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 ≥ 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, 1.9% (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 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 hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes during the early years of the disease, others with marked hyperglycaemia 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...
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. 

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.

TYPES

There are mainly two types of chemical analysis:
1. Qualitative (Identification)
2. Quantitative (Estimation)

Pharmaceutical Analysis:

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

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

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

PROCEDURE FOR BIOLOGICAL SAMPLE EXTRACTION

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

Dilution followed by injection--Solid Phase extraction--Protein precipitation--Filtration Liquid-liquid extraction.
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 BIOLOGICAL 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 form 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 mobile phase at time at which a specific analyte elutes (comes out of the column) is called the retention time. [23]

The choice of solvents, additives and gradient 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]

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, carbamamide, chlorpropanamide, dapagliflozin, empagliflozin, glibenclamide, glibornuride, gliclazide, glimepiride, glipizide, gliquidone, glisoxepide, glyburide, glycopyramide, linagliptin, metformin, miglitol, nateglinide, pioglitazone,
repaglinide, rosiglitazone, saxagliptin, sepalagliptin, sitagliptin, teneligliptin, tolazamide, tolbutamide, vildagliptin, and voglibose; “validation”, “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>
<thead>
<tr>
<th>Table 1: List of Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr.no.</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>1.</td>
</tr>
</tbody>
</table>
| 2.    | Detector system  | SPD-10A
| 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.
Selection of wavelength:

![UV Spectrum of Lobeglitazone](image)

**Figure 1:** UV SPECTRUM OF LOBEGLITAZONE

**ANALYTICAL METHOD DEVELOPMENT BY RP-HPLC**

**TRAIL 1:**

**Table no 2:** Chromatographic Parameter used for Trail 1

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

**Table no 3:** Result of Parameters found in Trail 1 of lobeglitazone.

**Trial -1 data of lobeglitazone**

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T.Plate#</th>
<th>Resolution</th>
<th>k’</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.152</td>
<td>5905868</td>
<td>469766</td>
<td>96.1463</td>
<td>11660.01</td>
<td>22.585</td>
<td>3.491</td>
<td>1.05</td>
<td>29</td>
</tr>
</tbody>
</table>

**Trial -2 data of lobeglitazone**

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T.Plate#</th>
<th>Resolution</th>
<th>k’</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2.809</td>
<td>5971474</td>
<td>714882</td>
<td>98.3201</td>
<td>2481.415</td>
<td>3.215</td>
<td>0.511</td>
<td>1.467</td>
<td>3.387</td>
</tr>
</tbody>
</table>
3. DEVELOPED HPLC METHOD OF LOBEGLITAZONE

![Figure 2: developed HPLC method of lobeglitazone](image)

**Table 4:** method development data of lobeglitazone

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k’</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.252</td>
<td>6021419</td>
<td>527718</td>
<td>96.7408</td>
<td>3154.818</td>
<td>8.783</td>
<td>2.284</td>
<td>1.302</td>
<td>3.48</td>
</tr>
</tbody>
</table>

Method development at 251 nm wavelength

**Table 5:** Result of parameters at 251 nm of lobeglitazone

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate</th>
<th>Resolution</th>
<th>k’</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>4.252</td>
<td>6021419</td>
<td>527718</td>
<td>96.7408</td>
<td>3154.818</td>
<td>8.783</td>
<td>2.284</td>
<td>1.302</td>
<td>3.48</td>
</tr>
</tbody>
</table>

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:**

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Solution</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blank</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>lobeglitazone Standard</td>
<td>5.017</td>
</tr>
<tr>
<td>3</td>
<td>lobeglitazone Sample</td>
<td>4.958</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k’</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.123</td>
<td>6369545</td>
<td>585249</td>
<td>97.3388</td>
<td>3249.158</td>
<td>8.039</td>
<td>1.221</td>
<td>1.35</td>
<td>7.038</td>
</tr>
</tbody>
</table>
Table 8: Intraday results of lobeglitazone 100 ppm.

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Conc. (ppm)</th>
<th>Peak area</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>100 ppm</td>
<td>6291354</td>
<td>62994.16913</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>6297177.333</td>
<td>6369545</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>6254633</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>100 ppm</td>
<td>6169810.333</td>
<td>6189645</td>
<td>0.290%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>6154895</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>6051770.667</td>
<td>6045779</td>
<td>0.233%</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>100 ppm</td>
<td>6041674</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interday (Intermediate) Precision

Table 9: Interday precision study of lobeglitazone

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k'</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4.121</td>
<td>5963531</td>
<td>552631</td>
<td>96.024</td>
<td>3368.208</td>
<td>7.794</td>
<td>2.149</td>
<td>1.369</td>
<td>3.267</td>
</tr>
</tbody>
</table>

Table 10:

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Conc. (ppm)</th>
<th>Peak area</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>100 ppm</td>
<td>62994.16913</td>
<td>6369545</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>6297177.333</td>
<td>6267354</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>6254633</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>100 ppm</td>
<td>5916.767136</td>
<td>5963531</td>
<td>0.099%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>5916.767136</td>
<td>5962511</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100ppm</td>
<td>5925211</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>100 ppm</td>
<td>6825.513412</td>
<td>5934273</td>
<td>0.115%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>5926629.667</td>
<td>5924473</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 ppm</td>
<td>5921143</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B) Repeatability

The method repeatability was performed by preparing five different sample preparations. The results obtained are given below in table:
Figure 3: repeatability study of lobeglitazone

Table 11: Repeatability data of lobeglitazone 100 ppm

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k’</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.245</td>
<td>657087</td>
<td>567075</td>
<td>96.7881</td>
<td>2981.307</td>
<td>7.729</td>
<td>2.247</td>
<td>1.353</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Table 12: Repeatability results of lobeglitazone 100 ppm

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Drug Name; lobeglitazone</th>
<th>Peak Area (Conc- 100ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6570087</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6565688</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6563666</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6560567</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>6565002</td>
<td></td>
</tr>
<tr>
<td>Std Dev.</td>
<td>3990.903908</td>
<td></td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.061%</td>
<td></td>
</tr>
</tbody>
</table>

LINEARITY

Table 13: HPLC analysis of 50 ppm of lobeglitazone

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k’</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.764</td>
<td>300423</td>
<td>206397</td>
<td>90.4109</td>
<td>2498.444</td>
<td>8.251</td>
<td>2.491</td>
<td>1.15</td>
<td>3.857</td>
</tr>
</tbody>
</table>

Table 14: HPLC analysis of 25 ppm of lobeglitazone

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k’</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.759</td>
<td>146767</td>
<td>105631</td>
<td>93.6673</td>
<td>2752.371</td>
<td>8.768</td>
<td>1.674</td>
<td>1.142</td>
<td>6.388</td>
</tr>
</tbody>
</table>

HPLC analysis of 12.5 ppm of lobeglitazone

Table 15: HPLC analysis of 12.5 ppm of lobeglitazone

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k’</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4.527</td>
<td>789889</td>
<td>59993</td>
<td>89.2623</td>
<td>2767.643</td>
<td>7.932</td>
<td>1.545</td>
<td>1.167</td>
<td>3.81</td>
</tr>
</tbody>
</table>
Table 16: HPLC analysis of 6.25 ppm of lobeglitazone

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k'</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4.446</td>
<td>393358</td>
<td>29206</td>
<td>82.905</td>
<td>2445.515</td>
<td>8.292</td>
<td>1.492</td>
<td>1.078</td>
<td>6.482</td>
</tr>
</tbody>
</table>

Table 17: HPLC analysis of 3.12 ppm of lobeglitazone

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k'</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.218</td>
<td>193573</td>
<td>16142</td>
<td>62.675</td>
<td>2805.347</td>
<td>7.031</td>
<td>1.408</td>
<td>1.121</td>
<td>3.429</td>
</tr>
</tbody>
</table>

Table 18: Linearity data of lobeglitazone

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Concentration (µg/ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>3024238</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>1512219</td>
</tr>
<tr>
<td>3</td>
<td>12.5</td>
<td>751170</td>
</tr>
<tr>
<td>4</td>
<td>6.25</td>
<td>395679</td>
</tr>
<tr>
<td>5</td>
<td>3.12</td>
<td>187899</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k'</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.668</td>
<td>5469223</td>
<td>539189</td>
<td>96.828</td>
<td>2991.579</td>
<td>7.121</td>
<td>2.161</td>
<td>1.361</td>
<td>3.278</td>
</tr>
</tbody>
</table>
Effect of flow rate on lobeglitazone

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T.Plate#</th>
<th>Resolution</th>
<th>k'</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.268</td>
<td>6837991</td>
<td>571251</td>
<td>96.7675</td>
<td>2864.52</td>
<td>7.369</td>
<td>2.026</td>
<td>1.313</td>
<td>3.07</td>
</tr>
</tbody>
</table>

Effect of solvent composition on lobeglitazone

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

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

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T.Plate#</th>
<th>Resolution</th>
<th>k'</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3.494</td>
<td>6167909</td>
<td>656300</td>
<td>96.4582</td>
<td>2982.655</td>
<td>5.585</td>
<td>1.739</td>
<td>1.368</td>
<td>2.667</td>
</tr>
</tbody>
</table>

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

Table 23: effect of 252 nm wavelength on lobeglitazone

Table 24: effect of 248 nm wavelength on lobeglitazone

Table 25: Robustness data of LOBEGLITAZONE

<table>
<thead>
<tr>
<th>Variables</th>
<th>Drug name: lobeglitazone</th>
<th>tₚ (min)</th>
<th>k'</th>
<th>Tᵢ</th>
<th>Rs</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (+0.1 ml/min)</td>
<td></td>
<td>3.668</td>
<td>2.161</td>
<td>1.361</td>
<td>7.121</td>
<td>1.361</td>
</tr>
<tr>
<td>Flow rate (-0.1 ml/min)</td>
<td></td>
<td>4.268</td>
<td>2.026</td>
<td>1.313</td>
<td>7.369</td>
<td>2864.52</td>
</tr>
<tr>
<td>ACN-MeOH (50+2%)</td>
<td></td>
<td>3.494</td>
<td>1.739</td>
<td>1.368</td>
<td>5.585</td>
<td>2982.655</td>
</tr>
<tr>
<td>ACN-MeOH (50-2%)</td>
<td></td>
<td>3.99</td>
<td>2.132</td>
<td>1.317</td>
<td>7.65</td>
<td>3095.499</td>
</tr>
<tr>
<td>wavelength (+2 nm)</td>
<td></td>
<td>4.00</td>
<td>2.098</td>
<td>1.307</td>
<td>7.616</td>
<td>3166.67</td>
</tr>
<tr>
<td>wavelength (+2 nm)</td>
<td></td>
<td>3.999</td>
<td>2.1</td>
<td>1.314</td>
<td>7.607</td>
<td>3167.761</td>
</tr>
</tbody>
</table>

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
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](image)

**Table 26: Result of standard lobeglitazone**

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T.Plate#</th>
<th>Resolution</th>
<th>k’</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4.252</td>
<td>6021419</td>
<td>527718</td>
<td>96.7408</td>
<td>3154.818</td>
<td>8.783</td>
<td>2.284</td>
<td>1.302</td>
<td>3.48</td>
</tr>
</tbody>
</table>

**Table 27: peak area of std lobeglitazone**

<table>
<thead>
<tr>
<th>Drug name: lobeglitazone</th>
<th>Conc. (%)</th>
<th>Std (ppm)</th>
<th>Amount added (ppm)</th>
<th>Peak area</th>
<th>Recovery (ppm)</th>
<th>recovery (%)</th>
<th>Mean (%)</th>
<th>SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80%</td>
<td>10</td>
<td>8</td>
<td>10985672</td>
<td>18.24</td>
<td>101.33</td>
<td>101.07</td>
<td>0.5483</td>
<td>0.543</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>8</td>
<td>10984448</td>
<td>18.26</td>
<td>101.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>8</td>
<td>10888776</td>
<td>18.08</td>
<td>100.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>10</td>
<td>10</td>
<td>12008016</td>
<td>19.94</td>
<td>99.70</td>
<td>100.42</td>
<td>0.7626</td>
<td>0.759</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>10</td>
<td>12089692</td>
<td>20.07</td>
<td>100.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>10</td>
<td>12189311</td>
<td>20.24</td>
<td>101.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>120%</td>
<td>10</td>
<td>12</td>
<td>13089453</td>
<td>21.73</td>
<td>98.77</td>
<td>99.74</td>
<td>1.1096</td>
<td>1.113</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>12</td>
<td>13377541</td>
<td>22.21</td>
<td>100.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>12</td>
<td>13185891</td>
<td>21.89</td>
<td>99.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The% RSD was found less than 2% and in range of 0.60% to 0.85% drug accuracy studies of lobeglitazone marketed formulation
Figure 7: HPLC analysis of marketed formulation; LOBG from Glenmark

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

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k'</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4.053</td>
<td>612391</td>
<td>552761</td>
<td>32.9985</td>
<td>2879.136</td>
<td>4.628</td>
<td>2.107</td>
<td>1.314</td>
<td>4.134</td>
</tr>
</tbody>
</table>

Table 29: Drug recovery data of marketed lobeglitazone (LOBG)

<table>
<thead>
<tr>
<th>Drug name: lobeglitazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. (%)</td>
</tr>
<tr>
<td>80%</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>100%</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>120%</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T. Plate#</th>
<th>Resolution</th>
<th>k'</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.206</td>
<td>234155</td>
<td>29756</td>
<td>1.291</td>
<td>505.958</td>
<td>--</td>
<td>0</td>
<td>1.783</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.708</td>
<td>1584170</td>
<td>183932</td>
<td>87.342</td>
<td>512.433</td>
<td>1.945</td>
<td>0.41</td>
<td>1.165</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>11.002</td>
<td>125220</td>
<td>10470</td>
<td>0.6904</td>
<td>18437.6</td>
<td>9</td>
<td>29.699</td>
<td>8.12</td>
<td>0.819</td>
</tr>
</tbody>
</table>
| 4      | 12.096    | 1936382 | 128742   | 10.676  | 14856.5   | 1         | 3.034  | 9.03       | 1.103      | 1.112
Bio-analysis/drug recovery studies from human plasma

Table 31: Bio-Analysis Studies Of Lobeglitazone

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Area%</th>
<th>T.Plate#</th>
<th>Resolution</th>
<th>k'</th>
<th>Tailing F.</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.759</td>
<td>1313584</td>
<td>139523</td>
<td>35.2913</td>
<td>471.727</td>
<td>--</td>
<td>0</td>
<td>--</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.889</td>
<td>1211795</td>
<td>100867</td>
<td>32.5566</td>
<td>97.13</td>
<td>0.238</td>
<td>0.074</td>
<td>--</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2.935</td>
<td>104900</td>
<td>12101</td>
<td>2.8183</td>
<td>2679.415</td>
<td>2.106</td>
<td>0.669</td>
<td>1.263</td>
<td>9.064</td>
</tr>
<tr>
<td>4</td>
<td>4.11</td>
<td>730251</td>
<td>73697</td>
<td>19.6192</td>
<td>3791.324</td>
<td>4.759</td>
<td>1.336</td>
<td>1.334</td>
<td>1.999</td>
</tr>
<tr>
<td>5</td>
<td>10.03</td>
<td>361593</td>
<td>31806</td>
<td>9.1477</td>
<td>17427.71</td>
<td>20.743</td>
<td>4.703</td>
<td>1.156</td>
<td>3.519</td>
</tr>
</tbody>
</table>

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.

VI. REFERENCES

Mane PB, Antre RV, Oswal RJ. Antidiabetic drugs: An overview.


