Development and Validation of UV-Spectroscopic Method for Simultaneous Estimation of Lobeglitazone sulphate and Glimepiride in Bulk and Pharmaceutical Dosage Form

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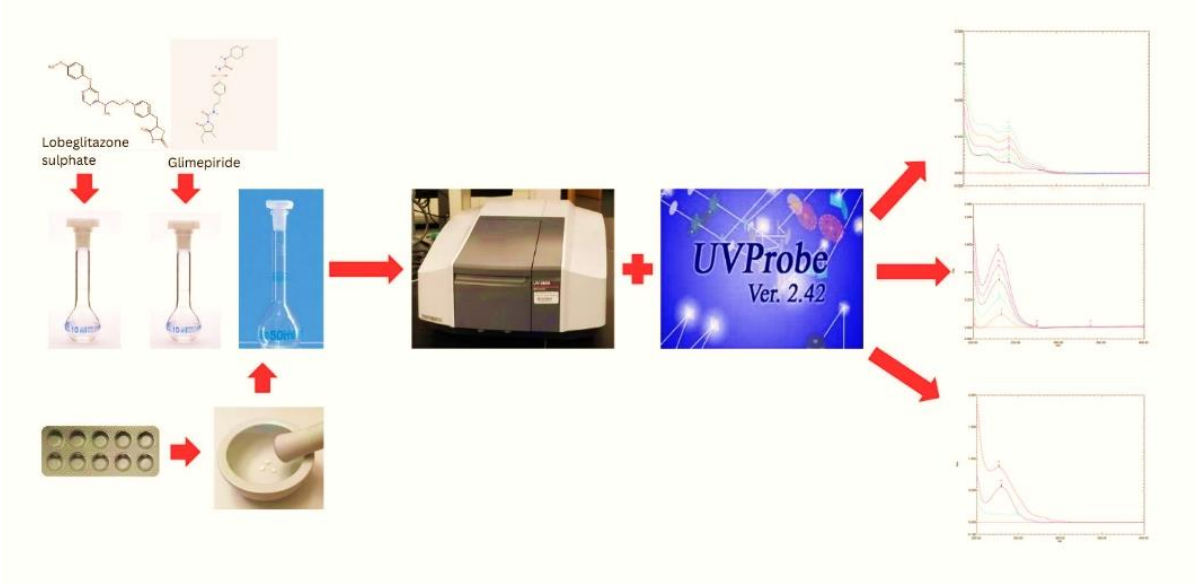
Abstract

A novel, simple, precise and accurate UV- Visible Spectrophotometric method was developed and validated for the simultaneous estimation of Lobeglitazone sulphate (LBG) and Glimepiride (GMP) in bulk and tablet dosage form. The combined formulation was approved for treating Type 2 Diabetes Mellitus (T2DM) by CDCSO. The method employed with organic-aqueous mobile phase i.e. acetonitrile: water (80:20% v/v). Absorbance was measured at 248 nm for LBG and 228 nm for GMP, along with evaluating the isobestic point at 251nm for simultaneous detection. The method demonstrated excellent linearity in the concentration range of 1–5 µg/ml for LBG and 2–10 µg/ml for GMP, with correlation coefficients (R^2) of 0.996 and 0.998 respectively. The limit of detection (LOD) for both drugs were 0.33µg and 0.5µg respectively. The limit of quantification (LOQ) of both drugs were 1.01µg and 1.5µg respectively. Based on the values of LOQ and LOD the linearity was fixed and shown the R^2 values within the limits, confirming the proposed method's sensitivity. Precision studies including intraday, interday, and repeatability assessments yields %RSD values less than 1%, confirming the method's reliability and repeatability. Recovery studies showed mean recoveries close to 99-101%, indicating high accuracy.

Keywords: Lobeglitazone sulphate, Glimepiride, UV- Visible Spectrophotometer, Method Development and Validation, Simultaneous Estimation.

Graphical Abstract

Lobeglitazone sulphate and Glimepiride pure API was dissolved into the solvent accordingly to the needed concentration. Its tablets dosage form was taken in the mortar and pestle crushed into the fine powders. Then weighted the required amount of powder needed based the equivalent weight calculation and dissolved in the same solvent. The solutions absorbance was checked with the help of UV Spectroscopy and data were obtained.



1. Introduction

The combination of Lobeglitazone sulphate and Glimepiride was approved by CDSCO (Central Drugs Standard Control Organization) on 23.05.2023. The combined dosage form is used as an antihyperglycemic agent. Lobeglitazone sulphate (LBG) is a solid white powder having a molecular formula of $C_{24}H_{24}N_4O_5S$, and its IUPAC name is 5- {[4- [2- [[6-(4-methoxy phenoxy) pyrimidin-4-yl]-methylamino] ethoxy] phenyl] methyl}-1,3-thiazolidine-2,4-dione, shown in figure 1 (A). LBG is the thiazolidinedione class of drug. It mainly actions as an insulin sensitiser with attaching & triggering gamma Peroxisome Proliferator-Activated Receptors (γPPAR) inside fatty cells. Through activating γPPAR and helps in attaching of insulin at fatty cells, LBG diminish elevated blood sugar levels, lesser haemoglobin A1C stages, in addition advance fat and liver outlines. LBG is a pure α-PPAR agonist used in the dealing of T2DM. Glimepiride (GMP) is a solid white powder having a molecular formula of $C_{24}H_{34}N_4O_5S$, and its IUPAC name is 4-ethyl-3-methyl-N-[2-{4-((4-methyl cyclohexyl) carbamoyl sulfamoyl) ethyl)-5-oxo-2H-pyrrole-1-carboxamide, shown in Figure 1 (B). GMP is the 2nd generation sulfonylurea class drug used for the managing T2DM by progresses glycaemic control. Sulfonylurea's pharmacological action is stimulus of the discharge of hypoglycaemic agent particles origin of beta cells by hindering ATP-sensitive potassium channels (KATP channels) and beginning depolarisation of the β-cells. Sulfonylurea occasionally categorised as a 3rd group sulfonylurea since it has higher replacements than further 2nd generation sulfonylureas. It is operative in falling starvation plasma sugar, postprandial sugar, and glycated haemoglobin levels and is measured to beneficial, profitable curing choice for handling T2DM. From the literature survey, it has been concluded that insufficient procedures were testified for the assessment of LBG independently, several methods are existing for the simultaneous estimation of GMP combined with other drugs. Very few methods are reported for Lobeglitazone sulphate and Glimepiride combined drugs in HPLC, and no methods were reported by UV. This study focuses on developing a rapid, sensitive and accurate UV-Visible spectroscopy technique for simultaneous assessment of LBG and GMP in pure API and Tablet. The created process was validated accordingly to the ICH Q2 (R1) guidelines which says the

validation parameter. The combined tablet dosage form of LBG and GMP was available in name of LOBG-G1 manufactured by Glenmark Pharmaceuticals.

Fig 1 (A): Lobeglitazone sulphate structure.

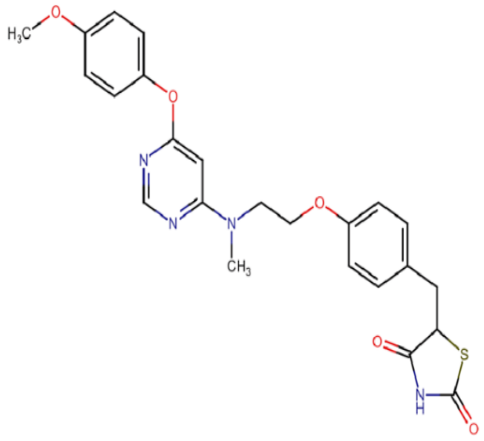
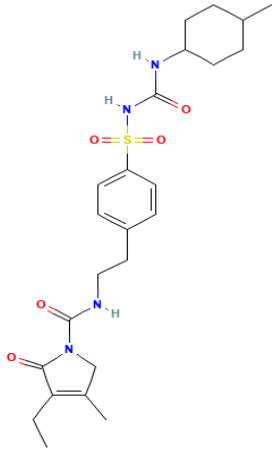


Fig 1 (B): Glimepiride structure



2. Substances and Procedure

2.1 Compounds and Components:

A pure API sample of LBG was generously provided as a gift by Akums Drugs and Pharmaceuticals Ltd., Delhi, India, while GLP was received as a free sample from an anonymous company. The commercial product of LBG and GMP was bought from the market, named LOBG G1, holding Lobeglitazone sulphate (0.5 mg) and Glimepiride (1mg), manufactured by GLENMARK Pharmaceuticals Ltd., Acetonitrile AR grade - Sigma Aldrich. Milli Q Water.

2.2 Instruments:

A double beam UV- visible spectrophotometer (Shimadzu, model-1650PC) having 2 coordinated quartz cells by a 1 cm pathlength were used for spectral measurement. UV probe 2.42 software was installed onto the UV-visible spectrophotometer. Ultrasonic Cleaner Sonicator were used to dissolve the sample in solvent. A micro balance series of Sartorious BSA224S was used to weigh the drugs.

2.3 Solubility Studies / Determine the Diluent:

The solubility of Lobeglitazone sulphate and Glimepiride were determined in various solvents. Solubility is determined by taking 10 mg of drug in a test tube and adding drop by drop (0.1ml) of solvents and shaking few minutes. Solvent was added until the drug dissolved completely. Solvent was measured by the required quantity. Solubility data for individual drug are showed in Table 1.

Table 1: Solubility Studies of individual Drugs

S. No.	SOLVENTS	LOBEGLITAZONE SULPHATE	GLIMEPIRIDE
1.	Acetonitrile	Soluble	Soluble with sonication
2.	Methanol	Soluble	Sparingly Soluble
3.	Water	Slightly Soluble	Insoluble
4.	Ammonia	Soluble	Slightly Soluble
5.	THF	Sparingly Soluble	Soluble
6.	Chloroform	Sparingly Soluble	Sparingly Soluble
7.	Toluene	Sparingly Soluble	Sparingly Soluble
8.	DMSO	Sparingly Soluble	Slightly soluble
9.	Ethanol	Soluble	Sparingly Soluble
10.	Methanol + water	Soluble	Sparingly Soluble
11.	Acetonitrile + water	Well Soluble	Soluble

From the Solubility Studies, it was confirmed that both Lobeglitazone sulphate and Glimepiride are soluble in Acetonitrile and water. The absorbances of the two drugs were found to be higher in Acetonitrile: Water in the ratio of 80:20 v/v.

2.4 Preparations:

2.4.1 Diluent:

To prepare 100 mL of 80:20 v/v ratio of Acetonitrile: water, accurately measure 80ml of acetonitrile and 20ml of Milli Q water using by measuring cylinder and transfer into a beaker. Mix thoroughly and sonicate for 20 mins.

2.4.2 Drug Stock Solution:

1 mg of LBG and GMP standards were measured and moved into 10 ml measuring flask separately, and capacity was filled up to 10 ml by Acetonitrile: water (80:20 v/v) to liquify them entirely. The end concentration of LBG and GMP were 100 µg/ml.

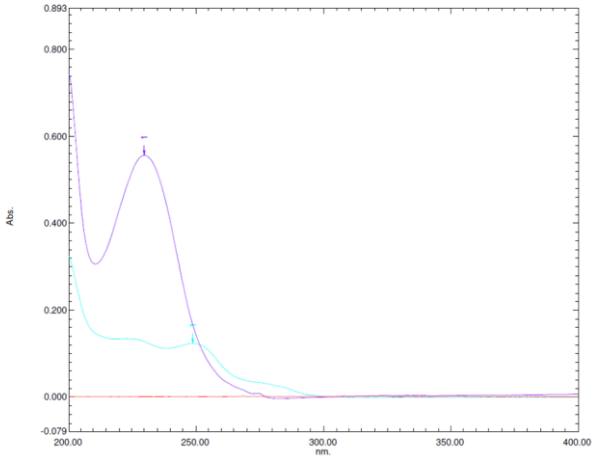
2.4.3 Drug Standard solutions:

Aliquots of 1ml from each stock solution 100µg/ml were shifted to a 10ml measuring bottle and capacity was occupied up to the mark with the solvent system Acetonitrile: water (80:20 v/v) to prepare 10 µg/ml of each drug.

2.4.4 Identification of Wavelength:

The wavelength for the study was investigated by scanning the standard spectra of LBG and GMP between 200 - 400 nm using Acetonitrile: water (80:20 v/v) as blank. The spectrum for LBG and GMP was scanned, and the maximum absorbance for LBG and GMP seemed to be 248nm and 228nm, respectively. The Isobestic point was obtained at 251nm, as shown in Figure 2.

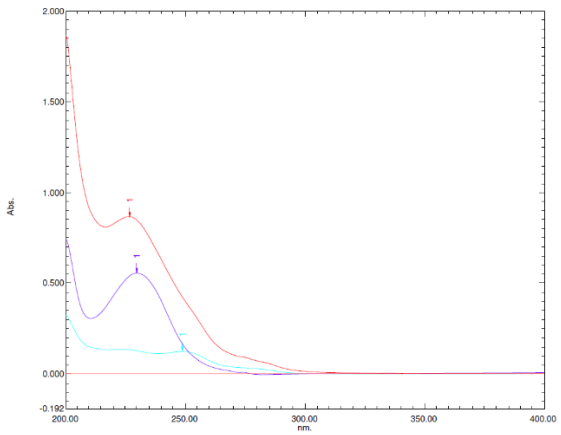
Figure 2: Superimposed spectra of LBG and GMP



2.4.6 Sample:

The 20 tablets of formulation (LOBG-G1 holding 0.5 mg of Lobeglitazone sulphate and 1 mg of Glimepiride) were weighed, containing quantity equal to approximately 2.5 mg of LBG and 5 mg of GMP, were moved into a 50mL measuring flask and 2/3rd was filled by solvent system. The mix was sonicated 20 mins, chilled to the room temperature and diluted up to the line with Acetonitrile: water (80:20 v/v) and sieved through a whatman filter paper. 1 ml aliquot was withdrawn into a 10 ml measuring flask and diluted up to line with Acetonitrile: water (80:20 v/v). The solution contains 5 µg/mL of LBG and 10 µg/mL of GMP. Absorbance's were analysed at 248 nm and 228 nm and the amount of LBG and GMP were calculated using simultaneous equation method and the overlay spectra obtained compared with standards is shown in Figure 3.

Figure 3: Superimposed spectra of formulation with Standards



2.5 Vierodt's Method:

This system of study was focused by occupy of drugs at the wavelength supreme of each other. 2 wavelengths of 248 nm and 228 nm were designated as the λmax of 2 drugs for the progress of the simultaneous calculations. The absorbance of LBG and GMP were analysed, and absorption factor values were estimated at 2 wavelengths. The concentration of 2 drugs in the combination can be quantified using the following equations:

$$\boldsymbol{Cx} = \frac{\mathbf{A2\ ay1 - A1\ ay2}}{\mathbf{ax2\ ay1 - ax1\ ay2}}$$

$$\boldsymbol{Cy} = \frac{\mathbf{A2\ ax1 - A1\ ax2}}{\mathbf{ax2\ ay1 - ax1\ ay2}}$$

Were,

Cx, Cy is the concentration of Lobeglitazone sulphate and Glimepiride, respectively.

A1, A2 are the absorbance of formulation at 248 nm and 228 nm respectively,

ax1, ax2 are the absorptivity of Lobeglitazone sulphate at 248 nm and 228 nm respectively,

ay1, ay2 are the absorptivity of Glimepiride at 248 nm and 228 nm respectively.

The absorption factor of each was planned by using the following formula,

$$\boldsymbol{Absorptivity} = \frac{\boldsymbol{absorbance}}{\boldsymbol{concentration}}$$

3. Outcomes and Discussion

3.1 Linearity and Range:

The linearity elicits that concentration of the sample is directly proportional to absorbance results. The developed method should be linear within the range. The upper and lower limit of the selected linearity concentration denotes as range.

3.1.1 Lobeglitazone sulphate:

LBG was linear at the range of 1-5 µg/ml concentration. From Standard solution, aliquots of LBG 1, 2, 3, 4, 5 ml was pipetted & poured into a 10 ml measuring bottle and filled up to the line by the solvent Acetonitrile: water (80:20 v/v) having concentration of 1, 2, 3, 4, 5 µg/ml respectively and shown in Figure 4(A).

The absorbance of this mixture was noted at wavelengths 248 nm and 228 nm. The calibration curve was designed using concentration vs absorbance. The absorbance is given in Table 2. Double analyses were performed on each concentration. The regression equations and correlation coefficient (r^2) for LBG at wavelengths 248 nm and 228 nm were found and represented in Figure 4(B) and 4(C), respectively.

3.1.2 Glimepiride:

GMP was linear at the range of 2-10 µg/ml concentration. From Standard solution, aliquots of GMP 0.2, 0.4, 0.6, 0.8, 1 ml was pipetted & poured into a 10 ml measuring bottle

and filled to the line with the solvent Acetonitrile: water (80:20 v/v) having concentration of 2, 4, 6, 8, 10 µg/ml respectively and shown in Figure 5(A).

The absorbance of this mixture was noted at wavelengths 228 nm and 248 nm. The calibration curve was designed using concentration vs absorbance. The absorbance is given in Table 3. Double analyses were performed on each concentration. The regression equations and correlation coefficient (r^2) for GMP at wavelengths 228 nm and 248 nm were found and represented in Figure 5(B) and 5(C), respectively.

Table 2: Linearity of absorbance of LBG

S.No.,	Concentration (µg/ml)	Absorbance	
		248 nm	228 nm
1.	1	0.051	0.032
2.	2	0.079	0.059
3.	3	0.104	0.081
4.	4	0.133	0.104
5.	5	0.167	0.131
6.	**Correlation Coefficient (R^2)**	**0.99693**	**0.99860**

Figure 4: (A) Superimposed spectra of LBG; (B) Linearity graph of LBG at 248 nm; (C) Linearity graph of LBG at 228 nm.

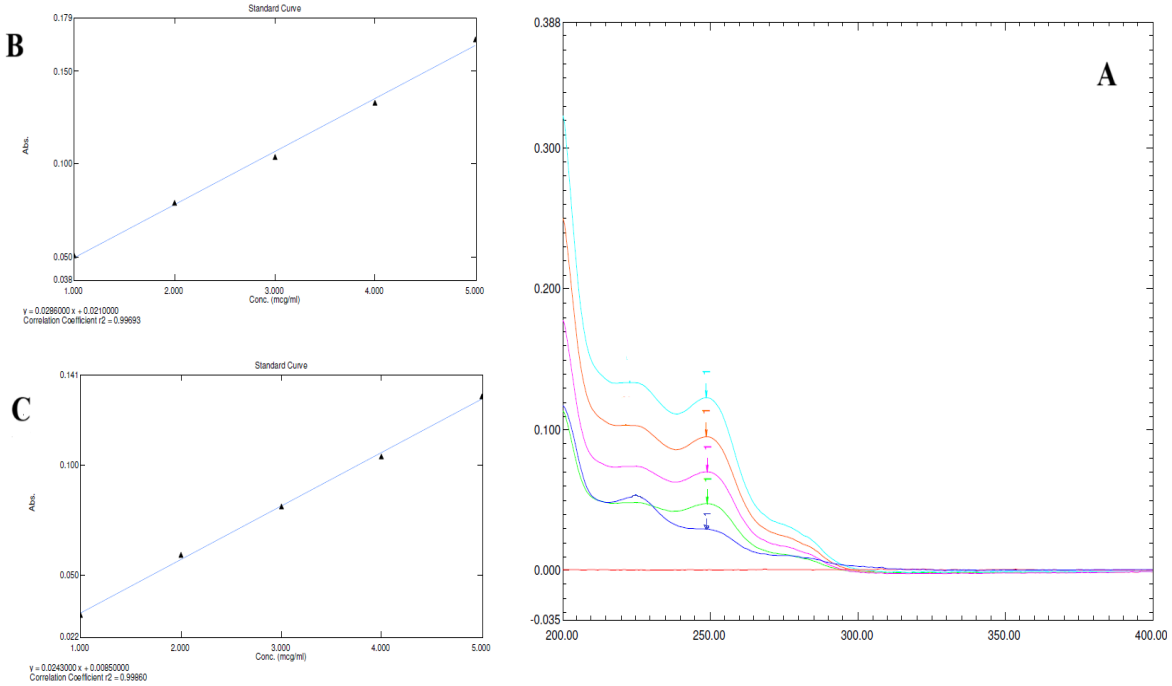
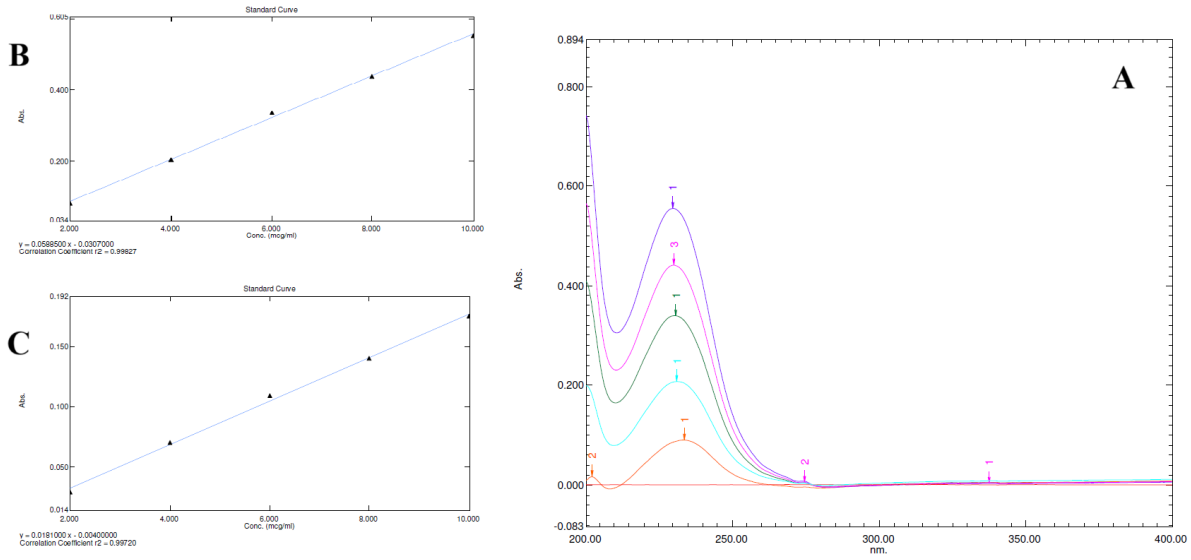


Table 3: Linearity absorbance of GMP

S. No.,	Concentration (µg/ml)	Absorbance	
		248 nm	228 nm
1.	2	0.029	0.082
2.	4	0.070	0.203
3.	6	0.109	0.336
4.	8	0.140	0.438
5.	10	0.175	0.553
6.	**Correlation Coefficient (R^2)**	**0.99720**	**0.99827**

Figure 5: (A) Superimposed spectra of GMP; (B) Linearity graph of GMP at 228 nm; (C) Linearity graph of GMP at 248 nm



3.2 Precision:

The precision of an analytical method is the exactness between separate test outcomes when the method is used continually to several specimens of homogeneous samples. It provides a signal of random mistake results and is expressed as relative standard deviation. Precision should be measured using a minimum of three determinations per concentration. The precision of the analytical method was demonstrated by Intra-day, Inter-day, and Repeatability.

3.2.1 Intraday precision

In an intra-day dissimilarity learning, 9 various mixtures of the identical concentration i.e., (3, 4, 5 µg/ml of LBG) & (6, 8, 10 µg/ml of GMP), were analysed three different times in a day and the absorbance is noted. From the absorbance results mean, standard deviation and %RSD were calculated and given in the following table 4.

Table 4: Intraday Precision of LBG and GMP

S. No.	Concentration (µg/ml)		Absorbance				% RSD			
	LBG	GMP	LBG	GMP	LBG	GMP	LBG	GMP	LBG	GMP
			248 nm		228 nm		248 nm		228 nm	
1.	3	6	0.103	0.108	0.082	0.335	0.961	0.917	0.709	0.454
			0.104	0.109	0.081	0.336				
			0.105	0.110	0.081	0.338				
2.	4	8	0.135	0.142	0.102	0.437	0.863	0.709	0.970	0.474
			0.133	0.140	0.104	0.438				
			0.133	0.141	0.103	0.441				
3.	5	10	0.165	0.174	0.133	0.557	0.602	0.571	0.757	0.477
			0.167	0.175	0.131	0.553				
			0.166	0.176	0.132	0.552				

3.2.2 Interday Precision:

In inter-day dissimilarity studies, 9 various solutions of identical concentration i.e., (3, 4, 5 µg/ml of Lobeglitazone sulphate) & (6, 8, 10 µg/ml of Glimepiride), were analysed 3 times for the 3 following days and %RSD was calculated and given in following table 5.

Table 5: Interday Precision of LBG and GMP

S. No.	Concentration (µg/ml)		Absorbance				% RSD			
	LBG	GMP	LBG	GMP	LBG	GMP	LBG	GMP	LBG	GMP
			248 nm		228 nm		248 nm		228 nm	
1.	3	6	0.103	0.108	0.082	0.335	0.558	0.934	0.698	0.746
			0.103	0.106	0.083	0.337				
			0.104	0.107	0.083	0.340				
2.	4	8	0.135	0.142	0.102	0.437	0.735	0.967	0.980	0.681
			0.136	0.143	0.101	0.440				
			0.137	0.145	0.103	0.443				
3.	5	10	0.165	0.174	0.133	0.557	0.916	0.869	0.859	0.720
			0.167	0.176	0.135	0.560				
			0.168	0.177	0.135	0.565				

3.2.3 Repeatability:

In repeatability studies, solution of the same concentration (3 µg/ml of Lobeglitazone sulphate) & (6 µg/ml of Glimepiride) was analysed six times repeatedly, %RSD were calculated and given in the succeeding Tables 6 & 7.

Table 6: Repeatability of Lobeglitazone sulphate

Concentration (µg/ml)	**Absorbance**		**% RSD**	
	248 nm	**228 nm**	**248 nm**	**228 nm**
3	0.104	0.081	0.608	0.502
	0.103	0.082		
	0.105	0.081		
	0.104	0.081		
	0.104	0.081		
	0.104	0.081		

Table 7: Repeatability of Glimepiride

Concentration (µg/ml)	**Absorbance**		**% RSD**	
	248 nm	**228 nm**	**248 nm**	**228 nm**
6	0.109	0.336	0.580	0.243
	0.108	0.335		
	0.110	0.334		
	0.109	0.335		
	0.109	0.336		
	0.109	0.336		

3.3 Limit of Detection:

LOD is a degree of lowermost concentration of a mixture that can be constantly detected, but is not necessarily quantified under the stated experimental conditions. The detection limit was calculated from the calibration curve, using the slope and the intercept values and is given in Table 8. The limit of detection can be computed by,

LOD = 3.3 * SD / Slope

Somewhere, SD = Standard Deviation of intercept values

3.4 Limit of Quantification:

LOQ is lowermost concentration of a mixture that can be consistently measured by adequate exactness and correctness. Quantification limit was intended from the calibration curve, using the slope and the intercept values and is given in Table 8. The limit of quantification can be computed by,

LOD = 10 * SD / Slope

Table 8: LOQ and LOD of LBG and GMP

PARAMETER	DRUGS			
	LBG		GMP	
	248 nm	228 nm	248 nm	228 nm
LOD	0.33	0.22	0.63	0.5
LOQ	0.99	0.68	1.93	1.5

3.5 Accuracy:

The performed method's accurateness was assessed by execution of recovery studies at the 80, 100, and 120% levels. The evaluated results were described in Table 9.

Table 9: Accuracy of LBG and GMP

Drugs	Spiked level	% Recovery	% RSD
LBG	80 %	99.1	0.588
	100 %	100.5	
	120 %	99.5	
GMP	80 %	99.3	0.805
	100 %	101.1	
	120 %	100.9	

4. Conclusion

The UV Spectroscopic Method was developed by using Acetonitrile: water (80:20 v/v). These solutions were scanned in the UV-Visible region 200-400nm. It was found that Lobeglitazone sulphate showed a maximum absorbance at 248 nm, and Glimepiride showed at 228 nm. For the study, the λmax at 248 nm and 228 nm of LBG and GMP were taken for the "Simultaneous Equation Method", respectively. The simultaneous equation was obtained using Cx and Cy, which were determined by Vierodt's method. The method was validated by using ICH guidelines for the following parameters: Linearity & Range, Precision, LOD and LOQ, and Accuracy.

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