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BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF LOBEGLITAZONE IN HUMAN PLASMA

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ABSTRACT

A simple, rapid, reliable, precise, accurate, sensitive and selective analytical method for the estimation of lobeglitazone. In human plasma and using as an internal standard (IS). Lobeglitazone is a novel thiazolidinedione (TZDs) based peroxisome proliferator-activated receptor (PPAR) agonist, used for the management of type-2 diabetes. After mixing the A, dissolved in acetonitrile, with a plasma sample containing lobeglitazone, The method was developed using acetonitrile-methanol-water (6:3:1, v/v) 10 µL of supernatant was injected into the HPLC system. The method showed good linearity Subsequently, serial dilutions of five different concentrations ranging between 3.12–50 ppm were made, ultrasonicated and then analysed as per the chromatographic condition in section 5.x. for Plasma ($r^2 \geq 0.9996$). The mean percent extraction recovery of lobeglitazone was 90.8 % for plasma. Freshly prepared stock solution of lobeglitazone (100 ppm) was analysed were tested and evaluated. The intra-day precision of plasma ranged from 0.233 ,0.290, 1% (RSD), respectively, and the inter-day precision of plasma ranged from 1.5 to 0.115 and 0.99, 1%, respectively. This method is simple, sensitive, and applicable for the pharmacokinetic study of lobeglitazone in human plasma. Most of the plasma concentrations of lobeglitazone were below the LLOQ because the lobeglitazone is extensively metabolized. The method was developed using acetonitrile-methanol-water (6:3:1, v/v). The peaks obtained for the drug of interest by the present method was symmetrical in nature with acceptable tailing factor and from the plasma endogenous proteins by Protein precipitation Extraction. The retention time of lobeglitazone was shorter and proves that the method is rapid.

I. INTRODUCTION

Analysis is found in almost every branch whether it is data analysis, market analysis or pharmaceutical analysis. Among these the pharmaceutical analysis deals with the quality of pharmaceutical products and ultimately life of the consumer. Another requirement for quality drug is that the regulatory and government agencies became stringent in case of poor-quality drug products. Quality of any drug product can be known by a series of tests starting from the testing of raw material, intermediates and finished products etc.

II. ANTI-DIABETIC MEDICATIONS IN GENERAL

Antidiabetic drugs are medicines developed to stabilise and control blood glucose levels amongst people with diabetes. Type 1 diabetes is a disease in which the body does not make enough insulin to control blood sugar levels. Type 1 diabetes was previously called insulin-dependent diabetes or juvenile diabetes. Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. type 2 diabetes during the early years of the disease, others with marked hyperglycemia and especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Uncontrolled diabetes may lead to stupor, coma and if not treated death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome [1,2,3]

Diabetes mellitus [4,5]: Diabetes is a chronic disease in which the body does not make or properly use insulin, a hormone that is needed to convert sugar, starches, and other food into energy by moving glucose from blood into the cells.

1. No insulin is being produced,
2. Insulin production is insufficient, and/or
3. The body is resistant to the effects of insulin.

Type I: Insulin-Dependent Diabetes Mellitus (IDDM), Juvenile onset diabetes mellitus: **Type II:** Non-Insulin-Dependent Diabetes Mellitus (NIDDM), maturity onset diabetes mellitus: In all type 1 cases, circulating insulin

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levels are low or very low and patients are more prone to ketosis. Patients with type 1 diabetes need to take insulin daily. This type is less common and has a low degree of genetic predisposition. [6,7]

INTRODUCTION TO DRUG ANALYSIS

Pharmaceutical Analysis is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter.

Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air[8,9] .

TYPES

There are mainly two types of chemical analysis:

1. Qualitative (Identification)
2. Quantitative (Estimation)

Pharmaceutical Analysis [10 ,11]:

Analytical methods are used for product research, product development, process control and chemical quality control purposes. Each of the techniques used in chromatographic or spectroscopic, have their own special features and deficiencies, which must be considered.

Need for pharmaceutical Analysis:

- ❖ New Drug Development
- ❖ Method Validation as for ICH Guidelines
- ❖ Research in Pharmaceutical Sciences
- ❖ Clinical Pharmacokinetic Studies

III. BIOANALYSIS IN PHARMACEUTICAL DRUG DEVELOPMENT [12]

Bioanalytical methods play a major role in estimating the drugs, interferences, metabolites from various matrices such as pure drug, dosage form, intermediates and biological fluids. Drug assay technology is now sufficiently advanced for it to be possible to measure the plasma concentration of majority of drugs used in clinical practices. They are useful to measure plasma concentration of drugs to confirm adequate dosage, to identify signs of possible drug toxicity, the response of patients to drug therapy and drug interactions.

Therapeutic efficacy of the particular drug can be known by bioanalysis. In pharma field bioanalysis plays a significant role. Bioanalysis involves the following steps.

- ❖ Selection and collection of biological fluid
- ❖ Preparation of sample -Analyte extraction from biological matrix
- ❖ Analyte detection done by various methods

DIFFERENT TERMINOLOGY USED IN BIOANALYTICAL DEPARTMENT [13]

Accuracy, Analyte, Analytical run, biological matrix, Calibration Standard, Internal standard, Matrix effect etc**.**

PROCEDURE FOR BIOLOGICAL SAMPLE EXTRACTION [14-15]

A sample can be cleaned up before analysis and/or concentrated to increase detection using a procedure called sample preparation. This process is referred to as "bioanalytical sample preparation" when samples are biological fluids like plasma, serum, or urine.

Different extraction techniques include liquid-liquid extraction, solid phase extraction, protein precipitation or denaturation, and dehydration techniques.

When samples are biological fluids such as plasma, serum or urine, this technique is described as bioanalytical sample preparation.

GENERAL TECHNIQUES FOR SAMPLE PREPARATION [16,17,18,19]

Dilution followed by injection--Solid Phase extraction--Protein precipitation--Filtration Liquid-liquid extraction.

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ANALYTICAL METHODS FOR QUANTITATIVE DETERMINATION OF DRUGS IN BIOLOGICAL FLUIDS

Chromatographic methods are mostly used and important for the estimation of drugs in biological samples. Chromatography derived from "chroma" means "color" and "graphein" means "to write". Separation of required analyte from the compounds by using the mobile phase and stationary phase is known as chromatography. Advanced methods for separation of samples from biological fluids are HPLC and their hyphenated methods like LC-MS; GC-MS [20].

DRUG ESTIMATION IN BIOLOGICL SAMPLES BY:

1. UV VISIBLE ABSORPTION SPECTROSCOPY: [21,22]

UV-Visible spectrometry: Spectroscopy is the study of matter's characteristics as a result of its interactions with various forms of electromagnetic radiation. Spectrometric Techniques refers to a broad category of analytical methods based on atomic and molecular spectroscopy. The measuring of the intensity of radiation using a photoelectric transducer or other forms of electronic equipment is referred to as spectrometry and spectrometric procedures.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

High-performance liquid chromatography (or High-pressure liquid chromatography, HPLC) is a specific from of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds. HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that move the mobile phase through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interaction between the stationary phase, the molecular being analysed and the solvents used. The sample to be analysed is introduced in small volume to the stream of mobi The time at time at which a specific analytic elutes (comes out of the column) is called the retention time.[23]

The choice of solvents, additives and gradsient depend on the nature of the stationary phase and the analytic.[24]

HPLC is one of the types of Chromatography. In modern pharmaceutical industries, HPLC is the major and integral analytical tool applied in all stages of drug discovery, development, and production[25]

INSTRUMENTATION OF HPLC [26-27]

Solvent delivery system …Pumps …Sample injection system ... Column …. Detectors … Data system

Analytical Method Validation:[28,29,30,31]The validity of a specific method should be demonstrated in laboratory experiment using sample or standards that are similar to the unknown samples that will be routinely analysed chromatographic method need to be validation before the first routine use.to obtained the most accurate results, all of the variables of the method should be considered, such a sampling, sample preparation, chromatographic separation, detection and data evaluation using the sample matrix as that of the intended sample. Parameters for Method Validation:

- Precision
- Accuracy
- Specificity
- Linearity
- Range
- Detection Limit
- Quantitation Limit
- Ruggedness
- Robustness

SYSTEMATIC LITERATURE SEARCH

A systematic review of the literature involving validated methods for quantitation of oral antidiabetic agent combinations in human blood was conducted. Systematic reviews guarantee that all publications on a particular subject will be included in the review. No restrictions regarding the analytical technique or publication date were imposed. For the search strategy, the descriptors were employed: “oral antidiabetic” and generic and brand name of the drugs acarbose, acetohexamide, alogliptin, buformin, canagliflozin, carbutamide, chlorpropamide, dapagliflozin, empagliflozin, glibenclamide, glibornuride, gliclazide, glimepiride, glipizide, gliquidone, glisoxepide, glyburide, glycopyramide, linagliptin, metformin, miglitol, nateglinide, pioglitazone,

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repaglinide, rosiglitazone, saxagliptin, septagliptin, sitagliptin, teneligliptin, tolazamide, tolbutamide, vildagliptin, and voglibose; "validtion", "quantitation", "therapeutic monitoring", "plasma", "blood", "serum", "human plasma", "human blood", "human serum" and the name of each analytical technique, combined with the Boolean operators "AND" and "OR". The search was conducted in the Medline, Web of Science and Scopus databases and was completed in October 2015. In addition, reference lists of the records were searched manually to retrieve any further articles. Records in non-Roman characters were excluded from the systematic review.

MATERIALS AND EQUIPMENTS

1.Active Drug:

lobeglitazone gifted by samples from Yarrow chem Ltd.

2.Solvents and Chemicals:

Methanol (HPLC Grade), Water (HPLC Grade), Human Plasma, Acetonitrile

Table 1: List of Instrument

Sr.no.	Name of Instrument	Model
1.	HPLC	AGILENT 1100 HPLC
2.	Detector system	SPD-10A$_{VP}$
3.	Analytical Column	Zodiac-100 C8 (5µm; 150 x 4.6 mm ID)
4.	Software	Chemstation Software
5.	Injector system	Rheodyne 20µl loop capacity manual injector
6.	UV spectrophotometer	Shimadzu UV 1800 Spectrophotometer (Japan corporation)
7.	pH meter	Mettler-Toledo
8.	Analytical Balance	Wenser high precision balance
9.	Sonicator	Labman®Ultra sonicator

Equipment:

1. Grade A certified Glassware's
2. Nylon Filter

IV. RESULT AND DISCUSSION

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

A bioanalytical RP-HPLC method was developed for the lobeglitazone. The chromatographic conditions were stabilized in order to provide a good performance of the assay. The standard solutions were prepared and chromatograms were recorded. The study proposes a method for the determination of lobeglitazone in human plasma by using RP-HPLC.

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Selection of wavelength:

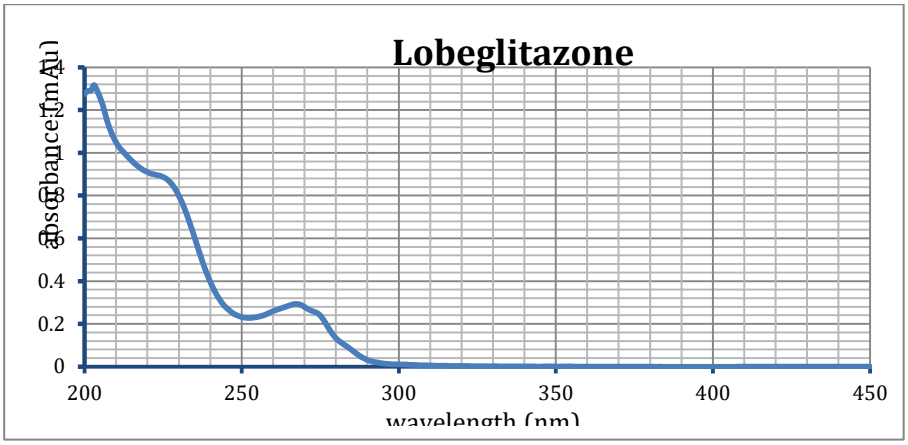

Figure 1: UV SPECTRUM OF LOBEGLITAZONE

ANALYTICAL METHOD DEVELOPMENT BY RP-HPLC

TRAIL 1:

Table no 2: Chromatographic Parameter used for Trail 1

Analytes	Lobeglitazone (100)
Mobile Phase	solvent A; 15mM ammonium acetate (AA) solvent B; acetonitrile-methanol (90:10, v/v)
Column	Zodiac-100 C8 (5µm; 150 x 4.6 mm ID.)
Column Temperature	27^{0}C
Flow rate	1ml/min
Elution mode	Gradient
Elution Program	0-3 mins, 30% B; 3-15 mins, 80% B
Injection volume	20µl
Λmax	lobeglitazone-251nm.

Table no3: Result of Parameters found in Trail 1 of lobeglitazone.

Trial -1 data of lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	9.152	5905685	469766	96.1463	11660.01	22.585	3.491	1.05	29

Trial -2 data of lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
3	2.809	5971474	714882	98.3201	2481.415	3.215	0.511	1.467	3.387

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3.DEVELOED HPLC METHOD OF LOBEGLITAZONE

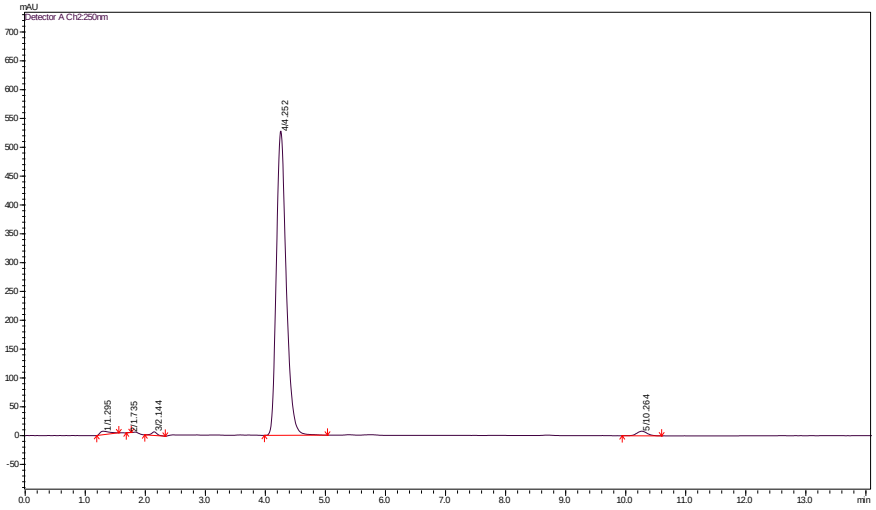

Figure 2: developed HPLC method of lobeglitazone

Table 4: method development data of lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	4.252	6021419	527718	96.7408	3154.818	8.783	2.284	1.302	3.48

Method development at 251 nm **wavelength**

Table 5: Result of parameters at 251nm of lobeglitazone

Analyte	**Ret. Time**	**Area**	**Height**	**Area%**	**T.Plate**	**Resolution**	**k'**	**Tailing F.**	**Separation**
1	4.252	6021419	527718	96.7408	3154.818	8.783	2.284	1.302	3.48

B. METHOD VALIDATION

1. SPECIFICITY:

The specificity of method was performed by comparing the chromatogram of blank, standard and sample. The retention time found are stated below.

Table 6:

Sr no.	**Solution**	**Retention time**
1.	Blank	0
2.	lobeglitazone Standard	5.017
3.	lobeglitazone Sample	4.958

2.PRECISION

In RP-HPLC, nine replicate injections of the prepared solution were injected as per the method and the results are given below in the following table. Freshly prepared stock solution of lobeglitazone (100 ppm) was analyzed thrice within the same day (intraday precision) and three successive days (intermediate precision) were tested and evaluated. Furthermore, their mean, standard deviation and relative standard deviation (RSD) were calculated which should be less than 2% as per the ICH guidelines.

Table 7: intraday precision study of lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.	Separation
1	4.123	6369545	585249	97.3388	3249.158	8.039	1.221	1.35	7.038

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Table 8: Intraday results of lobeglitazone 100 ppm.

Drug Name: lobeglitazone					
Sr no.	**Conc. (ppm)**	**Peak area**	**Mean**	**SD**	**%RSD**
1.	**100ppm**	6369545	6297177.333	62994.16913	1%
	100 ppm	6267354			
	100ppm	6254633			
2.	**100 ppm**	6189645	6169810.333	17889.67594	0.290%
	100ppm	6164891			
	100ppm	6154895			
3.	**100 ppm**	6067859	6051770.667	14083.27406	0.233%
	100 ppm	6045779			
	100 ppm	6041674			

Interday (Intermediate) Precision

Table 9: Interday precision study of lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
4	4.121	5963531	552631	96.024	3368.208	7.794	2.149	1.369	3.267

Table10:

Drug Name: lobeglitazone					
Sr no.	**Conc. (ppm)**	**Peak area**	**Mean**	**SD**	**%RSD**
1.	**100 ppm**	6369545	6297177.333	62994.16913	1%
	100 ppm	6267354			
	100 ppm	6254633			
2.	**100 ppm**	5963531	5959617.667	5916.767136	0.099%
	100 ppm	5962511			
	100ppm	5952811			
3.	**100 ppm**	5934273	5926629.667	6825.513412	0.115%
	100 ppm	5924473			
	100 ppm	5921143			

B) Repeatability

The method repeatability was performed by preparing five different sample preparations. The results obtained are given below in table:

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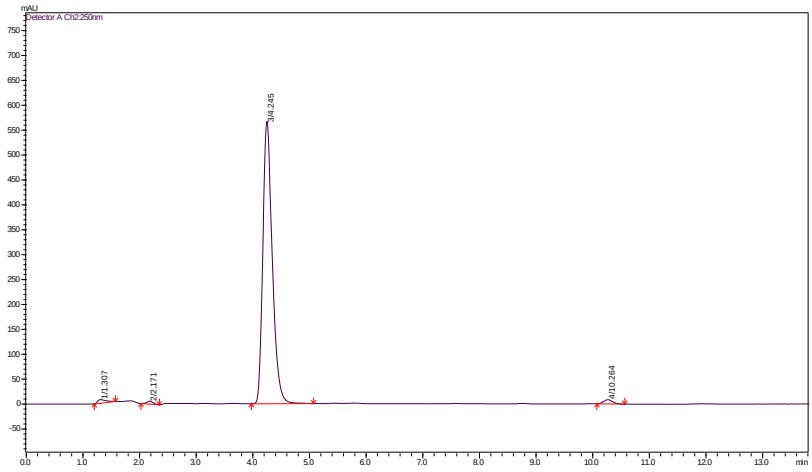

Figure 3: repeatability study of lobeglitazone

Table 11: Repeatability data of lobeglitazone 100 ppm

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	4.245	6570087	567075	96.7881	2981.307	7.729	2.247	1.353	3.4

Table 12: Repeatability results of lobeglitazone 100 ppm

Sr no.	Drug Name; lobeglitazone
	Peak Area (Conc- 100ppm)
1	6570087
2	6565688
3	6563666
4	6560567
Mean	**6565002**
Std Dev.	**3990.903908**
RSD (%)	**0.061%**

LINEARITY

Table 13: HPLC analysis of 50 ppm of lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	4.764	3004238	206397	90.4109	2498.444	8.251	2.491	1.15	3.857

Table 14: HPLC analysis of 25 ppm of lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	4.759	1467672	105631	93.6673	2752.371	8.768	1.674	1.142	6.388

HPLC analysis of 12.5 ppm of lobeglitazone

Table 15: HPLC analysis of 12.5 ppm of lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
4	4.527	789889	59993	89.2623	2767.643	7.932	1.545	1.167	3.81

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Table 16: HPLC analysis of 6.25 ppm of lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
3	4.446	393358	29206	82.9051	2445.515	8.292	1.492	1.078	6.482

Table 17: HPLC analysis of 3.12 ppm of lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	4.218	193573	16142	62.6754	2805.347	7.031	1.408	1.121	3.429

Table 18: Linearity data of lobeglitazone

Drug name: lobeglitazone		
Sr no.	**Concentration (µg/ml)**	**Area**
1	50	3024238
2	25	1512219
3	12.5	751170
4	6.25	395679
5	3.12	187899
Regression Equation		y=60361.3462x+4739.9167
Correlation coefficient (R^2)		0.9999
LOQ		3.04
LOD		1.05

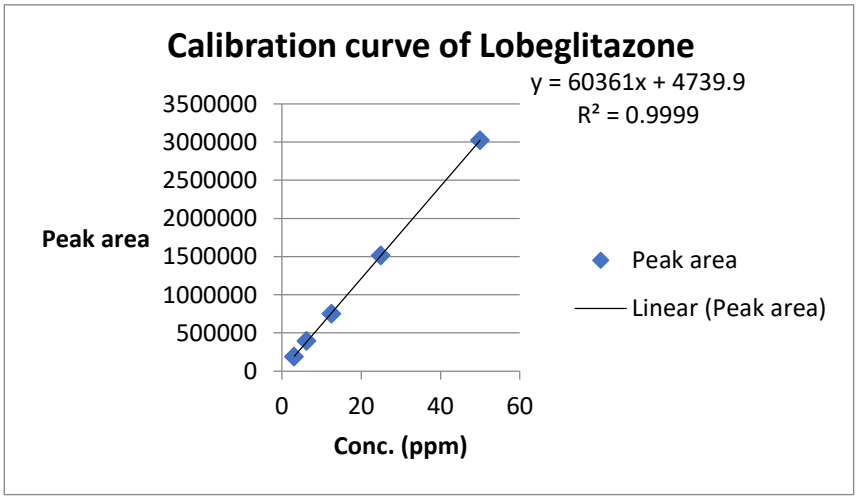

Figure 4: Calibration curve of lobeglitazone

ROBUSTNESS

Robustness of HPLC method represents its ability to remain unaffected by small but deliberate variations in separation parameters to ascertain its reliability during routine analysis. In this method, robustness was established by making deliberate changes in the flow rate, concentration and wavelength.

a) Effect of flow rate

Table 19: effect of flow rate 1.1 ml/min on lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	3.668	5469223	539189	96.8287	2991.579	7.121	2.161	1.361	3.278

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Table 20: effect of flow rate 0.9 ml/min on lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	4.268	6837991	571251	96.7675	2864.52	7.369	2.026	1.313	3.07

Effect of solvent composition on lobeglitazone

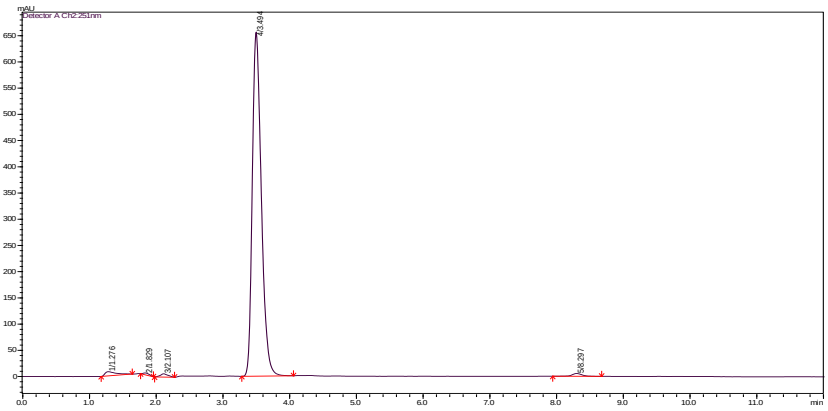

Figure 5: effect of organic solvent (B) of 52% on lobeglitazone

Table 21: effect of organic solvent (B) of 52% on lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
4	3.494	6167909	656300	96.4582	2982.655	5.585	1.739	1.368	2.667

Table 22: effect of organic solvent (B) of 48% on lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	3.99	6168163	579409	96.698	3095.499	7.65	2.132	1.317	3.21

Effect of wavelength on lobeglitazone

Table 23: effect of 252 nm wavelength on lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	4	5663704	529429	96.8114	3166.67	7.616	2.098	1.307	3.202

Table 24: effect of 248 nm wavelength on lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	3.999	5826634	544234	96.5321	3167.761	7.607	2.1	1.314	3.203

Table no 25: Robustness data of LOBEGLITAZONE

Variables	Drug name: lobeglitazone				
	t_R (min)	k'	T_f	Rs	N
Flow rate (+0.1 ml/min)	3.668	2.161	1.361	7.121	1.361
Flow rate (-0.1 ml/min)	4.268	2.026	1.313	7.369	2864.52
ACN-MeOH (50+2%)	3.494	1.739	1.368	5.585	2982.655
ACN-MeOH (50-2%)	3.99	2.132	1.317	7.65	3095.499
wavelength (+2 nm)	4.000	2.098	1.307	7.616	3166.67
wavelength (+2 nm)	3.999	2.1	1.314	7.607	3167.761

ACCURACY

Percentage drug accuracy of three different concentrations; 80%, 100% and 120% Exactly 5 tablets of LOBG® 0.5 manufactured by Glenmark pharmaceuticals Ltd. consisting 0.5 mg of lobeglitazone was weighed and the

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average weight was calculated. They were mixed and crushed to fine powder into the mortar and pestle. An accurately weighed amount of the finely powdered equivalent to 7 mg was dissolved in 7 ml of acetonitrile-methanol-water (6:3:1, v/v). It was then ultrasonicated for 5-10 mins and then filtered through 0.45µ nylon filter. Furthermore, serial dilutions were made in accordance to get the final concentration 100 ppm of lobeglitazone. The solution was then sonicated and analysed as per the chromatographic condition mentioned in section 5.x.

Standard lobeglitazone:

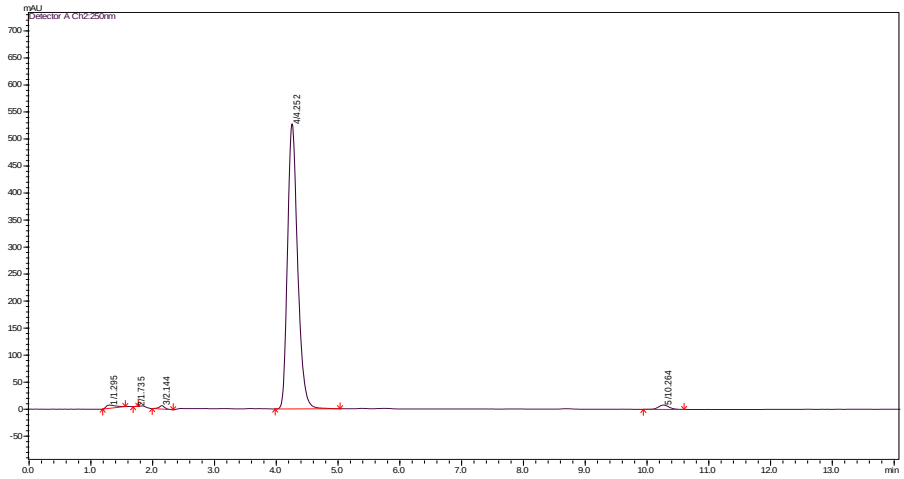

Figure 6: Drug accuracy studies of std lobeglitazone

Table 26: Result of standard lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
4	4.252	6021419	527718	96.7408	3154.818	8.783	2.284	1.302	3.48

Table 27: peak area of std lobeglitazone

Drug name: lobeglitazone								
Conc. (%)	**Std (ppm)**	**Amount added (ppm)**	**Peak area**	**Recovery (ppm)**	**ecovery (%)**	**Mean (%)**	**SD**	**% RSD**
80%	10	8	10985672	18.24	101.33	101.07	0.5483	0.543
	10	8	10998448	18.26	101.44			
	10	8	10888776	18.08	100.44			
100%	10	10	12008016	19.94	99.70	100.42	0.7626	0.759
	10	10	12089692	20.07	100.35			
	10	10	12189311	20.24	101.22			
120%	10	12	13089453	21.73	98.77	99.74	1.1096	1.113
	10	12	13377541	22.21	100.95			
	10	12	13185891	21.89	99.5			

The% RSD was found less than 2%and in range of 0.60%to 0.85%

drug accuracy studies of lobeglitazone marketed formulation

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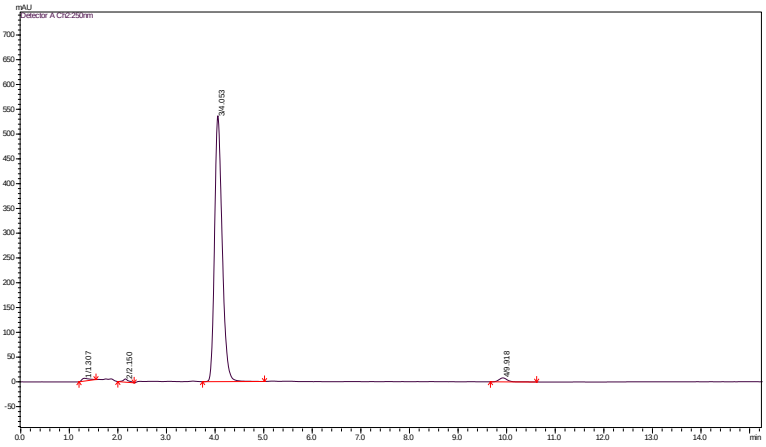

Figure 7: HPLC analysis of marketed formulation; LOBG from Glenmark

Table 28: Result of marketed formulation of lobeglitazone (LOBG tablet) from Glenmark

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
3	4.053	612391	552761	32.9985	2879.136	4.628	2.107	1.314	4.134

Table29: Drug recovery data of marketed lobeglitazone (LOBG)

Drug name: lobeglitazone								
Conc. (%)	**Std (ppm)**	**Amount added (ppm)**	**Peak area**	**Recovery (ppm)**	**ecovery (%)**	**Mean (%)**	**SD**	**% RSD**
80%	10	8	1098365	17.93	99.61	99.72	1.2286	1.232
	10	8	1086825	17.74	98.55			
	10	8	1113671	18.18	101			
100%	10	10	1235541	20.17	100.85	100.73	1.1793	1.171
	10	10	1247842	20.37	101.85			
	10	10	1218954	19.90	99.5			
120%	10	12	1333655	21.77	98.95	98.23	0.666	0.678s
	10	12	1322678	21.59	98.13			
	10	12	1315688	21.48	97.63			

The%RSD was found less than 2%and in range of 0.60%to 0.85%

FIXED CHROMATOGRAPHIC CONDITION

Table 30: Chromatogram of blank plasma

Peak #	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	1.206	234155	29756	1.291	505.958	--	0	1.783	0
2	1.708	15841705	138392 7	87.3425	512.433	1.945	0.416	1.165	0
3	11.002	125220	10470	0.6904	18437.69	29.699	8.123	0.819	19.517
4	12.096	1936382	128742	10.6761	14856.5	3.034	9.03	1.103	1.112

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- **Bio-analysis/drug recovery studies from human plasma**

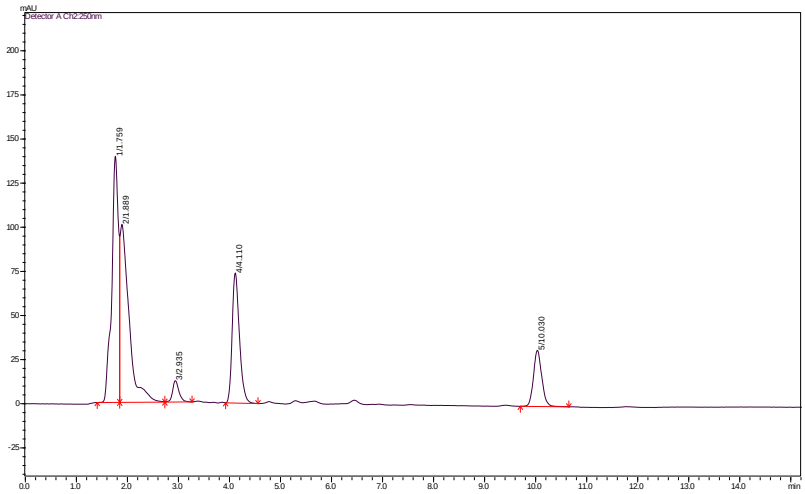

Figure 8: bio-analysis studies of lobeglitazone

Table 31: Bio-Analysis Studies Of Lobeglitazone

Peak#	Ret. Time	Area	Height	Area%	T.Plate#	Resolution	k'	Tailing F.	Separation
1	1.759	1313584	139523	35.2913	471.727	--	0	--	0
2	1.889	1211795	100867	32.5566	97.13	0.238	0.074	--	0
3	2.935	104900	12101	2.8183	2679.415	2.106	0.669	1.263	9.064
4	4.11	730251	73697	19.6192	3791.324	4.759	1.336	1.334	1.999
5	10.03	361593	31806	9.7147	17427.71	20.743	4.703	1.156	3.519

V. CONCLUSION

A Bioanalytical method was developed for the estimation of lobeglitazone in Human Plasma by HPLC method and was validated. Lobeglitazone is an antidiabetic drug from the class called thiazolidinedione. The method was developed using acetonitrile-methanol-water (6:3:1, v/v). The peaks obtained for the drug of interest by the present method was symmetrical in nature with acceptable tailing factor and from the plasma endogenous proteins by Protein precipitation Extraction. The retention time of lobeglitazone was shorter and proves that the method is rapid. All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The method was validated with respect to accuracy, precision, linearity and robustness.

The results of linearity, intraday and interday precision study and capability of the extraction method were within the limits of Bioanalytical method development. The method was linear with a correlation coefficient of acceptable agreement, which is suitable for the estimation of lobeglitazone human plasma.

The method developed can be used in therapeutic drug monitoring units, bioequivalence and bioavailability studies, pharmacokinetic and toxicology studies of lobeglitazone.

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