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Research Article

A STUDY OF DEVELOPMENT AND VALIDATION OF A METHOD FOR SIMULTANEOUS ESTIMATION OF LOBEGLITAZONE SULFATE AND GLIMEPIRIDE USING REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN DOSAGE FORM AND CHARACTERIZATION OF DEGRADANTS USING LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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ABSTRACT

Objectives: A novel, speedy, and accurate high-performance liquid chromatography approach has been established for quantitatively analyzing lobeglitazone sulfate and glimepiride.

Methods: In this method, the drugs were separated using Inertsil Octadecylsilyl (ODS) 250 × 4.6 mm, 5 µm column with a flow rate of 1 mL/min. The buffer consists of 1 mL of formic acid, which is dissolved in 1 L Milli-Q water, and a mobile phase consisting of a 50:50 (v/v) mixture of 0.1% formic acid and acetonitrile. The 260 nm wavelength was used for detection.

Results: Lobeglitazone sulfate concentrations ranging from 12.5 µg/mL to 75 µg/mL and glimepiride concentrations from 25 µg/mL to 150 µg/mL show good linearity in the suggested approach. The limit of detection and limit of quantification values were 0.3 µg/mL, 1.0 µg/mL and 0.15 µg/mL, 0.5 µg/mL for glimepiride and lobeglitazone sulfate, respectively. All the parameters of accuracy, specificity, and method precision were all within the acceptable limit.

Conclusion: In the forced degradation studies, the degradation products were characterized by the use of liquid chromatography–mass spectrometry. The current method was found to be simple, economical, suitable, and validated according to the International Council for Harmonization guidelines.

Keywords: International Council for Harmonization guide lines, Reversed-phase-high-performance liquid chromatography, Liquid-chromatography-mass spectrometry, Characterization.

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INTRODUCTION

The antidiabetic [1] lobeglitazone belongs to the thiazolidinedione family of medications and goes by the brand names Duvie and Chong Kun Dang. By binding to peroxisome proliferator-activated receptors (PPARs) in adipose tissue, the compound enhances cellular sensitivity to insulin. It acts as an agonist for both PPARα [2] and PPARγ [3,4], thereby contributing to its overall insulin-sensitizing effect [5]. To help regulate blood glucose levels, lobeglitazone is prescribed to individuals with type 2 diabetes mellitus [6,7]. It is compatible with both metformin and other diabetes medications. In terms of key membrane transporters, lobeglitazone interacts with three of the six that the Food and Drug Administration has identified OATP1B1 [8], OAT3 [9], and MDR1 [10,11]. A substrate of rodent OATP1B2 [12] was lobeglitazone *in vitro*. Lobeglitazone had interactions with CYP1A2 [13], 2C9 [14], and 2C19 [15]. Atorvastatin blocked the lobeglitazone's distribution to the liver in rats.

For the treatment of type 2 diabetes, doctors often give glimepiride, an antidiabetic drug belonging to the sulfonylurea family. Because metformin has been shown to be both safe and effective, it is considered a second-line therapy. In addition to making dietary and physical activity changes, glimepiride should be taken at the same time. It takes 3 h for the effects to kick in after oral administration and continue for roughly a day after that. Headache, nausea, and vertigo [16] are common adverse effects. Low blood sugar [17] is one of the worst adverse effects that may occur. It is advised against using this product if you are pregnant or nursing. Primarily, it achieves its effects by enhancing the pancreas's insulin secretion. Other possible side effects include gastrointestinal [18] problems, allergic responses [19]. Hypoglycemia is more likely to occur in the first few weeks of therapy. In rare cases, glimepiride may cause abnormalities in blood production, such as thrombocytopenia [20], leucopenia [21], and hemolytic anemia [22]. It is recommended to limit alcohol intake and sun exposure as they might amplify negative effects. Fig. 1 shows the chemical structures of lobeglitazone sulfate and glimepiride. The aim of this study was to develop a rapid, simple, and robust isocratic reversed-phase high-performance liquid chromatography (RP-HPLC) method that offers improved resolution or a shorter run time compared to existing methods and to comprehensively characterize the degradation products formed under the International Council for Harmonization (ICH)-recommended stress conditions using liquid chromatography–mass spectrometry (LC-MS).

The research published here use high-performance liquid chromatography (HPLC) to measure lobeglitazone sulfate and glimepiride. To yet, there have been four ultraviolet (UV) methods [23-26], three HPLC [27-29], and one ultra-performance liquid chromatography (UPLC) resolution [30] report available. Very few articles were reported in the last few decades for determining the lobeglitazone sulfate and glimepiride using HPLC, UV, and UPLC. We encountered problems such as long runtime, preparation of samples, and mobile phases, which were very costly

in previous methods. However, our developed method is validated as per ICH guidelines and has a shorter run time. It is more precise, less costly, and possesses good linear calibration curves, accuracy, limit of detection (LOD), and limit of quantification (LOQ). We developed a single method for the estimation of two drugs (lobeglitazone sulfate and glimepiride) and its degradants were characterized using LC-MS.

METHODS

Materials

Acetonitrile was supplied by Merck (India) Ltd. in Worli, Mumbai, India. The active pharmaceutical ingredients (APIs) used as standards for lobeglitazone sulfate (purity-99.98%) and glimepiride (purity-99.99%) were acquired from Zydus Cadila Healthcare Ltd, Ahmedabad. We acquired water from Milli-Q and formic acid from local vendor.

Equipment

- HPLC: Waters Alliance e2695 chromatographic apparatus with a quaternary pump, photodiode array (PDA) detector, and Empower-2.0 chromatographic software was used.
- LC-MS: The degradants were analyzed using a SCIEX QTRAP 5500 mass spectrometer fitted with a positive-ion electrospray ionization (ESI) source. Ion spray parameters include a source temperature of 550°C, a drying gas temperature range of 120–250°C, a pressure of 55 psi, drying gas flow rate 5 mL/min, a declustering potential of 40V, an entrance potential of 45V, an exit potential of 15V, a capillary voltage of 5500V, and a dwell time of 1 s. Nitrogen was used as a collision gas.

Chromatographic conditions

The approach was developed and validated using instruments from a Waters Alliance HPLC system. The data were processed using Empower 2.0 software. The experiment used an Inertsil ODS column with dimensions of 250 mm × 4.6 mm and a particle size of 5 µm. Isocratic elution was used to purify the chemical. The mobile phase consisted of a 50:50 ratio of acetonitrile and 0.1% formic acid buffer solution. A flow rate of 1.0 mL/min was set for the pump. A wavelength of 260 nm was used for UV detection. An injection volume of 10 µL was used.

Preparation of 0.1% formic acid buffer

Dissolve 1 mL of formic acid in 1 L of Milli-Q water and filter it through a 0.45 µ filter paper.

Preparation of mobile phase

0.1% formic acid: acetonitrile (50:50).

Diluent

Acetonitrile

Preparation of standard solution (concentration of glimepiride-100 µg/mL and lobeglitazone sulfate-50 µg/mL): Accurately weigh approximately 5 mg of lobeglitazone sulfate and 10 mg of glimepiride working standards into a 10 mL volumetric flask. Add 7 mL of diluents and sonicate until the drugs are dissolved. Fill the flask up to the mark with diluent. This is called a stock solution. The concentration of stock solution is lobeglitazone sulfate-500 µg/mL and glimepiride-1000 µg/mL.

Transfer 1 mL of the standard stock into a 10 mL volumetric flask and fill it up with diluents until it reaches the mark.

Making the test solution

To make a sample solution with 50 µg/mL of lobeglitazone sulfate and 100 µg/mL of glimepiride, 76 mg of the sample (containing 0.5 mg of lobeglitazone sulfate and 1 mg of glimepiride) was dissolved in 10 mL of diluents.

Wavelength optimization

The lobeglitazone sulfate and glimepiride solution absorption spectrums were scanned and recorded using a PDA detector of 200–400 nm. By looking at the spectrum, we can see that lobeglitazone sulfate and glimepiride have the highest absorbance at 260 nm. Hence, the choice was made to use a wavelength of 260 nm for method validation.

RESULTS AND DISCUSSION

Optimization of method and standard solution concentration

Initial trials with a C8 column and 0.1% perchloric acid: acetonitrile (60:40) resulted in peak tailing. Switching to a C18 column and using 0.1% formic acid: acetonitrile (50:50) significantly improved peak symmetry and resolution. Based on these tests, the Inertsil ODS column (250 × 4.6 mm, 5 µ) with a PDA detector produced rather good peak shapes. In this method, a 50:50 combination of buffer and acetonitrile was used as mobile phase, with a flow rate of 1.0 mL/min and a column temperature of room temperature. Tabulated in Table 1 are the ideal parameters for the established and verified HPLC procedure.

System suitability

The HPLC was started after adding the standard solution to the system, and the system suitability parameters were determined to be within an acceptable range. The average relative standard deviation (RSD) peak regions were used to calculate the RSD percentage. The RSD obtained an acceptable proportion of identical injections. The results are shown in Fig. 2 and Table 2 [31,32].

Specificity

The placebo effect was studied through a research effort. To perform the test, we prepared equal amounts of API and placebo with the test concentration in the test method and then injected the samples into the HPLC. No interference was found in the chromatograms of the placebo solution and empty cell solution, where lobeglitazone sulfate and glimepiride were retained. Blank chromatogram is shown in Fig. 3.

Table 1: Optimized HPLC method conditions

S. No.	Parameter	Method conditions
1	Column	Inertsil ODS 250×4.6 mm, 5 µm
2	Flow rate	1 mL/min
3	Wave length	260 nm
4	Injection volume	10 µL
5	Run time	5 min
6	Mobile phase	0.1% formic acid: ACN 50:50

HPLC: High-performance liquid chromatography, ACN: Acetonitrile

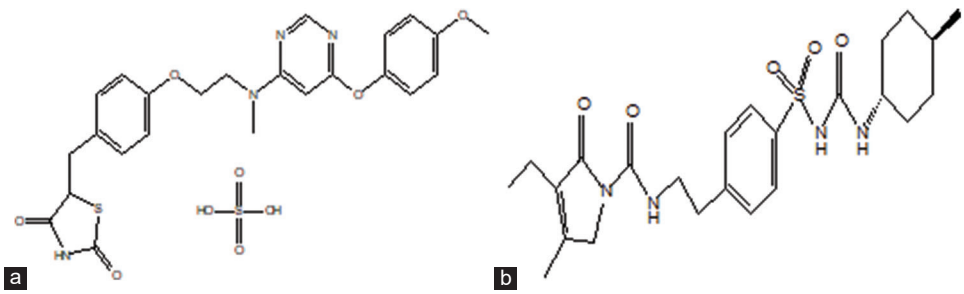

Fig. 1: Structural representations of (a) lobeglitazone sulfate and (b) glimepiride

Placing blank or placebo samples at the retention periods of glimepiride or lobeglitazone sulfate did not reveal any chromatographic interference.

Linearity

For lobeglitazone sulfate, linearity concentrations of 12.5 µg/mL–75 µg/mL were prepared, while for glimepiride, they ranged from 25 µg/mL to 150 µg/mL. The regression models derived for quantifying Lobeglitazone sulfate are presented here Y=25604.22x+1249.79 (CC-0.99992) and Glimepiride Y=27081.32x+11605.61 (CC-0.99992), respectively. Table 3 shows the results, and Fig. 4 depicts the linearity plots [33].

Robustness

No notable variation in RSD is seen in robustness, even if there is a modest flow rate fluctuation of 0.1 mL and an organic solvent content of 10% in its chromatographic condition [34]. Table 4 displays the results.

Precision

By analyzing both the process of preparing the sample solution and the final findings, the procedure's accuracy may be determined. The RSD was determined using data from at least six separate determinations, which were used to verify repeatability [35]. The following arguments are advanced using the information in Table 5. Fig. 5 displays the chromatogram of the method's accuracy.

Intermediate precision

Two independent days, with separate analysts and devices, were used to sample six identical solutions. For each peak position, we calculated the mean and percentage RSD. Table 6 contains the results.

LOD and LOQ

We used the calibration curve approach to compute the LOD and LOQ. The LOD and LOQ of the drugs were determined using a well-established RP-HPLC method, which included injecting standard solutions of varying concentrations. The LOD and LOQ were determined. The LOD and LOQ concentrations of lobeglitazone sulfate were 0.15 µg/mL and 0.5 µg/mL, respectively, whereas for glimepiride, the corresponding values were 0.30 µg/mL and 0.1 µg/mL.

Accuracy

The recovery studies at three distinct levels (80%, 100%, and 120%) validated the method's accuracy. We made preparations with varying amounts of lobeglitazone sulfate (40, 50, and 60 µg/mL) and

Table 2: Results of system precision

S. No.	System suitability parameter	Acceptance criteria	Lobeglitazone sulfate		Glimepiride	
			Mean	Standard deviation	Mean	Standard deviation
1	Retention time	NLT 2.0	3.716	0.0026	2.344	0.0032
2	USP tailing	NMT 2.0	1.07	0.0163	0.89	0.0163
3	USP plate count	NLT 3000	7891	12.3721	16724	22.7369
4	USP resolution	NLT 2.0	9.79	0.0261	-	-

Mean±standard deviation (n=6). NLT: Not less than, NMT: Not more than, USP: United states pharmacopeia

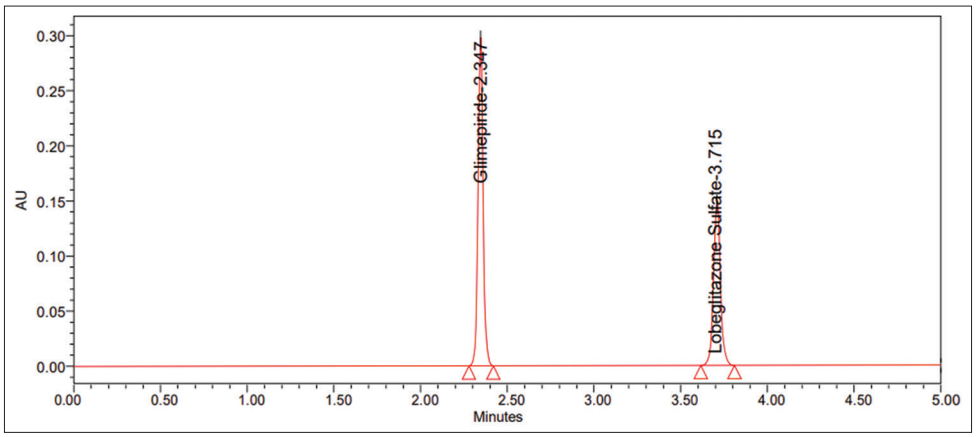

Fig. 2: Chromatogram of system suitability

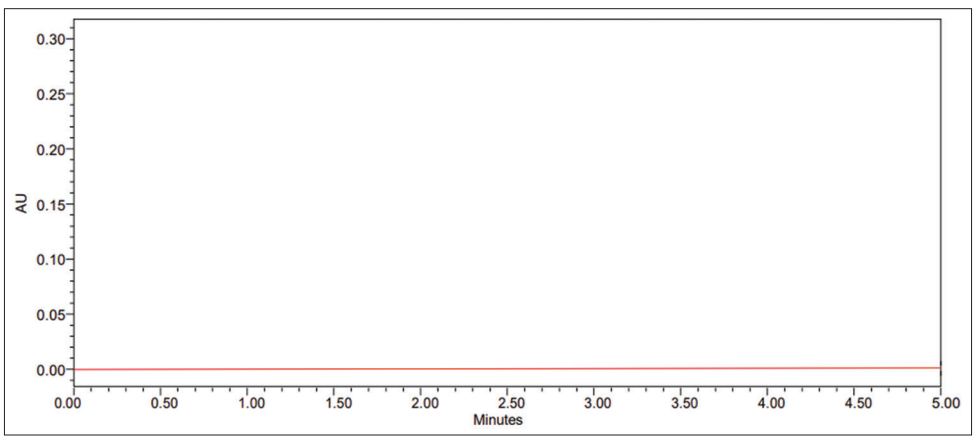

Fig. 3: Chromatogram of blank

glimepiride (80, 100, and 120 µg/mL). The recovery rates ranging from 98% to 102% were discovered. Tables 7 and 8 show the results of the accuracy tests for glimepiride and lobeglitazone sulfate, respectively.

Table 3: Results of linearity

S. No.	Lobeglitazone sulfate		Glimepiride	
	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area
Linearity-1	12.50	319374	25.00	678551
Linearity-2	25.00	647748	50.00	1374994
Linearity-3	37.50	948122	75.00	2053545
Linearity-4	50.00	1295497	100.00	2752649
Linearity-5	62.50	1604871	125.00	3381210
Linearity-6	75.00	1914245	150.00	4057984
Slope	25604.22		27081.32	
Intercept	1249.79		11605.61	
CC	0.99992		0.99992	

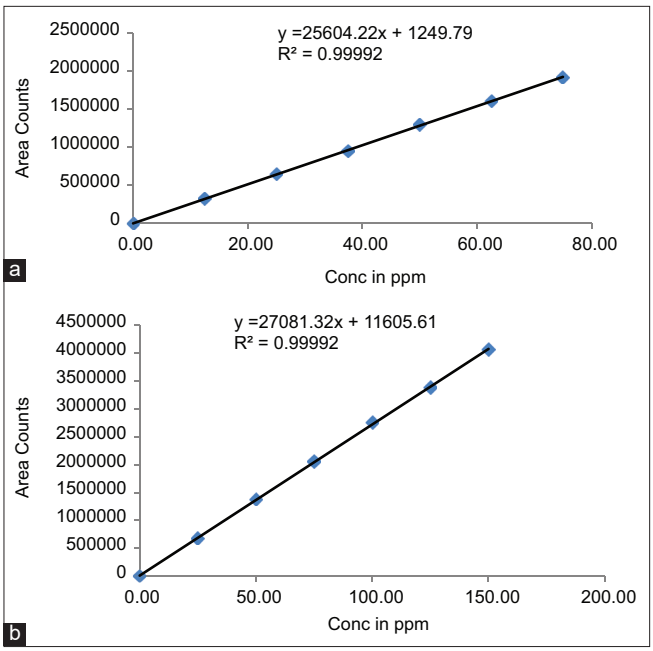

Fig. 4: Calibration plots of (a) lobeglitazone sulfate and (b) glimepiride

Degradation effects

Since the suggested strategy works well for stability studies as well as release ones, it may be considered a superior methodology overall. The ICH-mandated forced degradation research includes heat degradation, acid-base reactions, oxidation, reduction, and more. Despite the presence of damaged peaks, the pharmaceuticals being tested remained stable during the stress testing, depending on the kind of chromatography used. We checked forced degredation (FD) initially for 15 min, we got the results if it is not reach the point to increase the time points. However, we also used 1N solutions.

Acid degradation

To a 10-mL volumetric flask, transfer 76 mg of sample. Then, add 1 mL of 1N HCl. Allow the mixture to stand for 15 min. After 15 min, add 1 mL of 1N sodium hydroxide and fill up to the diluent mark. Before injecting the solution into the HPLC system, filter it using a syringe filter. There were two degradation products (DP1 and DP4).

Alkali degradation

Weigh 76 mg of sample and transfer to a 10 mL volumetric flask. 1 mL of 1N NaOH was added, and the mixture was allowed to sit for 15 min. Fill to the line with 1 mL of 1N HCl after 15 min. Before injecting the solution into the HPLC system, filter it using a syringe filter. We got one product of degradation, DP5.

Peroxide degradation

To a 10-mL volumetric flask, transfer 76 mg of sample. Then, add 1 mL of a 10% hydrogen peroxide solution and fill the rest of the way with diluents. Before injecting the solution into the HPLC system, filter it using a syringe filter. A pair of degradation products DP2 and DP6 was generated.

Reduction degradation

To a 10-mL volumetric flask, transfer 76 mg of the sample. Then, add 1 mL of a 10% sodium bisulfite solution and fill the rest of the way with diluents. Before injecting the solution into the HPLC system, filter it using a syringe filter. No products of deterioration were produced.

Thermal degradation

The standards were set in an oven at 105°C for 6 h. Using these standards, a standard solution was prepared and injected into HPLC system. Only one degradation product (DP3) was formed.

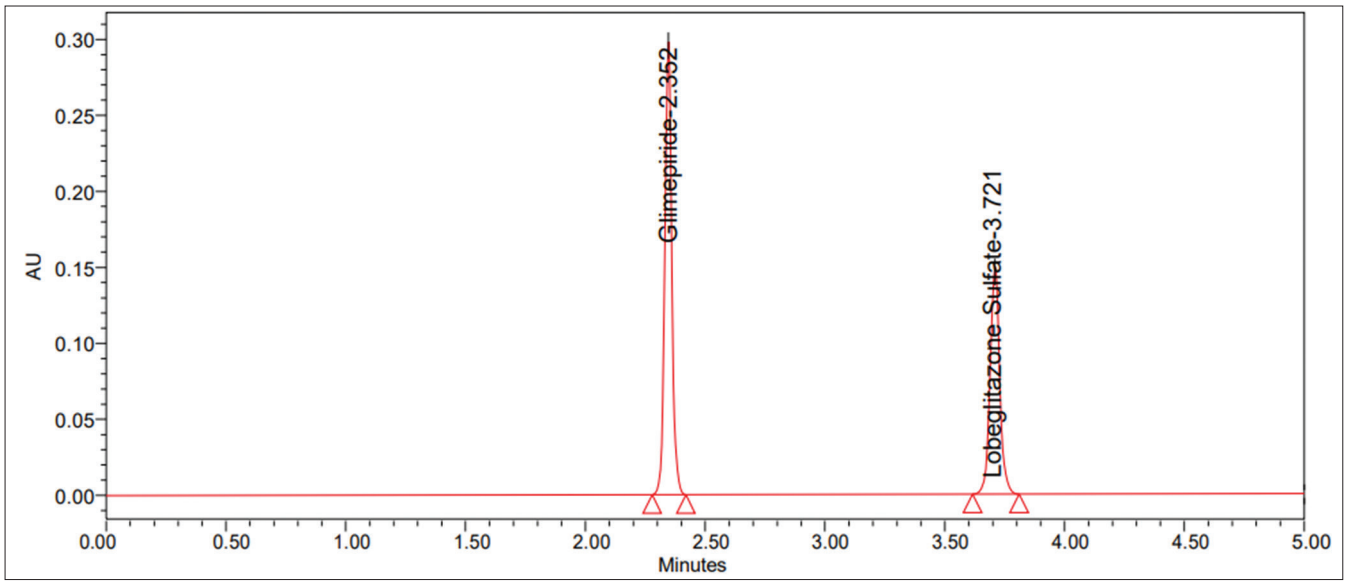

Fig. 5: Chromatogram of method precision

Table 4: Results of robustness

S. No.	Parameter name	Lobeglitazone sulfate			Glimepiride		
		Mean	Standard deviation	%RSD	Mean	Standard deviation	%RSD
1	Flow (0.9 mL/min)	100.3	0.513	0.51	100.5	0.624	0.62
2	Flow (1.1 mL/min)	100.5	0.50	0.50	100.2	0.611	0.61
3	Organic solvent (+10%) (55:45)	100.6	0.529	0.53	100.1	0.651	0.65
4	Organic solvent (−10%) (45:55)	100.4	0.794	0.79	100.3	0.70	0.70

RSD: Relative standard deviation. Mean±standard deviation (n=3)

Table 5: Results of method precision

S. No.	Lobeglitazone sulfate			Glimepiride		
	Conc. (µg/mL)	Area	Percentage assay	Conc. (µg/mL)	Area	Percentage assay
1	50	1292704	100.6	100	2754949	100
2		1288469	100.2		2729907	99
3		1290101	100.4		2761142	100.2
4		1279545	99.5		2768787	100.5
5		1275077	99.2		2737719	99.3
6		1281646	99.7		2748897	99.7
Mean			99.9	Mean		99.8
Standard deviation			0.5502	Standard deviation		0.5636

Mean±standard deviation (n=6)

Table 6: Results of intermediate precision

S. No.	Lobeglitazone sulfate		Glimepiride	
	Area counts	Percentage assay	Area counts	Percentage assay
1	1297547	100.9	2748747	99.6
2	1291801	100.5	2770122	100.4
3	1283766	99.9	2759947	100.0
4	1279450	99.5	2775411	100.6
5	1274989	99.2	2767049	100.3
6	1290452	100.4	2745457	99.5
Mean		100.1	Mean	100.1
Standard deviation		0.6470	Standard deviation	0.4457

Mean±standard deviation (n=6)

Table 7: Results of the accuracy of lobeglitazone sulfate

S. No.	Accuracy (%)	Percentage recovery	Mean percentage recovery
1	80	99.3	99.3
2	80	99.4	
3	80	99.1	
4	100	100.9	100.6
5	100	100.7	
6	100	100.3	
7	120	99.9	99.0
8	120	98.8	
9	120	98.3	

Mean±(n=3)

Table 8: Results of the accuracy of glimepiride

S. No.	Accuracy (%)	Percentage recovery	Mean percentage recovery
1	80	98.4	99.1
2	80	99.5	
3	80	99.4	
4	100	98.9	98.9
5	100	98.9	
6	100	99.1	
7	120	98.8	98.8
8	120	98.7	
9	120	98.9	

Mean±(n=3)

Photo degradation

The sample solution was set in photo stability chamber for 24 h. The resultant solution was injected into the HPLC system. No degradation products were formed.

Hydrolysis degradation

To a 10-mL volumetric flask, transfer 76 mg of sample. Then, add 1 mL of HPLC-grade water and fill the rest of the way with diluents. Before injecting the solution into the HPLC system, filter it using a syringe filter. No degradation products or deterioration were produced.

Table 9 shows the outcomes of forced degradation. These degradation samples are characterized using LC-MS.

Collision-induced dissociation of lobeglitazone sulfate and glimepiride

- Scheme 1: The process of fragmentation of degradation product 1 of m/z-428.0921 was observed under acidic degradation conditions of glimepiride. At m/z-262.0179 ($C_8H_{12}N_2O_2$ loss), m/z-157.9553 (C_8H_{10} loss), and m/z-93.9934 (SO_2 loss), the spectrum shows a large number of material ions. MS and precise mass measurements both lent credence to the suggested structures.
- Scheme 2: Under the peroxide degradation condition of glimepiride, the second degradation product fragmented at m/z-395.1151. At m/z-272.0467 ($C_7H_{11}NO$ loss), m/z-213.046 (CH_3NO_2 loss), m/z-149.0841 (SO_2 loss), and m/z-78.047 (C_3H_7NO loss), the spectrum shows an abundance of product ions. Precise mass measurements, MS/MS analyses validated the suggested structures.
- Scheme 3: The mechanism of fragmentation of degradation product 3, which has been seen under circumstances of thermal degradation of glimepiride, with a mass/charge ratio of 370.1311. At m/z-271.0627 ($C_5H_{11}NO$ loss), m/z-200.0256 (C_3H_7NO loss), m/z-142.0089 (CH_4N_2O loss), and m/z-78.047 (SO_2 loss), the spectrum displays a large number of product ions. In conjunction with precise mass measurements, the MS/MS tests validated the suggested structures.
- Scheme 4: The acid degradation of lobeglitazone sulfate circumstances that led to the fragmentation of m/z-490.0384 degradation product 4. At m/z-392.071 (H_2SO_4 loss), m/z-277.0982 ($C_3H_3NO_2S$ loss), m/z-165.1154 ($C_4H_3ClN_2$ loss), and m/z-59.0735 (C_7H_8O loss), the spectrum shows abundant ions of substances. The MS/MS tests, when combined with precise mass measurements, validated the suggested structures.
- Scheme 5: The alkali degradation conditions of lobeglitazone sulfate that led to the fragmentation of degradation product 5 at m/z-564.0985. The spectrum shows a large number of substance ions at m/z-466.1311 (H_2SO_4 loss), m/z-351.1583 ($C_3H_3NO_2S$ loss),

Table 9: Forced degradation results

Degradation condition	% Degradation of lobeglitazone sulfate	% Degradation of glimepiride
Unstressed degradation	0	0
Acid degradation	10.2	14.8
Alkali degradation	12.2	1.2
Peroxide degradation	14.3	12.4
Reduction degradation	2.4	0.4
Thermal degradation	0.8	10
Photolytic degradation	0.3	1.4
Hydrolysis degradation	1.5	0.3

m/z-245.1164 (C_7H_8O loss), and m/z-153.0902 (C_6H_6O loss). The MS/MS tests, when combined with precise mass measurements, validated the suggested structures.

- Scheme 6: Product 6 of the degradation process, with the mass/energy shift of 359.0787, may have been fragmented in a peroxide environment. Spectra show a high concentration of substance ions at m/z-261.1113 (H_2SO_4 loss), m/z-169.0851 (C_6H_6O loss), and m/z-96.0324 (C_3H_9NO loss). The MS/MS tests, when combined with precise mass measurements, validated the suggested structures.

Fig. 6 shows the mass spectra of DPs and Fig. 7 shows the fragmentation mechanisms of all schemes and the results were shown in Tables 10 and 11.

Table 10: LC-MS/MS data of glimepiride and its degradation products and some major fragments

Degradation product	Molecular formula	Calculated mass	Observed mass	Error	Major fragment ions
Glimepiride	$C_{24}H_{34}N_4O_5S$	490.225	490.2254	0.815952	100.0257, 161.2874, 259.8942, 330.5297, 382.3106
DP1	$C_{17}H_{21}ClN_4O_5S$	428.0921	428.0927	1.401568	94.9939, 158.9560, 263.0184
DP2	$C_{17}H_{21}N_3O_6S$	395.1151	395.1158	1.771636	79.0477, 150.0849, 214.0464, 273.0471
DP3	$C_{15}H_{22}N_4O_5S$	370.1311	370.1315	1.080698	79.0475, 143.0095, 201.0263, 272.0632

LC-MS/MS: Liquid chromatography with tandem mass spectrometry, DP: Degradation products

Table 11: LC-MS/MS data of lobeglitazone sulfate and its degradation products and some major fragments

Degradation product	Molecular formula	Calculated mass	Observed mass	Error	Major fragment ions
Lobeglitazone sulfate	$C_{24}H_{26}N_4O_9S_2$	578.1141	578.1148	1.210834	170.9638, 232.2976, 309.3861, 383.6143, 435.2817, 518.8820
DP4	$C_{17}H_{19}ClN_4O_7S_2$	490.0384	490.0391	1.428459	60.0739, 166.1160, 278.0987, 393.0716
DP5	$C_{23}H_{24}N_4O_9S_2$	564.0985	564.0993	1.418192	154.0907, 246.1168, 352.1590, 467.1318
DP6	$C_{13}H_{17}N_3O_7S$	359.0787	359.0795	2.227924	97.0329, 170.0855, 262.1120

LC-MS/MS: Liquid chromatography with tandem mass spectrometry

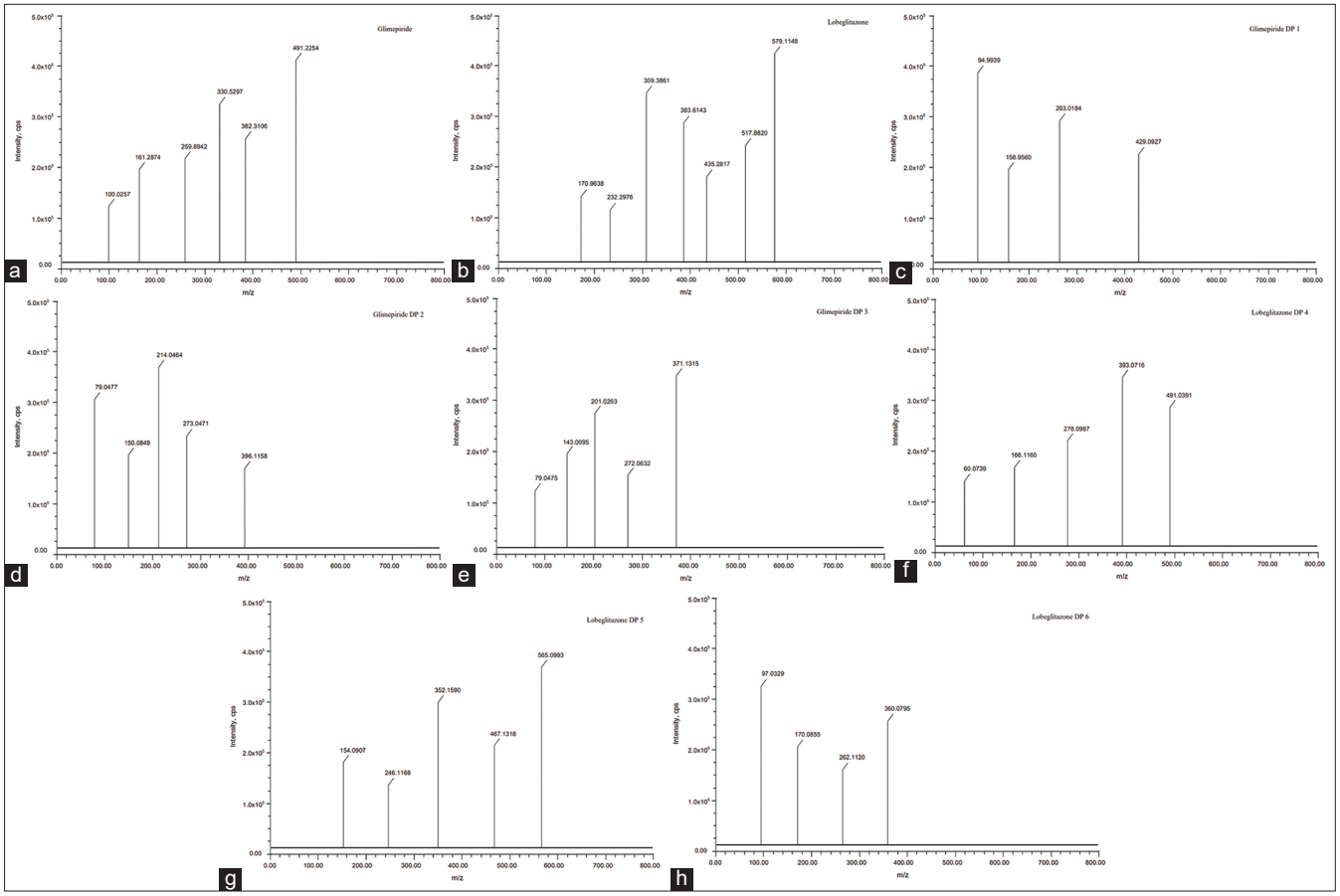

Fig. 6: Mass spectras of (a) glimepiride (b) Lobeglitazone sulfate (c) DP1 (D) DP2 (e) DP 3 (f) DP4 (g) DP5 (h) DP6. DP: Degradation products

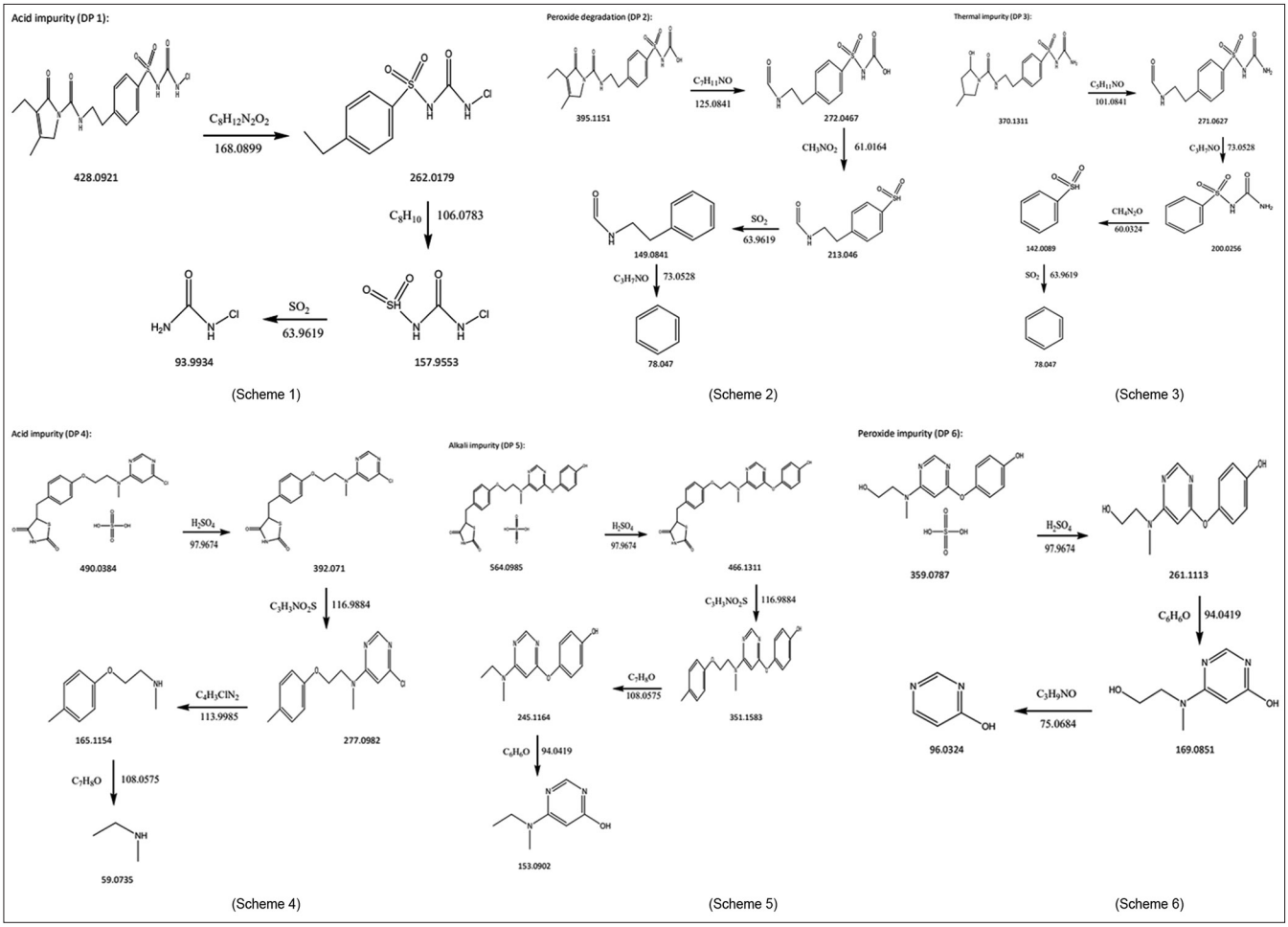

Fig. 7: Fragmentation mechanisms of all schemes. Scheme 1: Fragmentation mechanism of DP 1. Scheme 2: Fragmentation mechanism of DP 2. Scheme 3: Fragmentation mechanism of DP 3. Scheme 4: Fragmentation mechanism of DP 4. Scheme 5: Fragmentation mechanism of DP 5. Scheme 6: Fragmentation mechanism of DP 6. DP: Degradation products

CONCLUSION

A straightforward, fast, and accurate RP-HPLC technique for the API quantification of lobeglitazone sulfate and glimepiride will be developed. The drug's reaction to several environmental conditions, including acidity, basicity, hydrolysis, oxidation, reduction, sun stress, and heat stress, was studied. When subjected to hydrolysis, reduction, and light conditions, the drugs remained stable, but under the remaining degradation conditions, they were unstable. Using isocratic RP-HPLC, a method has been devised for the precise and selective measurement of lobeglitazone sulfate and glimepiride. The regression line equations discovered in the peak region allow for the accurate prediction of medication concentrations ranging from 12.5 to 75 µg/mL for lobeglitazone sulfate and 25–150 µg/mL for glimepiride. The medications, lobeglitazone sulfate and glimepiride, were able to be identified in a timely, accurate, and exact manner using a technique that successfully demonstrated their effectiveness.

AUTHOR'S CONTRIBUTION

Ravi Ch.: Literature review, methodology, data curation, writing-original draft, and evaluation; Kiran Kumar K; Literature review, writing original draft, review and editing, supervision, evaluation, and visualization; Krishna Veni G; writing original draft; review and inspect the results.

CONFLICTS OF INTEREST

The authors express no conflicts of interest.

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