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Development and Validation of UV Spectrophotometric methods for simultaneous estimation of Lobeglitazone Sulfate and Glimepiride in combined dosage form

Dhara Patel[a], Jeel Dobariya*[a], Prasanna Pradhan[a], Grishma Patel[a], Dhananjay Meshram[a]

[a]*Department of Pharmaceutical Quality Assurance, Pioneer Pharmacy College, Sayajipura, Vadodara, India.,*
**Corresponding author: patel.dhara.j@gmail.com*

Two UV spectrophotometric methods have been developed for accurately analyzing Lobeglitazone Sulfate and Glimepiride in combined dosage form, used in the treatment of type 2 Diabetes Mellitus. Method I, known as the simultaneous equation method (Vierodt's Method), relies on measuring the absorption at 250 nm for Lobeglitazone Sulfate and 227 nm for Glimepiride, their respective λ_{max} values. Method II involves the second order derivative method, where the absorbance of Lobeglitazone Sulfate is measured at 297 nm (zero-crossing point of Glimepiride), and that of Glimepiride is measured at 259 nm (zero-crossing point of Lobeglitazone Sulfate). Both methods exhibit linearity within specified concentration ranges: 3-13 $\mu g.mL^{-1}$ for Lobeglitazone Sulfate and 6-26 $\mu g.mL^{-1}$ for Glimepiride, using methanol as the solvent. The accuracy of these methods was confirmed through recovery studies, yielding results within the range of 98-102% for both drugs. Precision was evaluated through repeatability and intermediate precision studies, demonstrating % RSD values below 2%, indicating high precision. A comparison between the two methods using the F-test showed no significant difference. Statistical validation according to ICH Q2 R1 guideline confirmed the reliability of the results obtained from both methods.

Keywords: Lobeglitazone sulfate; Glimepiride; Simultaneous equation method; Second order derivative spectrophotometry; Validation.

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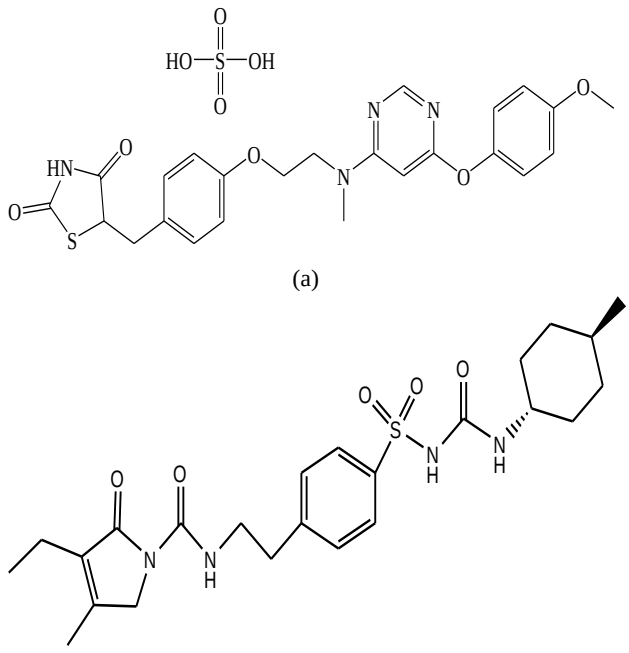
Introduction

Lobeglitazone Sulfate (LBG) is a medication belonging to the thiazolidinedione class, serves as an antidiabetic agent (Figure 1(a)). Its main mechanism involves enhancing insulin sensitivity by engaging Peroxisome Proliferator-Activated Receptors (PPAR) gamma found in adipose tissue. Through this activation, lobeglitazone facilitates insulin's binding to adipose cells, leading to decreased blood glucose levels, improved HbA1C levels, and better lipid and liver profiles. (1,2)

Glimepiride (GLP) is a second-generation sulfonylurea drug utilized in managing type 2 Diabetes Mellitus (T2DM) (Figure 1(b)), aids in regulating blood sugar levels. Its mechanism involves triggering the release of insulin from pancreatic beta cells by inhibiting ATP-sensitive potassium channels (KATP channels), which results in beta cell depolarization. Consequently, glimepiride enhances insulin secretion and enhances peripheral tissue responsiveness to insulin, leading to heightened glucose uptake and decreased plasma glucose and HbA1C levels. (3,4)

This combination medication of LBG and GLP comprises two antidiabetic components. LBG functions as an insulin sensitizer by attaching to PPAR receptors within fat cells, thereby enhancing their sensitivity to insulin. On the other hand, GLP reduces blood sugar levels by stimulating insulin production in the pancreas, a crucial substance for sugar breakdown in the body, and aiding in efficient insulin utilization (5,6).



(a)

(b)

Figure 1. Chemical structure of (a) LBG and (b) GLP

Extensive literature survey revealed various analytical methods were reported for estimation LBG and GLP individually and in combination with other drugs (7-31). However, no analytical method was reported for simultaneous estimation of both LBG and GLP. Therefore, there was interest in developing simple, accurate, precise,

and reproducible UV spectrophotometric methods for simultaneous estimation of LBG and GLP in combined dosage form. The developed methods underwent validation according to the guidelines outlined in the ICH [Q2 (R1)] (32).

Experimental section

Instrumentation

UV-Visible double beam spectrophotometer (Shimadzu-1800 Japan) equipped with UV Probe 2.33 software was employed to record all absorption spectra (spectral bandwidth of 2 nm, wavelength accuracy of 0.5 nm, and utilized a pair of 10 mm matched quartz cells within the range of 200-400 nm). Analysis was conducted using UV Probe 2.33 software. Additionally, an electronic balance (Shimadzu ATX-200) was utilized in the experiment.

Reagents and Chemicals

A pure sample of LBG was generously provided as a gift by Akums Drugs and Pharmaceuticals Ltd., Delhi, India, while GLP was received as free sample from anonymous company. methanol utilized in the experiment was of analytical grade.

Selection of solvent

Solubility testing of LBG and GLP was conducted with various solvents including distilled water, methanol, acetonitrile, 0.1 M NaOH, and 0.1 M HCl. Both drugs exhibited solubility in methanol, thus methanol was chosen as the preferred solvent.

Preparation of Standard solution

A precisely measured amount of 25 mg of LBG and 25 mg of GLP was separately transferred into distinct 25 mL volumetric flasks. 10 ml Methanol was added to each flask, shake it to dissolve the drugs, and then diluted with methanol to the mark, resulting in a stock solution with a concentration of 1000 µg.mL^{-1}. Subsequently, a 2.5 mL aliquot was pipetted from the above stock solution into another 25 mL volumetric flask and diluted to the mark with methanol to obtain a stock solution with a concentration of 100 µg.mL^{-1}.

Preparation of Test solution

Twenty tablets, each containing 0.5 mg of LBG, and 1 mg of GLP were weighed. The tablets were finely powdered in a mortar, and an amount equivalent to the content of one tablet was accurately weighed and transferred into a 100 mL standard volumetric flask containing 50 mL of methanol. The mixture was then sonicated for 10 minutes and then diluted with methanol up to the mark, resulting in a stock solution with concentration of 5, 10 µg.mL^{-1}of LBG and GLP, respectively.

Procedure for determination of wavelength for measurement

0.5 mL of LBG stock solution (100 µg.mL^{-1}) and 1.0 mL of GLP stock solution (100 µg.mL^{-1}) were transferred into two separate 10 mL volumetric flasks. Methanol was added to each flask to reach the mark, resulting in concentrations of 5 µg.mL^{-1} for LBG and 10 µg.mL^{-1} for GLP. Both solutions were then scanned within the wavelength range of 200-400 nm against methanol as the blank. The obtained spectra revealed maximum absorbance wavelengths of 250 nm and 227 nm for LBG and GLP, respectively (Figure 2).

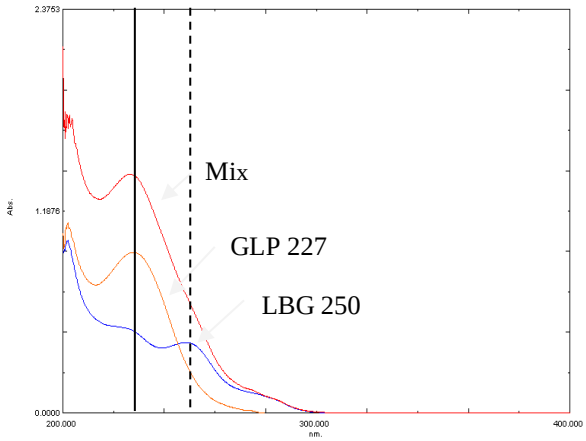


Figure 2. Overlaid spectra of LBG (5 µg.mL^{-1}) and GLP (10 µg.mL^{-1})

Method I: Simultaneous equation method (Vierodt's)

The simultaneous equation method relies on measuring the absorption of drugs (X and Y) at wavelengths corresponding to the maximum absorbance of the other drug. For this method, wavelengths 250 nm and 227 nm were selected, representing the λ_{max} of LBG and GLP, respectively. Absorbances were recorded at these wavelengths, and absorptivities (A1%1cm) for both drugs and both wavelengths were determined as the mean of six independent determinations. The concentrations in the sample were calculated using the following equations:

$$C_{LBG} = (A_2\, ay_1 - A_1\, ay_2) / (ax_2\, ay_1 - ax_1\, ay_2) \quad \text{(Equation 1)}$$

$$C_{GLP} = (A_1\, ax_2 - A_2\, ax_1) / (ax_2\, ay_1 - ax_1\, ay_2) \quad \text{(Equation 2)}$$

A_1 and A_2 represent the absorbances of the mixture on 250 nm and 227 nm, respectively. ax_1 and ax_2 are the absorptivities of LBG at λ_1 (250 nm, i.e., the λ_{max} of LBG) and λ_2 (227 nm, i.e., the λ_{max} of GLP), respectively. Similarly, ay_1 and ay_2 are the absorptivities of GLP at λ_1 and λ_2, respectively. C_{LBG} and C_{GLP} represent the concentrations of LBG and GLP, respectively. Figure 3 displays the overlay spectra of both drugs in a 1:2 ratio, and the criteria for achieving maximum precision (absorbance ratio $(A_2/A_1)/ax_2/ax_1$ and ay_2/ay_1) using this method were calculated. It was found that these criteria fell outside the range of 0.1-2.0, which is satisfactory for both drugs.

Method II: Second order derivative spectrophotometric method

For the second order derivative spectrophotometric method, precise volumes of LBG ranging from 3 to 13 µg.mL^{-1} were withdrawn from its stock solution (100 µg.mL^{-1}) and transferred into a series of 10 mL volumetric flasks. These were then diluted to the mark with methanol and thoroughly mixed. Similarly, accurate volumes of GLP ranging from 6 to 26 µg.mL^{-1}were withdrawn from its working solution (100 µg.mL^{-1}), transferred into separate 10 mL volumetric flasks, diluted to the mark with methanol, and mixed well.
Upon evaluating the derivative order spectra of both LBG and GLP from first to fourth derivative, it was determined that the second order derivative spectra with a δλ of 16 and a scaling factor of 1000 was suitable. From the overlaid second-order derivative spectra of LBG (5 µg.mL^{-1} and GLP (10 µg.mL^{-1}), the zero crossing points (ZCP) of LBG and GLP were identified. The selected wavelength for the ZCP of LBG was 259 nm, whereas GLP exhibited absorbance at this point. Conversely, the ZCP of GLP was found to be 297 nm, where LBG showed absorbance (Figure 3).
The absorbances versus concentrations were plotted in quantitative mode to generate working curves. By extrapolating the absorbance values of the sample solution from these curves, the concentrations of the corresponding drugs were determined. Both drugs demonstrated adherence to Beer's Law.

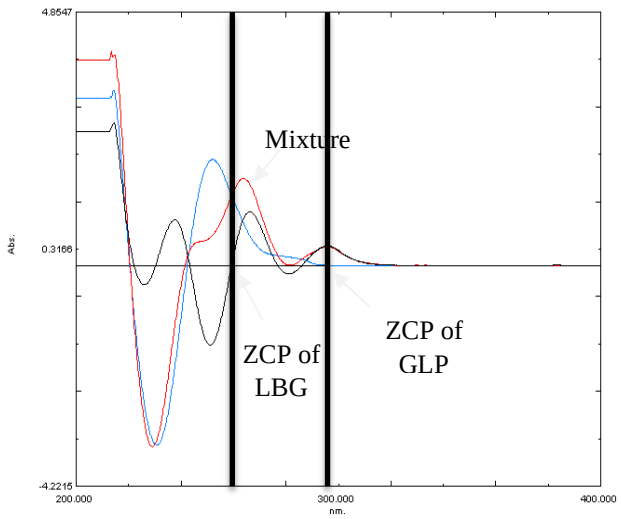


Figure 3. Overlaid second order derivative spectra of LBG (5 µg.mL^{-1}), GLP (10 µg.mL^{-1}) and Mixture (5 + 10 µg.mL^{-1}).

Validation Parameters

Validation was conducted in accordance with ICH guidelines (ICH Q2 (R1)).

Accuracy

In order to evaluate potential interference from excipients present in combined dosage form, experiments were conducted using the standard addition method. Known quantities of LBG and GLP to a predetermined concentration of the test solution. The quantities of standards recovered were then calculated, providing the mean recovery with upper and lower limits of %RSD.

Precision

Repeatability: Instrument's precision was assessed by repeatedly scanning and measuring the absorbance of solutions (n = 6) containing LBG and GLP, without altering the parameters of the proposed spectrophotometric methods.
Intermediate Precision: Intraday and inter day precision were evaluated in terms of %RSD. The experiments were conducted three times within a single day for intraday precision and on three different days for inter day precision. Concentration values for both intraday and inter day precision were determined separately three times, and %RSD values were calculated.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the criteria of 3s/m and 10s/m, respectively. Where, 's' represents the standard deviation of the intercept (n = 3) of the sample, while 'm' denotes the slope of the corresponding calibration curve.

Statistical Analysis

Statistical analysis was conducted to evaluate the impact of two methods on the simultaneous determination of LBG and GLP through the utilization of F-test.

Results and Discussion

Method I: Simultaneous equation method (Vierodt's)

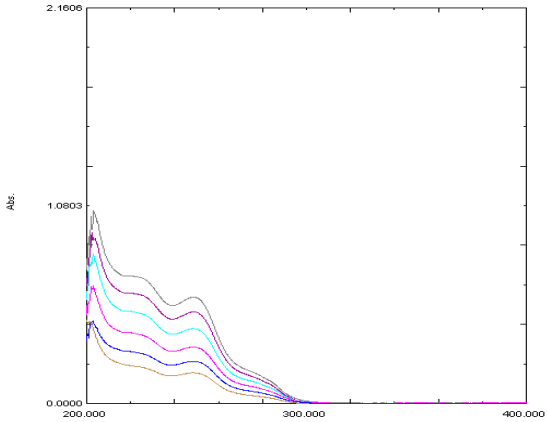
It relies on measuring the absorbance of both drugs, LBG and GLP, at their respective λ_{max} values. Wavelengths 250 nm and 227 nm were chosen, corresponding to the λ_{max} of LBG and GLP, respectively. Figure 4 displays the overlaid spectra of both drugs in a 1:2 ratio. Absorbances were recorded at these selected wavelengths, and specific absorptivities (A1%1cm) for both drugs on both wavelengths were determined as mean of six independent determinations (Table 3). The calculation of A1%1cm is carried out using equation 3.

$$A = a.b.c \qquad \text{(Equation 3)}$$

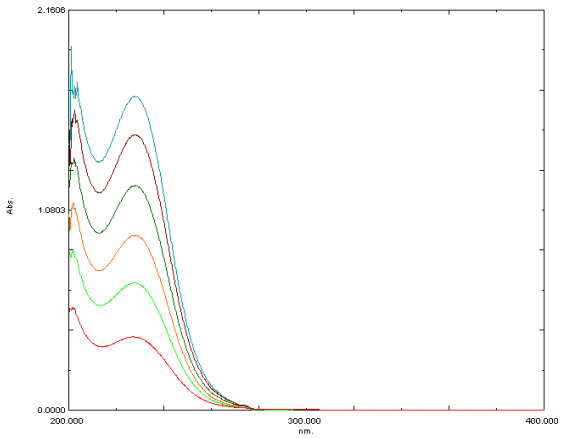
Where:
A = absorbance,
a = specific absorptivity,
b = path length 1 cm,
c = concentration of absorbing species in g.100 mL^{-1}.

Table 1. Absorptivities at 250 nm and 227 nm

At 250 nm		At 227 nm	
ax_1	676.56	ax_2	582.04
ay_1	668.37	ay_2	179.92



(a)

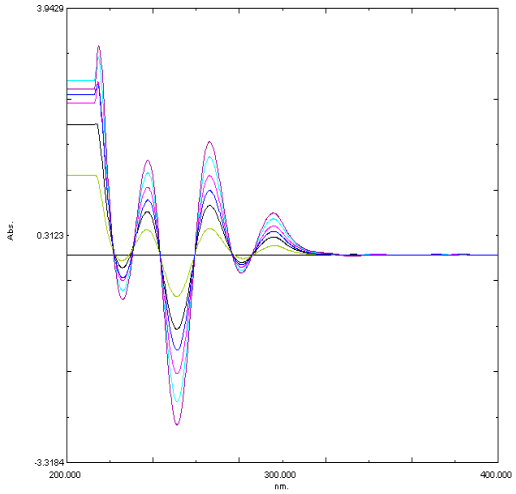


(b)

Figure 4. (a) zero order overlain spectra of LBG (3-13 µg.mL^{-1}); (b) zero order overlain spectra of GLP (6-26 µg.mL^{-1}).

Method II: First order derivative Spectrophotometric method

In comparison to zero order spectra, second derivative spectra offer greater resolution, as illustrated by the zero-crossing points depicted in Figures 3 and 5. These figures demonstrate overlaid second order derivative spectra for LBG and GLP. At 259 nm, LBG having zero-crossing point allows for the determination of GLP, while at 297 nm, GLP having zero crossing point allows for the determination of LBG.



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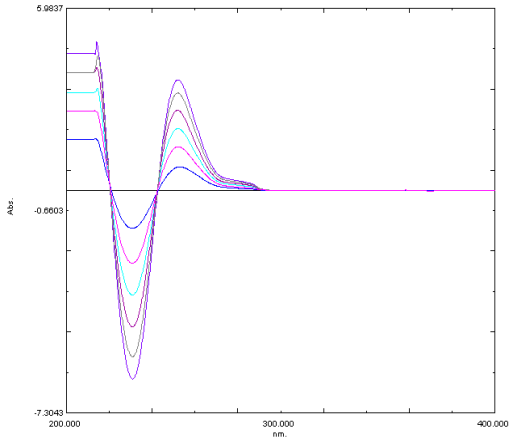

(b)

Figure 5. Overlaid second order derivative spectra of (a) LBG (3-13 µg.mL^{-1}) and (b) GLP (6- 26 µg.mL^{-1}).

Application of proposed methods for analysis of LBG and GLP in combined dosage form

Method I: This method involved recording the zero-order spectrum of the test solution and measuring absorbance at 250 nm and 227 nm to determine LBG and GLP concentrations, respectively. The concentrations of LBG and GLP in the combined dosage form were then determined using the simultaneous equation method. The percentage assay values are provided in Table 2.

Method II: This method involved recording the second order spectrum of the test solution and measuring absorbance at 297 nm and 259 nm for the estimation of LBG and GLP, respectively. The concentrations of LBG and GLP in the combined dosage form were determined using the second order derivative method. The percentage assay values are also presented in Table 2. Furthermore, Table 2, Table 3, and Table 4 display the results of the assay, accuracy studies, and a summary of various validation parameters for the methods, respectively.

Table 2. Assay results for tablets using proposed methods.

Formulation	Proposed methods	Label claim (mg)		Amount of drug found (mg)		% Label Claim Assay (n=3) ± SD	
		LBG	GLP	LBG	GLP	LBG	GLP
LOBE-G1	METHOD I	0.5	1	0.501	1.006	100.33 ± 1.00	100.66 ± 1.28
	METHOD II	0.5	1	0.502	1.001	100.53 ± 0.77	100.12 ± 0.42

Table 3. Application of the standard addition technique to analysis of LBG and GLP in combined dosage form by the proposed methods.

Method	Drugs	Amount present ($\mu g.mL^{-1}$)	Amount added ($\mu g.mL^{-1}$)	Total amount of drug ($\mu g.mL^{-1}$)	Amount found ($\mu g.mL^{-1}$)	% Recovery ± SD (n=3)	% RSD
Method I	LBG	2.5	2	4.5	4.51	98.50 ± 1.18	1.18
			2.5	5	5.01	98.80 ± 1.00	1.00
			3	5.5	5.54	99.66 ± 0.80	0.08
			4	9	9.00	99.50 ± 1.25	1.25
	GLP	5	5	10	10.05	100.60 ± 1.06	1.06
			6	11	11.05	100.50 ± 0.67	0.67
Method II	LBG	2.5	2	4.5	4.53	100.50 ± 1.06	1.06
			2.5	5	5.00	98.80 ± 1.46	1.46
			3	5.5	5.54	100.66 ± 1.04	1.04
			4	9	4.03	100.75 ± 0.61	0.61
	GLP	5	5	10	5.01	100.20 ± 1.09	1.09
			6	11	6.03	100.50 ± 0.32	0.32

Table 4. Summary of validation parameters by developed method.

Parameters		Method I		Method II	
		LBG	GLP	LBG	GLP
Working wavelength (nm)		250 nm	227 nm	297 nm	259 nm
Concentration range (µg.mL^{-1})		3-13	6-26	3-13	6-26
Slope		0.055	0.065	0.050	0.0952
Intercept		0.020	0.021	0.007	0.0358
Correlation Coefficient (r^2)		0.997	0.999	0.999	0.999
LOD (µg.mL^{-1})		0.401	0.104	0.066	0.084
LOQ (µg.mL^{-1})		1.217	0.316	0.201	0.257
Precision	Repeatability (n=6) %RSD	1.72	0.73	0.70	1.05
	Intraday (n=3) %RSD	0.78-1.25	0.53-0.69	0.44 - 0.78	0.35 - 1.04
	Interday (n=3) %RSD	0.87-1.56	0.72-0.94	1.45 - 1.69	0.53 - 1.11
Accuracy (%)	80%	98.50 ± 1.18	99.50 ± 1.25	100.50 ± 1.06	100.75 ± 0.61
	100%	98.80 ± 1.00	100.60 ± 1.06	98.80 ± 1.46	100.20 ± 1.09
	120%	99.66 ± 0.80	100.50 ± 0.67	100.66 ± 1.04	100.50 ± 0.32
% Label claim Assay ± SD (n=3)		100.33 ± 1.00	100.66 ± 1.28	100.53 ± 0.77	100.12 ± 0.42

Table 5. F-test for LBG and GLP

LBG	Variable 1	Variable 2	GLP	Variable 1	Variable 2
Mean	100.33	100.53	Mean	100.66	100.12
Variance	0.01507	0.00687	Variance	0.00168	0.00032
Observations	5	5	Observations	5	5
df	4	4	df	4	4
F	2.19		F	5.25	
P(F<=f) one-tail	0.232741902		P(F<=f) one-tail	0.068608	
F Critical one-tail	6.388232909		F Critical one-tail	6.388232909	

A statistical comparison between the developed simultaneous equation method and the second order derivative spectrophotometric method was conducted using an F-test (Table 5). The calculated F-value was found to be less than the critical F-value of 6.38 for both LBG and GLP. This suggests that there is no significant difference observed in the assay results between the two methods.

Conclusions

Two spectrophotometric techniques, namely the simultaneous equation method and the second order derivative method, were devised to concurrently determine LBG and GLP in combined dosage form. The methods demonstrated precision and accuracy, as evidenced by validation outcomes. Application of the developed methods for LBG and GLP estimation in dosage form proved successful. F-test outcomes revealed no substantial variance between assay results obtained from both techniques. Consequently, the proposed methods offer a cost-effective and straightforward approach for routine analysis of LBG and GLP using instrumentation that is relatively inexpensive and easy to operate.

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Conflict of interest

The authors declare no conflicts of interest. Sources of funding may

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