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Analytical Approach Towards Concurrent Quantification Of Lobeglitazone Sulfate And Dapagliflozin

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ABSTRACT:
Clinical studies have demonstrated that the combination of Lobeglitazone sulfate and Dapagliflozin is effective in improving body fat parameters in patients with type 2 diabetes mellitus. Despite their therapeutic relevance, no validated analytical method has yet been reported for the simultaneous qualitative and quantitative estimation of these drugs in combination. Therefore, the objective of the present study was to develop and validate simple, precise, accurate, and robust first-order derivative UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Lobeglitazone sulfate and Dapagliflozin in a synthetic mixture, in accordance with ICH Q2 (R2) guideline. Simple and sensitive first-order derivative ultraviolet visible (UV) spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods were developed and validated for the simultaneous estimation of Lobeglitazone sulfate and Dapagliflozin in a synthetic mixture. The UV method utilized methanol with zero-crossing measurements at 240 nm and 256 nm, while the RP-HPLC method employed a Kromstar C_{18} column with an Acetonitrile: Phosphate buffer mobile phase (70:30 % v/v) at 228 nm. Both methods showed good linearity, accuracy, precision, robustness, specificity and sensitivity as per ICH Q2 (R2) guideline, confirming their suitability for routine quality control analysis.

1 INTRODUCTION:
According to World Health Organization epidemiological data, the global prevalence of diabetes has increased markedly over recent decades and continues to rise. Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and associated microvascular and macrovascular complications. Combination therapy has emerged as an effective strategy to achieve optimal glycemic control in patients with type 2 diabetes mellitus. The combination of Lobeglitazone sulfate and Dapagliflozin has been evaluated in a

Phase III clinical trial [1], demonstrating that the therapy is safe, well tolerated, and effective in improving glucose concentrations and reducing body fat in patients with type 2 diabetes. Furthermore, the combination of thiazolidinediones (TZDs) and sodium–glucose cotransporter-2 (SGLT-2) inhibitors, which act through complementary mechanisms by improving insulin sensitivity and enhancing urinary glucose excretion, is expected to exert synergistic and compensatory effects, thereby offering an effective therapeutic approach for diabetes management [2]. In addition, studies investigating the effects of combined thiazolidinedione (TZD) and sodium–glucose cotransporter-2 (SGLT-2) inhibitor therapy on changes in body fat mass and metabolic phenotype are limited. A clinical investigation evaluated the effect of combination therapy with Dapagliflozin, an SGLT-2 inhibitor, and Lobeglitazone, a TZD, on the reduction of visceral fat, expressed as the abdominal visceral fat mass to abdominal subcutaneous fat mass ratio [3, 4]. Lobeglitazone sulfate *(5-[(4-((2-fluorophenyl)methoxy)phenyl)methyl]-2,4-thiazolidinedione sulfate)* is a thiazolidinedione antidiabetic agent that enhances insulin sensitivity via activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) [5], while Dapagliflozin *(1-[(4-chloro-3-(4-ethoxybenzyl)phenyl)methyl]-1H-pyrazol-3-yl)-β-D-glucopyranoside)* is a selective sodium–glucose co-transporter-2 (SGLT-2) inhibitor that lowers blood glucose by increasing urinary glucose excretion [11]; the Chemical structure of Lobeglitazone sulfate and Dapagliflozin were showed in Figure 1(A) and 1(B), respectively.

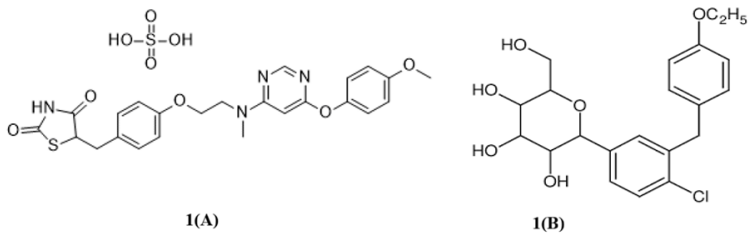


Figure 1: Chemical structure of 1(A) Lobeglitazone sulfate and 1(B) Dapagliflozin

Despite the availability of analytical methods for the individual estimation of these drugs, no validated analytical method has yet been reported for their simultaneous estimation. Therefore, the present study aims to develop and validate simple, accurate, and reproducible UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Lobeglitazone sulfate and Dapagliflozin in a synthetic mixture. A literature survey reveals that several analytical methods have been reported for the estimation of Lobeglitazone and Dapagliflozin either individually or in combination with other active pharmaceutical ingredients. For Lobeglitazone, reported methods include HPLC analysis in bulk and tablet dosage forms [5], a Quality by Design (QbD)–driven RP-HPLC method with Glimepiride [6], combination with Remogliflozin [7], and UV spectrophotometric methods for bulk and pharmaceutical dosage forms [8]. Review articles summarizing analytical methods for Lobeglitazone have also been published [9,10]. Similarly, various analytical methods have been reported for Dapagliflozin, including RP-HPLC methods for tablet dosage forms [11], stability-indicating RP-HPLC methods for bulk and pharmaceutical formulations [12], and simultaneous estimation with Metformin hydrochloride [13] and Saxagliptin [15]. A QbD-based RP-HPLC method for Dapagliflozin has also been developed [14]. Additionally, review articles covering analytical methods for Dapagliflozin in bulk and pharmaceutical formulations are available [16,17], along with HPTLC methods for bulk and tablet dosage forms [18].

Furthermore, bioanalytical methods such as liquid chromatography–tandem mass spectrometry (LC–MS/MS) have been reported for the determination of Lobeglitazone [19] and Dapagliflozin [20] in human plasma. Stability-indicating RP-HPLC and UV spectrophotometric methods have also been documented [21].
However, no analytical method has been reported for the simultaneous estimation of Lobeglitazone sulfate and Dapagliflozin in a synthetic mixture. Hence, the present study aims to develop and validate simple, economical, precise, and robust first-order derivative UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Lobeglitazone sulfate and Dapagliflozin, in accordance with ICH Q2 [R2] guideline [22].

2. EXPERIMENTAL MATERIALS AND TECHNIQUES

2.1 Chemicals and Reagents

Lobeglitazone sulfate and Dapagliflozin were obtained as gift samples from Glenmark Pharmaceuticals Limited, Mumbai & Stallion Pharmaceuticals Pvt. Ltd., Ahmedabad respectively. Methanol, acetonitrile (HPLC grade), potassium dihydrogen phosphate, and orthophosphoric acid were of analytical grade.

2.2 Instrumentation

The spectrophotometric measurements were performed using a UV-Visible spectrophotometer (Shimadzu-1900, UV Probe 2.7 version software) with a spectral bandwidth of 1 nm was employed for all spectroscopic measurements, using a pair of 1.0 cm matched quartz cells over the range of 200-400 nm. For chromatographic information acquisition and analysis, High-Performance Liquid Chromatography system Systronic RP-HPLC (SYS-LC-138) with UV Detector was utilized together. The pH of the buffer solution was observed utilizing the Chemi Line pH meter. The Scale-Tec analytical balance was utilized to weigh the samples. The HPLC mobile phase was subjected to sonication using an Sonicator- Digital Pro^{+}, PS-10A, (Broleo). Chromatographic separation was achieved using a Kromstar C_{18} column (250 × 4.6 mm, 5 µm).

2.3 Analytical Condition

In accordance with ICH Q2 (R2) [22] requirements, the analytical conditions for a simultaneous technique for the measurement of Lobeglitazone sulfate & Dapagliflozin in UV and HPLC were optimized and validated. For UV Spectroscopy Methanol was used as a Solvent. Detection wavelength (λmax) of LOBE and DAPA were 250 nm and 224 nm, respectively. The first-order derivative UV spectra were derived from the zero-order spectra using methanol as the solvent. Quantitative analysis was performed at the zero-crossing point (ZCP) of Lobeglitazone sulfate at 240 nm for the estimation of Dapagliflozin, and at the ZCP of Dapagliflozin at 256 nm for the estimation of Lobeglitazone sulfate. For RP-HPLC, Kromstar C_{18} (250 mm × 4.6 mm, 5 µm) was used in the procedure. The mobile phase consisted of ACN: Phosphate Buffer (pH 3 adjusted with 10% ortho phosphoric acid) (70:30 % v/v) 224 nm wavelength was selected for RP-HPLC, with 1 mL/min flow rate.

2.4 Preparation of Solutions:

2.4.1 Preparation of Stock Solution

Accurately weighed 10 mg of Lobeglitazone sulfate (LOBE) and 10 mg of Dapagliflozin (DAPA) were individually transferred into separate 100 mL volumetric flasks and dissolved in methanol. The solutions were sonicated to ensure complete dissolution, and the volume was made up to the mark with methanol to obtain standard stock solutions having a concentration of 100 µg/mL of LOBE and 100 µg/mL of DAPA, respectively.

2.4.2 Preparation standard solution

Pipetted out 0.1 ml solution of Lobeglitazone sulfate (100 µg/ml) and 2 ml standard stock solution of Dapagliflozin (100 µg/ml) into different 10 ml volumetric flask and diluted up to mark with Methanol to get the 1 µg/ml of Lobeglitazone sulfate and 20 µg/ml of Dapagliflozin.

2.4.3 Preparation of standard working solution

The concentration ranges of 0.5-2.5 µg/mL of LOBE and 10-50 µg/mL of DAPA produced from each stock solution, LOBE (0.05, 0.1, 0.15, 0.20 and 0.25 ml) and DAPA (1, 2, 3, 4 and 5 ml) were pipetted out in ten different 10 ml volumetric flasks and made up to mark with Methanol to obtained 0.5, 1.0, 1.5, 2.0 and 2.5 µg/mL of LOBE and 10, 20, 30, 40 and 50 µg/mL for DAPA, respectively. Under the optimized spectrophotometric conditions, the samples were analyzed using a 1 cm quartz cuvette in the UV spectrophotometer. Similarly, the optimized chromatographic conditions, 20 µL of each standard working solution was injected into the RP-HPLC system.

2.4.5 Preparation of 10% Orthophosphoric acid

10% orthophosphoric acid was prepared by diluting 1.0 ml of concentrated ortho phosphoric acid in 10 ml HPLC grade water.

2.4.5 Preparation of 10mM Phosphate Buffer

Accurately weighed 1.36 g of potassium dihydrogen phosphate (KH_2PO_4) was transferred to a 1000 mL volumetric flask, dissolved in approximately 800 mL of purified water, and sonicated until completely dissolved. The pH of the solution was adjusted to the acidic value 3 using 10% Orthophosphoric acid. The final volume was made up to 1000 mL with purified water and mixed well. The buffer solution was filtered through a 0.45 µm membrane filter prior to use.

3. METHODOLOGY

3.1 Method I: UV method

First Order Derivative Method was selected for simultaneous estimation of Lobeglitazone sulfate & Dapagliflozin in Synthetic Mixture. Each working standard solution was scanned individually over the wavelength range of 200-400 nm. In zero order UV spectra, Lobeglitazone sulfate exhibited an absorption maximum at 250 nm showed in Figure 2 (A), while Dapagliflozin showed an absorption maximum at 224 & 277 nm in Figure 2 (B). LOBE and DAPA standard stock solutions were prepared in Methanol at concentrations of 100 µg/mL and 100 µg/mL, respectively. A small amount of each stock solution was taken and placed into 10 mL volumetric flasks. Methanol was used to adjust the volumes to the mark, resulting in final concentrations of LOBE ranging from 0.5 to 2.5 µg/mL and DAPA ranging from 10 to 50 µg/mL. All zero-order absorption UV spectra were converted to first-order derivative UV spectra. Calibration functions were established by plotting first-order derivative absorbance against corresponding concentrations for each analyte. Appropriate volume, 0.10 mL of LOBE and 2.0 ml DAPA standard stock solution was transferred to two separate 10 mL volumetric flasks and the volume was adjusted to mark with methanol to get concentration 1.0 and 20 µg/mL, respectively. The solutions were scanned separately in the UV-region i.e., 400-200 nm. The zero-order UV absorption spectra of LOBE and DAPA in Methanol shown in Figure 2 (A) and 2 (B). The zero-order spectrum (Figure 3) was processed to obtain first-derivative spectrum. The two first derivative spectra were overlaid which showed that Lobeglitazone sulfate showed zero crossing at 240 nm, while Dapagliflozin showed zero crossing at 256 nm which showed in Figure 4. The determinations were made at 256 nm for Lobeglitazone sulfate (ZCP of Dapagliflozin) and 240 nm for Dapagliflozin (ZCP of Lobeglitazone sulfate). The Linearity overlay UV spectra of Lobeglitazone sulfate & Dapagliflozin in first order were presented in Figure 7 (A) and (B), respectively.

3.2 Method II: Reverse Phase High Performance Liquid Chromatography Method (RP-HPLC)

For RP-HPLC, the analysis was carried out using an isocratic elution technique using a mobile phase comprised of different mobile phases such as ACN: Phosphate Buffer (pH 3 adjusted with 10% ortho phosphoric acid) (70:30 % v/v) at a flow rate of 1 mL/min found better separation of both the drug peaks. Prior to usage, the solvents were filtered through a 0.45 µm filter and sonicated for 30 min. The stationary phase was a Kromstar C_{18} (250 mm × 4.6 mm, 5 µm), and the eluent was observed by a U.V Detector from 200 to 400 nm, alongside chromatograms extracted at 228 nm (figure 5). The calibration curves were prepared by measuring the peak areas of LOBE and DAPA and plotted their values against the pertinent concentrations. In accordance, the equations for linear regression were calculated.

3.3 Method Validation

The analytical procedures employed in this study were validated in accordance with the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), ICH Q2 (R2): *Validation of Analytical Procedures* [22]. Validation parameters evaluated included specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness [23-25].

3.3.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. Its results showed in figure 8, 9, 10.

3.3.2 Linearity and Range: (n=6)

The linearity of Lobeglitazone sulfate & Dapagliflozin was found to be in the range of 0.5-2.5 µg/mL and 10-50 µg/mL, respectively. Plot the calibration curve of peak area vs. concentration (µg/mL). Linearity of both the drugs were checked in term of slope, intercept and correlation coefficient.

3.3.3 Precision

The Intraday and Interday precisions also referred to as repeatability and intermediate accuracy, respectively were used to assess the precision of Methods I and II. The experiment was conducted on the same day and for the next three days for both Intraday and Interday precision, analysing freshly made solutions at concentrations of 0.5, 1.0, and 1.5 µg/mL of LOBE and 10, 20, and 30 µg/mL of DAPA. To assess intermediate precision, the mean absorbance (UV) and peak area (HPLC) were recorded for each set of experiments. For repeatability, 1.0 µg/mL of LOBE and 20 µg/mL of DAPA were used. The results were represented as a percentage Relative Standard Deviation (RSD), with a value of less than two considered acceptable. This meticulous approach

ensures a comprehensive evaluation of the precision of the analytical methods, providing confidence in the reliability and consistency of the results obtained for the concentrations of LOBE & DAPA in the tested solutions.

3.3.4 Limit of Detection (LOD)

Limit of detection can be calculated using following equation as per ICH guidelines.

$$\text{LOD} = 3.3 * \frac{\sigma}{S}$$

Where, σ = standard deviation of the calibration curve
S = slope of the calibration curve

3.3.5 Limit of Quantification (LOQ)

Limit of quantification can be calculated using following equation using the standard deviation of the Y-intercept (σ) and the mean slope (S) of the calibration curve according to ICH Q2 (R2) guideline.

$$\text{LOQ} = 10 * \frac{\sigma}{S}$$

3.3.6 Accuracy (Recovery study) (n=3)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50 %, 100 %, 150 % and the values were measured for Lobeglitazone sulfate (1.0 µg/mL) and Dapagliflozin (20 µg/mL). This performance was done in triplicate. The accuracy of the method was determined by calculating recovery of Lobeglitazone sulfate and Dapagliflozin by the standard addition method.

3.3.7 Assay as analysis of Synthetic Mixture

The synthetic mixture of Lobeglitazone and Dapagliflozin was prepared in the ratio of 1:20. A synthetic mixture equivalent to 50 mg was prepared by accurately weighing Lobeglitazone sulfate (0.5 mg) and Dapagliflozin (10 mg). Lactose (18 mg), starch (9.5 mg), Magnesium Stearate (1 mg), and MCC [Micro Crystalline Cellulose] (6 mg), Croscarmellose Sodium (5 mg) were used as excipients. All the components were transferred into a mortar and blended thoroughly using a pestle to obtain a homogeneous synthetic mixture. This mixture was transferred in 100 ml volumetric flask and allowed to sonicate and made up to mark with Methanol. This solution was filtered through Whatmann filter paper. The filtrate was diluted to the mark with Methanol. The mixture contains 5 µg/mL of Lobeglitazone sulfate and 100 µg/mL of Dapagliflozin.

3.3.7.1 Preparation of sample solution

Accurately 2 ml of the above [mixture solution of Lobeglitazone sulfate (5 µg/ml) and Dapagliflozin (100 µg/ml)] was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol. Final concentration of Lobeglitazone sulfate was 1 µg/ml and Dapagliflozin 20 µg/ml. then analysed using the previously described UV-spectrophotometric and chromatographic conditions. The concentrations of LOBE and DAPA were calculated using a regression equation.

3.3.8 Robustness

The robustness of analytical methods becomes evaluated to decide their ability to face up to minor variations in approach situations. For the HPLC technique, samples have been subjected to evaluation below changed situations, which include adjustments inside the flow rate (± 0.1 mL/min), detection wavelength (± 2 nm), and natural content material (± 2 %) inside the mobile segment. The resulting results on machine suitability parameters have been intently monitored. In the times of Methods I and II, distinct analysts conducted sample analyses to evaluate the robustness of the strategies.

3.3.9 System Suitability Tests

A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The test includes the Resolution, Column efficiency, Tailing factor and Theoretical plates.

4. RESULTS AND DISCUSSION:

4.1 Method I: UV Method

The first-order derivative UV spectrophotometric method provides distinct advantages over conventional zero-order UV techniques by enhancing spectral resolution and reducing baseline drift, thereby improving selectivity and accuracy. Measurement at zero-crossing wavelengths allows selective quantification of analytes in the presence of overlapping spectra without prior separation. In comparison with higher-order derivative methods, the first-order derivative approach offers a better signal-to-noise ratio, resulting in improved precision and reproducibility. Additionally, the method is simple, rapid, cost-effective, and requires minimal sample preparation, making it well suited for routine quality-control analysis of multicomponent pharmaceutical formulations.

4.1.1 Selection of wavelength for Lobeglitazone sulfate and Dapagliflozin

The remarkable absorbance of Lobeglitazone sulfate exhibited an absorption maximum at 250 nm [figure 2(A)], while Dapagliflozin showed an absorption maximum at 224 and 277 nm [figure 2(B)].

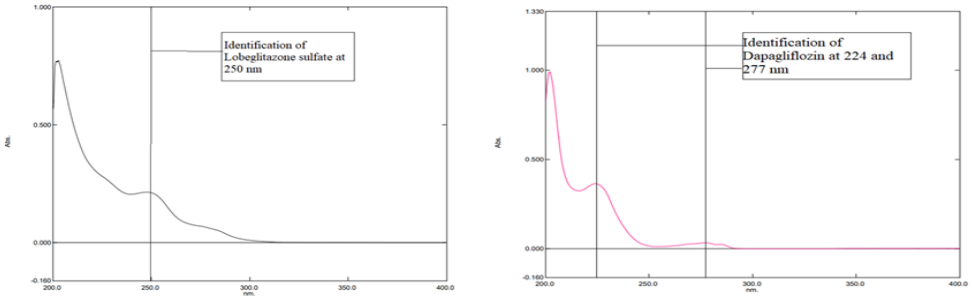


Figure 2: UV Spectrum of 2(A) Lobeglitazone sulfate (1 µg/mL) at 250 nm and 2(B) Dapagliflozin (20 µg/ml) at 224 & 277 nm

The zero-order UV absorption spectra of Lobeglitazone sulfate (1 µg/mL) and Dapagliflozin (20 µg/mL) in Methanol was showed in Figure 3.

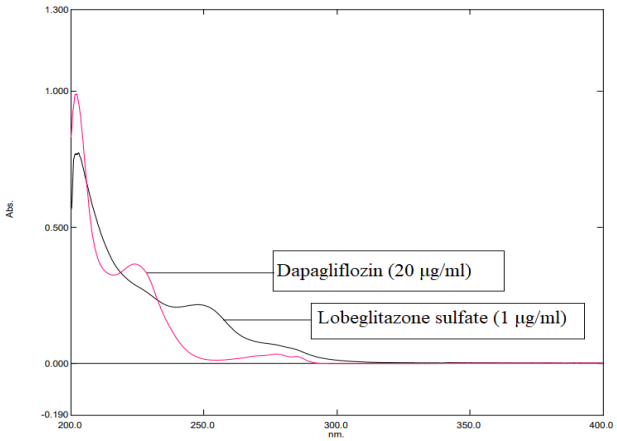


Figure 3: Overlain UV Spectra of Lobeglitazone sulfate (1 µg/ml) and Dapagliflozin (20 µg/ml) in Methanol (Zero Order)

4.1.2 First order derivative UV Method Development

The LOBE and DAPA overlapping absorption throughout the 200 - 400 nm range is shown by these spectra, which makes it more difficult to quantify the pharmaceuticals using traditional UV spectrophotometry without accounting for the overlap. The sum of the absorbances of the two compounds may be used to calculate the overall absorbance of a solution containing a combination of both at a certain wavelength. In situations where the levels of the two medicinal drugs overlap, the method entails figuring out the quantity of each drug using their zero-order spectra. The resulting absorbance spectra were derived to eliminate the interference of

absorbing species. The first derivative corresponding to each absorption spectrum of each drug was recorded, using Δλ = 2 nm and scaling factor 4. The amplitude values were measured at 256 nm (λ1) (ZCP of Dapagliflozin) for LOBE and 240 (λ2) (ZCP of LOBE) for DAPA showed in Figure 4.
To determine the wavelength for measurement, Lobeglitazone sulfate (1 µg/ml) and Dapagliflozin (20 µg/ml) solutions were scanned between 200-400 nm.

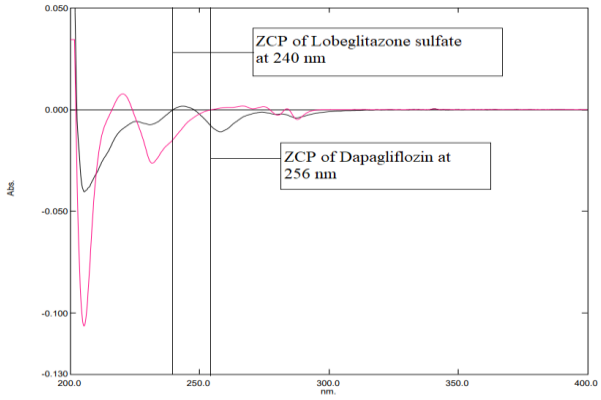


Figure 4: Overlain UV Spectra of Lobeglitazone sulfate (1 µg/ml) and Dapagliflozin (20 µg/ml) in Methanol (First Order)

4.2 Method II: RP-HPLC Method

Reverse-phase high-performance liquid chromatography (RP-HPLC) was selected for the analysis due to its high resolution, sensitivity, and reproducibility in the separation and quantification of compounds with varying polarity. The technique offers excellent peak symmetry, shorter analysis time, and superior compatibility with aqueous and organic mobile phases, making it particularly suitable for routine quality control and analytical applications. In addition, RP-HPLC provides high method robustness and ease of method optimization, while requiring minimal sample preparation. Owing to these advantages and its wide regulatory acceptance, RP-HPLC is extensively employed in pharmaceutical and analytical research, making it an appropriate and reliable choice for the present study.

C_{18} column was selected because it is least polar compare to C_4 and C_8 columns. C_{18} column allows eluting polar compounds more quickly compare to non-polar compounds.

4.2.1 Selection detection wavelength

The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 228 nm both drugs give good peak height and shape. So, 228 nm was selected for simultaneous estimation of Lobeglitazone sulfate and Dapagliflozin in synthetic mixture. Overlain UV spectra of Lobeglitazone sulfate (1 µg/ml) and Dapagliflozin (20 µg/ml) in Methanol showed in figure 5.

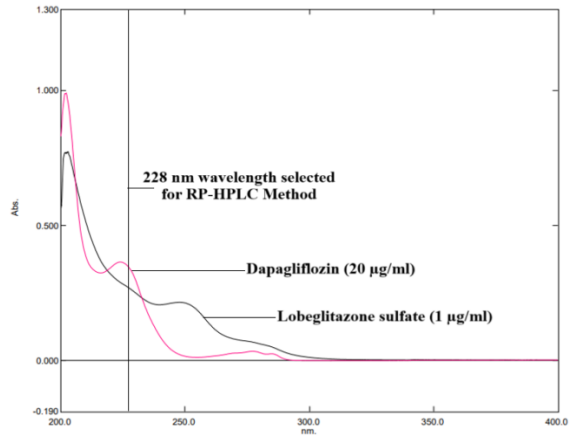


Figure 5: Overlain Zero Order UV Spectra of Lobeglitazone sulfate (1 µg/ml) and Dapagliflozin (20 µg/ml) in Methanol

4.2.2 Chromatography

The mobile phase ACN: Phosphate Buffer (pH 3 adjusted with 10% ortho phosphoric acid) (70:30 % v/v) was selected because it was found to ideally resolve the peaks with retention time 2.2 min and 4.5 min for Dapagliflozin and Lobeglitazone sulfate, respectively. Kromstar C_{18} (250×4.6 mm, 5 µm) column was used for separation of Lobeglitazone sulfate and Dapagliflozin with Flow rate of 1.0 ml/min. Figure 6 showed RP-HPLC Chromatogram of Lobeglitazone sulfate (1 µg/ml) and Dapagliflozin (20 µg/ml).

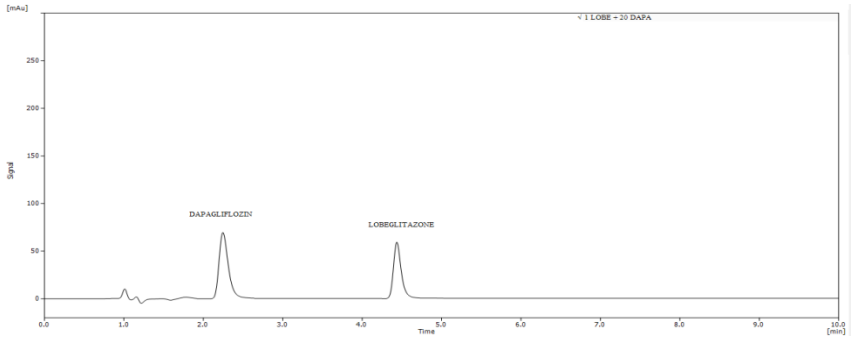


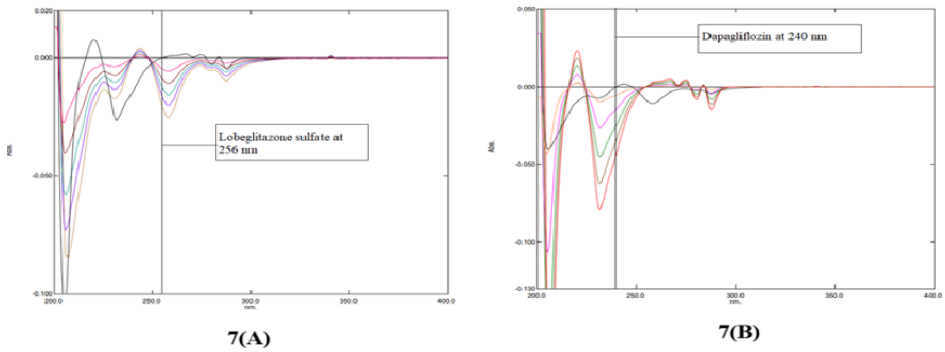
Figure 6: RP-HPLC Chromatogram of Lobeglitazone sulfate (1 µg/ml) and Dapagliflozin (20 µg/ml) in ACN: Phosphate Buffer (pH 3 adjusted with 10% ortho phosphoric acid) (70:30 %v/v) at 228 nm {Run Time: 10 min, Flow Rate: 1.0 ml/min}

4.3 VALIDATION OF THE PROPOSED METHODS

4.3.1 Validation Parameters of the UV Method

4.3.1.1 Linearity and range

For LOBE and DAPA, the absorbances ranged from 0.5-2.5 µg/mL at 256 nm and 10-50 µg/mL at 240 nm showed in Figure 7(A) and 7(B), respectively.



7(A) 7(B)

Figure 7: Overlain UV Spectra of 7(A)Lobeglitazone sulfate (0.5-2.5 µg/ml) at 256 nm and 7(B)Dapagliflozin (10-50 µg/ml) at 240 nm

A linear relationship was found and calibration curve was plotted for concentration vs. absorbance. For LOBE, the calibration curve equation y = 0.0074x + 0.0015, while for DAPA, it was y = 0.001x - 0.0047. Results showed that the correlation coefficient (R^2) was between 0.9978 and 0.9997 (Table 1).

Table 1: Linearity data of LOBE and DAPA by UV and RP-HPLC Method

Parameters	UV Spectrophotometry		RP-HPLC	
	LOBE at 256 nm	DAPA at 240 nm	LOBE at 256 nm	DAPA at 240 nm
Linearity Range	0.5-2.5 µg/mL	10-50 µg/mL	0.5-2.5 µg/mL	10-50 µg/mL
Regression Equation	y = 0.0074x + 0.0015	y = 0.001x - 0.0047	y = 286.25x - 47.652	y = 32.479x - 48.931
Correlation Coefficient	0.9978	0.9997	0.9994	0.9979
LOD	0.03	0.24	0.01	0.31
LOQ	0.11	0.73	0.04	0.95

4.3.1.2 Precision

In terms of precision, both Inter-day, Intraday and Repeatability measurements were conducted at three distinct concentrations 0.5, 1.0 & 1.5 µg/mL for LOBE and 10, 20 & 30 µg/mL for DAPA in triplicate over three consecutive days and on the same day. The absorbance of the same solutions was measured. For repeatability,

1.0 µg/mL for LOBE and 20 µg/mL for DAPA were measured. The resulting RSD values for Intraday, Inter-day precision, and Repeatability were showed in Table 2, respectively

Table 2: Precision data of Lobeglitazone sulfate and Dapagliflozin for UV method

Intraday precision					
Conc. (µg/mL)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
LOBE	**DAPA**	**LOBE**	**DAPA**	**LOBE**	**DAPA**
0.5	10	|-0.005| ± 0.000077	|-0.005| ± 0.000080	1.51	1.39
1.0	20	|-0.009| ± 0.000120	|-0.009| ± 0.000120	1.32	1.04
1.5	30	|-0.013| ± 0.000147	|-0.013| ± 0.000151	1.12	0.75
Interday precision					
Conc. (µg/mL)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
LOBE	**DAPA**	**LOBE**	**DAPA**	**LOBE**	**DAPA**
0.5	10	|-0.005| ± 0.000080	|-0.005| ± 0.000071	1.58	1.41
1.0	20	|-0.009| ± 0.000120	|-0.014| ± 0.000148	1.33	1.05
1.5	30	|-0.013| ± 0.000151	|-0.024| ± 0.000191	1.15	0.79
Repeatability					
Conc. (µg/mL)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
LOBE	**DAPA**	**LOBE**	**DAPA**	**LOBE**	**DAPA**
1.0	20	|-0.009| ± 0.000116	|-0.014|±0.000142	1.29	1.01

4.3.1.3 LOD and LOQ

The minimum detectable quantity of an analyte within a sample by an analytical method was determined to be 0.03 µg/mL for LOBE at 256 nm and 0.24 µg/mL for DAPA at 240 nm, The quantitation limit for a specific analytical method refers to the minimum quantity of the substance in a sample that can be accurately and precisely measured which was found to be 0.11 µg/mL for LOBE at 256 nm and 0.73 µg/mL for DAPA at 240 (Table 1). The low LOD and LOQ values obtained at the selected wavelengths indicated the adequate sensitivity of the proposed UV spectrophotometric method for the estimation of both drugs.

4.3.1.4 Accuracy

The accuracy of the technique recuperation accomplished by means of standard addition approach. To pre-analyzed pattern acknowledged quantity of general LOBE and DAPA spiked in extraordinary concentrations. The restoration was executed in three stages 50 %, 100 % and 150 % of LOBE and DAPA. Accuracy was carried out by the Recovery Studies (standard addition method). The results were stipulated in triplicate and the accuracy indicated by % recovery. For UV, The % Recovery was obtained in range of 99%-99.60% for Lobeglitazone sulfate and 99.06%-99.56% for Dapagliflozin were showed in Table 3.

Table 3: Recovery study data for UV and RP-HPLC Method

UV method						
Name of Drug	**% Level of recovery**	**Test Amount (µg/ml)**	**Amount of drug taken (µg/ml)**	**Total Std Amt (µg/ml)**	**Total amount Recovered (µg/ml)**	**% Mean Recovery ± SD(n=3)**
Lobeglitazone sulfate	50	1	0.5	1.5	1.485	99.00±1.1007
	100	1	1	2.0	1.982	99.13±1.2003
	150	1	1.5	2.5	2.49	99.60±1.3055
Dapagliflozin	50	20	10	30	29.72	99.06±1.5033
	100	20	20	40	39.67	99.17±1.5507
	150	20	30	50	49.78	99.56±1.5556
RP-HPLC Method						
Lobeglitazone sulfate	50	1	0.5	1.5	1.49	99.33±1.1014
	100	1	1	2.0	1.99	99.50±1.1193
	150	1	1.5	2.5	2.498	99.92±1.1571
Dapagliflozin	50	20	10	30	29.96	99.86±1.1322
	100	20	20	40	39.98	99.95±1.2328
	150	20	30	50	49.99	99.98±1.2421

4.3.1.5 Assay of synthetic mixture

From assay, Final concentration of Lobeglitazone sulfate was 1 µg/mL and Dapagliflozin 20 µg/mL were run into UV and the Percentage assay of Lobeglitazone sulfate and Dapagliflozin were found to be 99.66 % and 99.83 %, respectively. Its results showed in Table 4.

Table 4: Analysis of synthetic mixture for UV and RP-HPLC Method

UV- Method				
Name of Drug	**Amount in synthetic mixture (µg/ml)**	**Mean Amount found (µg/ml)**	**% Assay ± SD(n=3)**	**%RSD**
Lobeglitazone sulfate	1	0. 992	99.66 ± 0.058	0.059
Dapagliflozin	20	19.98	99.83 ± 0.057	0.058
RP-HPLC Method				
Lobeglitazone sulfate	1	0.998	99.80±1.103	1.104
Dapagliflozin	2	19.99	99.95±1.054	1.055

4.3.2 Validation Parameters of the RP-HPLC Method

4.3.2.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. It was proved by comparing the chromatogram of mobile phase, test preparation solution to show that there was no interference of mobile phase and excipients peaks with peak of Lobeglitazone sulfate and Dapagliflozin.

4.3.2.2 Linearity

The Peak Area of Lobeglitazone sulfate (0.5-2.5 µg/mL) and Dapagliflozin (10-50 µg/mL) at 228 nm were recorded. Calibration graphs were plotted between concentrations and peak areas. The regression equation of calibration curve was generated y = 286.25x - 47.652 for LOBE and y = 32.479x - 48.931 for DAPA. The correlation coefficient (R^2) values were observed to be 0.9994 and 0.9979. (Table 1).

4.3.2.3 Precision

Concentrations of 0.5, 1.0, and 1.5 µg/mL for LOBE and 10, 20, and 30 µg/mL for DAPA were selected for precision studies. On the same day, the peak area of the prepared solutions was measured at three different time intervals at the selected wavelength. Similarly, on the first, second, and third days, the peak area of the same solutions was measured to evaluate inter-day precision. Each solution was prepared and analyzed in triplicate. The relative standard deviation (RSD) values obtained for intra-day and inter-day precision are presented in Table 5.

Table 5: Precision study data for RP-HPLC method

Intraday precision					
Conc. (µg/ml)		**Mean peak area (mAu*sec) ± S.D (n=3)**		**%RSD**	
LOBE	**DAPA**	**LOBE**	**DAPA**	**LOBE**	**DAPA**
0.5	10	99.722±1.2185	302.084±3.0338	1.22	1.00
1.0	20	230.281±2.1834	588.191±4.5918	0.95	0.78
1.5	30	386.710±2.5180	898.331±5.1339	0.65	0.57
Interday precision					
Conc. (µg/ml)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
LOBE	**DAPA**	**LOBE**	**DAPA**	**LOBE**	**DAPA**
0.5	10	99.744±1.2409	302.058±3.0629	1.24	1.01
1.0	20	230.297±2.2066	588.256±4.6747	0.96	0.79
1.5	30	386.716±2.5599	898.297±5.1790	0.66	0.58
Repeatability					
Conc. (µg/ml)		**Mean Absorbance ±SD (n=3)**		**%RSD**	
LOBE	**DAPA**	**LOBE**	**DAPA**	**LOBE**	**DAPA**
1.0	20	229.586±2.2030	587.826±4.6926	0.96	0.80

4.3.2.4 Accuracy

The accuracy of the technique recuperation was decided change into accomplished by means of standard addition approach. To pre-analyzed pattern acknowledged quantity of general LOBE and DAPA spiked in extraordinary concentrations. The restoration was executed in three stages 50 %, 100 % and 150 % of fashionable LOBE and DAPA. The results were studied in triplicate and the accuracy changed into indicated by% recovery (Table 3). Accuracy was carried out by the Recovery Studies. For HPLC, The % Recovery was obtained in range of 99.33%-99.92% for Lobeglitazone sulfate and 99.86%-99.98% for Dapagliflozin were showed in Table 3. The mean percentage recovery values for both drugs were found to be within the ICH-accepted range of 98-102%, with low standard deviation. These results confirmed the accuracy, trueness, and reliability of the RP-HPLC method and indicated that excipients present in the synthetic mixture did not interfere with the estimation of either drug.

4.3.2.5 LOD and LOQ

LOD Values were found to be 0.01 and 0.31 µg/mL for Lobeglitazone sulfate and for Dapagliflozin, respectively. LOQ Values were found to be 0.04 and 0.95 µg/mL, respectively for Lobeglitazone sulfate and for Dapagliflozin. These results showed in Table 1.

4.3.2.6 Assay

From assay, Final concentration of Lobeglitazone sulfate was 1.0 µg/mL and Dapagliflozin 20 µg/mL were injected into HPLC System and The Percentage assay of Lobeglitazone sulfate and for Dapagliflozin were found to be 99.80 % and 99.95 %, respectively. Results showed in Table 4.

4.3.2.7 Robustness

Chromatographic analysis was used to analyze the effects of changes in analysts, and the results showed that there was no statistically significant difference in the% RSD of technique II. Additionally, small changes were performed to assess the robustness of the created HPLC procedures. The approaches robustness was demonstrated by the% RSD, which remained constant despite minor variations in flow rate, run time, and detection. It was determined that the created approaches were essential as a result showed in Table 6.

Table 6: Robustness data

Condition	Variation	Lobeglitazone sulfate	Dapagliflozin
		% Assay ± SD (n=3)	% Assay ± SD (n=3)
Flow rate (1 ml±0.2 ml/min)	0.8 ml/min	99.41±4.51	98.95±2.37
	1.0 ml/min	99.98±9.42	99.86±0.55
	1.2 ml/min	99.65±6.47	99.55±1.02
Detection wavelength (228 nm±2 nm)	226	98.74±1.12	99.25±9.45
	228	99.99±9.42	100.05±0.55
	230	98.78±2.04	99.65±3.76
Mobile Phase ACN: Phosphate Buffer (pH 3) (70:30 ±2 %v/v)	68:32	98.87±2.07	98.95±1.11
	70:30	99.95±9.42	99.94±0.55
	72:28	99.75±0.14	99.97±7.18

5. CONCLUSION:

The present study successfully demonstrates the development and validation of first-order derivative UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Lobeglitazone sulfate and Dapagliflozin in a synthetic mixture. Both methods were validated in accordance with the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q2 (R2) and showed excellent linearity, accuracy, precision, sensitivity, and reproducibility. The derivative UV spectrophotometric method provides a rapid and cost-effective alternative suitable for routine quality-control analysis, whereas the RP-HPLC method offers superior specificity and robustness, making it appropriate for advanced quality-control laboratories. Furthermore, the proposed methods can be extended for the analysis of marketed formulations and stability studies, highlighting their potential applicability in regulatory and industrial quality-control settings.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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