	4.26
Standard_Inj_04	5983916	3.173	7759	1.42	4.24
Standard_Inj_05	5983916	3.172	7739	1.38	
Mean	5987700.40	3.172			
SD	2910.8931	0.0022			
%RSD(NMT2)	0.5	0.07			

Table 3: System Suitability Study for Linagliptin

Theoretical plate ,resolution and Telling factor observed within acceptance criteria, also %RSD of replication injections for area and retention time observed within acceptance criteria , hence system is suitable for analysis of Linagliptin, Hence System suitability is justified.

Name	Area	RT (min)	TP(NLP2000)	TF(NMT2)	Resolution (NLT-2)
Standard_Inj_01	736837	3.800	10319	1.37	4.26
Standard_Inj_02	736743	3.795	10324	1.35	4.27
Standard_Inj_03	739805	3.800	10290	1.34	4.26
Standard_Inj_04	737267	3.795	10374	1.35	4.24
Standard_Inj_05	735356	3.795	10317	1.34	4.24
Mean	737202	3.797			
SD	1622.602	0.0027			
%RSD	0.22	0.07			

Table 4: System Suitability Study for Lobeglitazone

Theoretical plate ,resolution and Telling factor observed within acceptance criteria, also %RSD of replication injections for area and retention time observed within acceptance criteria , hence system is suitable for analysis of Lobeglitazone, Hence System suitability is justified.

Linearity

Linearity for Lobeglitazone And Linagliptin The linearity graph of average peak area at each level against the concentration in µg/ml is plotted and found to be straight line graph. This method proved to be linear between µg/ml of Linagliptin and Lobeglitazone, with a typical Linearity curve of correlation equation.

Con. (ppm or ug/ml)	Area
25.0	2993019
37.5	4493165
50.0	5959407
62.5	7425311
75.0	8943011
Correlation coficient	NLT 0.995
Intercept	29931
Slope	118657

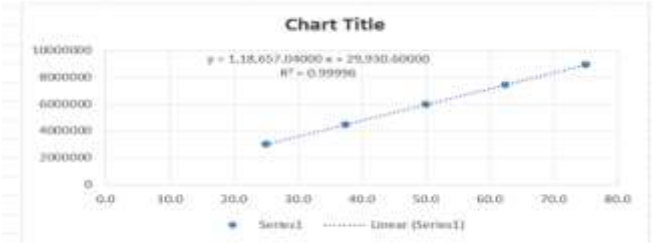


Fig: Linearity Curve for Linagliptin

Table 5: Calibration Standards Peak Area

Con. (ppm or ug/ml)	Area
2.50	365606
3.75	556174
5.00	732658
6.25	911370
7.50	1098819
Correlation coficient	NLT 0.995
Intercept	4277
Slope	145730

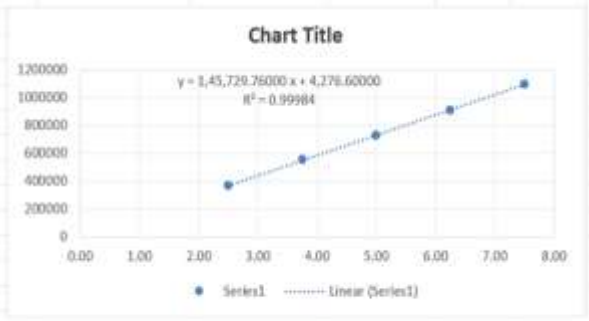


Fig: Linearity Curve for Lobeglitazon

Table 6: Calibration Standards Peak Area

Correlation coefficient observe within acceptance criteria, hence method is linear and linearity is justified.

LOD and LOQ

The LOD is the lowest concentration of the analyte that can be detected & LOQ is the lowest concentration that can be quantitatively measured based on the steyx and the slope. The LOD and LOQ were calculated using the following formulas: LOD 3.30/s and LOQ-100/%

Con. (ppm or ug/ml)	Area
25.00	19958250
37.50	29023390
50.00	38857564
62.50	48255312
75.00	57498797
STEYX	197751
SLOPE	754504
LOD (ug/ml)	0.86
LOQ (ug/ml)	2.62

Table 7: LOD & LOQ of Linagliptin

Con. (ppm or ug/ml)	Area
2.50	917839
3.75	1366071
5.00	1821418
6.25	2279434
7.50	2723200
STEYX	4110
SLOPE	361927
LOD (ug/ml)	0.04
LOQ (ug/ml)	0.11

Table 8: LOD &LOQ of Lobeglitazone

Accuracy

For accuracy studies, samples were prepared at three concentration levels: Low (LQC), Medium (MQC), and High (HQC) Quality Controls. Concentration of each injection was calculated and the standard deviation between the readings is calculated.

Name	Preparation	Area	Amount Added ug/ml	Amount recovered ug/ml	Recovery (97-103) %
Accuracy at 80%	Prep- 1	591048	4.10	4.01	97.77
Accuracy at 80%	Prep- 2	584981	4.00	3.97	99.19
Accuracy at 80%	Prep- 3	591734	4.10	4.01	97.89
Accuracy at 100%	Prep- 1	734077	5.00	4.98	99.58
Accuracy at 100%	Prep- 2	732365	4.90	4.97	101.37
Accuracy at 100%	Prep- 3	731845	5.00	4.96	99.27
Accuracy at 120%	Prep- 1	892533	6.10	6.05	99.24
Accuracy at 120%	Prep- 2	903701	6.10	6.13	100.48
Accuracy at 120%	Prep- 3	802416	6.00	5.98	99.75

	Mean % recovery	SD	% RSD (NMT 2)
Accuracy at 80 %	100.06	1.0206	1.02
Accuracy at 100 %	99.38	0.0206	0.68
Accuracy at 120 %	100.21	0.2387	0.24

Name	Preparation	Area	Amount Added ug/ml	Amount recovered ug/ml	Recovery (97-103) %
Accuracy at 80%	Prep- 1	2591048	4.10	4.01	99.77
Accuracy at 80%	Prep-	584981	4.00	3.97	99.197
Accuracy at 80%	Prep- 3	591734	4.10	4.01	97.89
Accuracy at 100%	Prep- 1	734077	5.00	4.98	99.58
Accuracy at 100%	Prep- 2	732365	4.90	4.97	101.37
Accuracy at 100%	Prep- 3	731845	5.00	4.96	99.27
Accuracy at 120%	Prep- 1	892533	6.10	6.05	99.24
Accuracy at 120%	Prep- 2	903701	6.10	6.13	100.48
Accuracy at 120%	Prep- 3	882416	6.00	5.98	99.75

	Mean % recovery	SD	% RSD (NMT 2)
Accuracy at 80 %	98.28	0.7866	0.80
Accuracy at 100 %	100.07	1.1340	1.13
Accuracy at 120 %	99.82	0.6241	0.63

Precision

Precision can be determined by two types: 1) Intraday precision 2) Interday precision

The preparation was injected into HPLC four times and mean peak area was calculated separately for each concentration and from that precision percentage RSD values were calculated.

Table :- Interday Precision Data of Linagliptin

Name	Preparations	% Assay
Day-1	prep-1	99.97
	prep-2	99.19
Day-2	prep-1	100.30
	prep-2	99.36
Mean		99.71
SD		0.5191
% RSD (NMT 2)		0.52

Table :- Interday Precision Data of Lobeglitazon

Name	Preparations	% Assay
Day-1	prep-1	99.99
	prep-2	99.77
Day-2	prep-1	99.08
	prep-2	99.12
Mean		99.49
SD		0.4595
% RSD (NMT 2)		0.46

Table 13: Intraday Precision Data of Linagliptin

Name	Preparations	% Assay
Set-1	prep-1	99.97
	prep-2	99.19
Set-2	prep-1	99.39
	prep-2	98.91
Mean		99.37
SD		0.4488
% RSD (NMT 2)		0.45

Table 13:Intraday Precision Data of Lobeglitazone

Name	Preparations	% Assay
Set-1	prep-1	99.99
	prep-2	99.77
Set-2	prep-1	98.81
	prep-2	100.63
Mean		99.80
SD		0.7541
% RSD (NMT 2)		0.76

Overall % RSD for Intraday and Interday results were observed within the acceptance criteria. Thus, the developed method is found to be precise, hence precision is justified.

Robustness

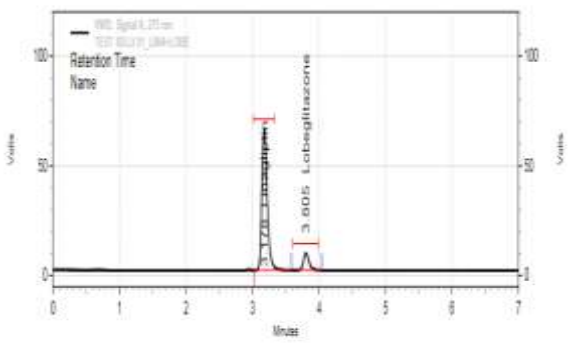
Robustness was attempted by deliberately changing the chromatographic conditions to evaluate the difference in Flow Rate and Buffer concentration. Robustness was studied for Linagliptin and Lobeglitazone, results obtained was displayed in following Tables.

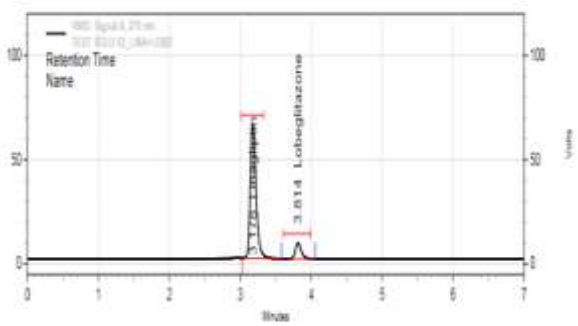
Name	Preparations	%Assay
Robustness change in method parameters		
Original method parameters	Test prep-1	99.97
Original method parameters	Test prep-2	99.19
Pump, Flow 1.1 ml/min	Test prep	99.94
Pump, Flow 0.9 ml/min	Test prep	98.88
MeOH:Buffer, 71:29	Test prep	99.51
MeOH:Buffer, 81:19	Test prep	100.48
Mean		99.66
SD		0.5829
%RSD (NMT 2)		0.58

Name	Preparations	%Assay
Robustness change in method parameters		
Original method parameters	Test prep-1	99.99
Original method parameters	Test prep-2	99.77
Pump, Flow 1.1 ml/min	Test prep	100.04
Pump, Flow 0.9 ml/min	Test prep	99.22
MeOH:Buffer, 71:29	Test prep	98.05
MeOH:Buffer, 81:19	Test prep	101.21
Mean		99.71
SD		1.0427
%RSD (NMT 2)		1.05

Overall % RSD of results with change in pump flow rate and change in mobile phase composition observed within acceptance criteria method is robust in terms of slight change in internal method parameters, hence Robustness is justified.

Analysis of Formulation:-





Conclusion

A simple, accurate, precise, and selective isocratic RP-HPLC method was successfully developed and validated for the simultaneous estimation of Linagliptin and Lobeglitazone in tablet dosage form. The method was validated as per ICH guidelines and demonstrated satisfactory results for key parameters such as linearity, accuracy, precision, specificity, LOD, LOQ, and robustness. The use of a cost-effective mobile phase (0.2% formic acid in HPLC-grade water) and detection at 275 nm (isosbestic point) ensured high sensitivity and clear peak resolution within a short retention time. The method is well-suited for routine quality control of formulated anti-diabetic combination tablets containing Linagliptin and Lobeglitazone.

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