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Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Lobeglitazone Sulphate and Dapagliflozin Propanediol Monohydrate in Synthetic Mixture

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ABSTRACT:

An accurate, precise and robust stability indicating RP-HPLC method has been developed for the estimation of Lobeglitazone sulphate and Dapagliflozin propanediol monohydrate in synthetic mixture. The chromatographic separation of the drug and its Degradants was done by using a Inert sustain C18 column (250 mm × 4.6 mm, 5 μ) and column oven set at 25 °C with a mobile phase consisting of methanol : phosphate buffer (pH 3.5) in 65:35%v/v ratio at a flow rate of 1.0 mL/min and an injection volume of 50 μL. Force degradation studies of samples and standard were conducted under alkaline, acidic, oxidative, thermal, and Photo degradation conditions, with analysis performed using the proposed method. The method exhibited the well separation of the degradation peaks from Analyte peaks, indicative of stability indicating nature of the method. The developed method was then validated in accordance with ICH guidelines for specificity, precision, linearity and range, limit of quantification, limit of detection, assay, and robustness, ensuring reliability and accuracy.

KEYWORDS: Lobeglitazone sulphate, Dapagliflozin propanediol monohydrate, RP-HPLC method, forced degradation, validation, stability-indicating

INTRODUCTION

Diabetes mellitus is a category of metabolic illnesses that cause hyperglycemia due to abnormalities in insulin secretion or activity (1). Diabetes mellitus refers to the excessive discharge of sweet urine. Diabetes can cause long-term damage to organs such as the eyes, kidneys, nerves, heart, and blood vessels due to chronic hyperglycemia (high blood sugar). Hyperglycemia results from insufficient insulin secretion or ineffective stimulation of target cells. Hyperglycemia causes high blood glucose levels that spill over into urine. Cells suffer from starvation due to poor glucose entry (2). Hyperglycemia symptoms include increased thirst and frequent urination. Chronic

hyperglycemia can harm the eyes, kidneys, nerves, heart, and blood vessels.(3)

CAUSES OF DIABETES MELLITUS

Main causes of diabetes mellitus are:

- 1) Genetic defects of beta-cell function
- 2) Genetic defects in insulin action.
- 3) Diseases of the exocrine pancreas. Endocrinopathies, (4)

Based on the therapeutic relevance of this Fixed-Dose Combination (FDC), it is essential to construct a strong, validated, and stability-indicating analytical technique for quality assurance in pharmaceutical development and

regular manufacture. Among every analytical method, Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) has been most extensively used because it is highly resolving, reproducible, sensitive, and versatile for estimations of a drug simultaneously present in complex matrices.

A review of the literature shows that numerous methods are reported for the individual estimation of Lobjeglitazone and Dapagliflozin, but few validated methods exist for their estimation together, especially in a combined or synthetic dosage form. Many UV-spectrophotometric methods are marred with considerable drawbacks such as low specificity, overlapping UV-spectra, and insufficient validation parameters. Additionally, Lobjeglitazone does not have a monograph in official pharmacopoeias like IP, BP, USP, and EP, which further justifies the requirement of a sound method.

A thorough review of literature reveals that despite many methods being available for individual estimation of Lobjeglitazone and Dapagliflozin, very few validated methods are available for their simultaneous estimation, especially in a synthetic or combined dosage form. Most UV- spectrophotometric methods published were plagued by major drawbacks such as low specificity, superposed absorption spectrum, and poor validation parameters . Furthermore, Lobjeglitazone has no monograph in common pharmacopoeias like IP, BP, USP, and EP, further highlighting the importance of a good method.

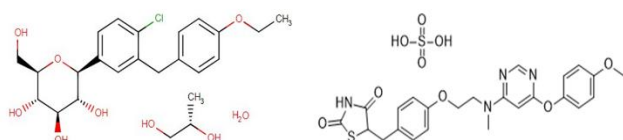


Figure 1 chemical structure of Dapagliflozin Propanediol monohydrate and Lobjeglitazone sulphate

Dapagliflozin Propanediol monohydrate and Lobjeglitazone Sulphate fixed dose combination tablet was approved by CDSCO to conduct phase III trials to Akmus Pharmaceuticals Ltd. As the drug combination is in phase 3 clinical trial stage, marketed formulation is not available yet. Therefore, synthetic mixture will be used for experimental work.

In pharmaceutical analysis, the precision and reliability of the analytical data depend largely on the type and quality of the instrument employed. Of these, High-Performance Liquid Chromatography (HPLC) is a robust and versatile method commonly used for the separation, identification, and quantitation of drug substances and impurities. HPLC

setups contain crucial parts including a solvent delivery system (pump), sample injector, analytical column, detector, and data handling system. The system runs at elevated pressure to drive the mobile phase through a packed column with fine particles to facilitate efficient separation based on analyte interactions with the stationary phase.

The present work made use of a Shimadzu LC 2010 CHT HPLC system, equipped with LC Solution software, UV detector, and a 100 µL auto-injector loop to facilitate reproducible and accurate injections. An Inertsustain C18 column (250 mm × 4.6 mm, 5 µ) was used to achieve the best resolution and peak shape. Other supporting equipment used were a Sartorius CP224S analytical balance (0.1 mg sensitivity) for precise weighing, LabMan melting point apparatus, Athena ultrasonic bath to degas solutions, Eppendorf micropipettes, Whatman 0.4 µm PVDF syringe filters, and a Patel Scientific hot air oven for thermally stable studies.

Every part of the HPLC system has a vital function: the pump supplies the mobile phase at a steady flow and pressure; the injector adds accurate volumes of sample; the stationary phase (C18 column) provides differential interaction and separation of analytes; the UV detector detects and measures components by absorbance; and the data system produces chromatograms utilized for further interpretation. Correct calibration, maintenance, and proper usage of this instrumentation are crucial for obtaining high-quality, reproducible results in day-to-day pharmaceutical quality control.

Synthetic Mixture Composition [11]:

Table 1 Composition of Active Ingredients

Sr. No.	Active Ingredients	Quantity
1	Dapagliflozin Propanediol monohydrate eq. to Dapagliflozin	10 mg
2	Lobjeglitazone Sulphate	0.5 mg

Table 2 Composition of Excipients

Sr. No.	Excipients	Quantity
1	Lactose hydrate	56.8mg
2	Croscarmellose cellulose	5.0 mg
3	Hydroxypropyl cellulose	39.2mg
4	Microcrystalline cellulose	10.0 mg
5	Povidone K-30	7 mg
6	Starch	6 mg
7	Magnesium Stearate	0.5 mg

RATIONALE

Type 2 diabetes mellitus is a chronic, progressive metabolic syndrome that usually requires lifelong pharmacological treatment to provide optimal blood glucose control. Though monotherapy will

initially meet the glycaemic target, it usually becomes inadequate over time as the disease advances and combination therapy has to be applied. The application of fixed-dose combinations (FDCs) of oral antidiabetic drugs has become quite popular owing to their capacity to enhance therapeutic effect, decrease pill burden, and increase patient compliance. In this regard, a new antidiabetic FDC of Lobeglitazone and Dapagliflozin formulated by Torrent Pharmaceuticals Ltd. has been approved by the Central Drugs Standard Control Organization (CDSCO) for Phase III clinical trials on November 15,

2023.

Among the techniques available for analysis, High-Performance Liquid Chromatography (HPLC) is most commonly accepted as the method of choice for the analysis of FDCs, because of its higher resolution, accuracy, reproducibility, and capacity to analyse complex pharmaceutical matrices. HPLC is most suitable for the simultaneous estimation of drug components in mixtures because of its high sensitivity and specificity.

A thorough literature search showed the existence of a pharmacopoeia monograph for Dapagliflozin Propanediol Monohydrate, but none such standardized procedure exists for Lobeglitazone sulphate. Also, there is only one reported UV spectrophotometric method for the simultaneous estimation of Lobeglitazone and Dapagliflozin. But UV methods suffer from the drawback of overlapping absorption spectra, which decrease their accuracy and reliability to a large extent.

To the best of our knowledge, no HPLC method has been validated so far for the simultaneous estimation of Lobeglitazone sulphate and Dapagliflozin Phosphate Monohydrate. Hence, the creation of a simple, rugged, accurate, and stability-indicating RP-HPLC method for their estimation in synthetic blends is both the need of the hour and essential. The current research was carried out with this objective.

AIM AND OBJECTIVES:

As there is no simultaneous HPLC assay method available for Lobeglitazone and Dapagliflozin, this study aims to

develop a cost-effective, validated analytical method suitable for simultaneous determination of Lobeglitazone and Dapagliflozin in synthetic mixture.

The main aim of the current work is to create and improve an easy, accurate, and robust RP-HPLC method for the simultaneous determination of Lobeglitazone and Dapagliflozin Propanediol Monohydrate in a spiked mixture.

Follow-up objectives are:

To prove the established method as per standard guidelines provided by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the United States Pharmacopeia (USP).

In order to assess the use of the procedure for quality control analysis by using routine

assays with a synthetic mix of the combination drug.

RP-HPLC METHOD DEVELOPMENT:

INSTRUMENTATION:

Liquid Chromatograph Shimadzu LC 2010 CHT was used, with LC Solution Software, UV detector, Autoinjector 100 μ L loop, Column Inertsustain C18 column (250 mm \times 4.6 mm, 5 μ), Analytical Balance (Sartorius CP224S, 0.1 mg sensitivity), Melting Point Apparatus (LabMan), Ultrasonic bath (Athena Technology), Micropipette (Eppendorf tube), Syringe filter, Whatman 0.4-micron PVDF, Hot Air Oven from Patel Scientific and all glassware from borosilicate were used.

MATERIALS AND CHEMICALS:

Lobeglitazone sulphate and Dapagliflozin Propanediol monohydrate working standard were obtained from Hema Pharmaceuticals, Ankeshwar. Water, HPLC Grade was obtained from Chronos. Methanol and Acetonitrile, HPLC Grade from Rankem; Trifluoroacetic acid AR Grade was obtained from ThermoFisher Scientific.

RP-HPLC METHOD DEVELOPMENT

Selection of Wavelength

In this choosing an analytical wavelength, the solutions of Lobeglitazone sulphate and Dapagliflozin propanediol monohydrate were prepared in methanol having concentration of 20 μ g/ml and 10 μ g/ml respectively. The solutions measured spectra overlapped when they were examined in the UV at 200nm – 400nm region. The process of selecting the wavelength involved taking into account

both the absorption point of interest and the findings from the literature review.

Preparation of Stock Solution

Preparation of standard stock solution of Lobeglitazone Sulphate (Solution A1)(1 mcg/ml). About 5 mg of Lobeglitazone sulphate working standard was accurately weighed and transferred into 500 ml volumetric flask. To this, 100 ml of methanol was added and dissolved by sonication. The solution was diluted up to the mark with methanol and used as a stock solution. Preparation of standard stock solution of Dapagliflozin propanediol monohydrate (Solution B1)(200ppm)(20 mcg/ml)

About 10 mg of dapagliflozin propanediol monohydrate working standard was accurately weighed and transferred into 50 ml volumetric flask. To this, 10 ml of methanol was added and dissolved by sonication. The solution was diluted up to the mark with diluent and used as a stock solution.

Mix standard solution (20 mcg/ml dapagliflozin+ 1 mcg/ml lobeglitazone):

Take out 1 ml of dapagliflozin stock and 1 ml of lobeglitazone stock by using pipette in 10 ml volumetric flask. Volume was made upto the mark with diluent and used as a standard solution. Mobile phase/ diluent Preparation of Dilute Orthophosphoric acid solution: Taken 7.0 ml of Orthophosphoric acid into 100 ml of water and mixed well. Preparation of buffer: 2.72 g of potassium dihydrogen orthophosphate dissolved in 1 L of MiliQ water by sonication. pH was adjusted 3.5 by diluted ortho-phosphoric acid (OPA) Mobile phase B: Acetonitrile

METHOD DEVELOPMENT

Analytical method validation is a careful process that is carried out to ensure that the established RP- HPLC method is valid for its intended use. During the current research work, validation was done based on the guidelines presented in ICH Q2(R2) and USP <1225> to guarantee global compliance and scientific reliability. The main validation parameters were specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantitation (LOQ), and assay. Every parameter was tested carefully to guarantee the precision, accuracy, and reproducibility of the method. Specificity was measured by confirming there was no interference from placebo and excipients, while linearity was proven for a broad concentration range for Dapagliflozin Propanediol monohydrate and Lobeglitazone Sulphate. Accuracy was

confirmed through recovery studies at more than one level of concentration, and precision was validated through repeatability and intermediate precision. Robustness was assessed by making small, intentional changes in chromatographic conditions, and the sensitivity of the method was ascertained from LOD and LOQ values. The assay of synthetic mixtures also confirmed the suitability of the method for routine quality control use. Successful validation of these parameters ensured that the method is not only sensitive and specific but also reproducible and reliable for pharmaceutical analysis.

RESULTS

Identification of Dapagliflozin Propanediol monohydrate and Lobeglitazone Sulphate:

Fourier Transform Infrared Spectroscopy of Lobeglitazone and Dapagliflozin was done. Spectral comparison with reference and structural interpretation confirmed received working standards are true to their nature.

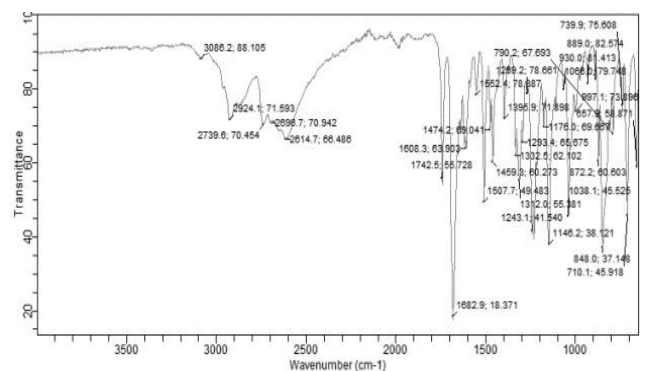


Figure 2 IR Spectrum of Lobeglitazone Sulphate

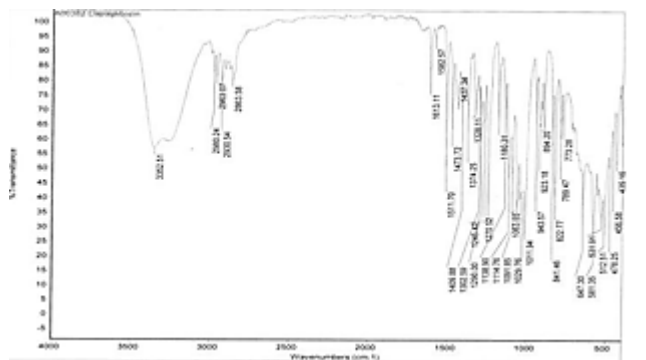


Figure 3 IR Spectrum of Dapagliflozin Propanediol monohydrate

Chromatography Parameters

The chromatographic separation was carried out in isocratic mode with a mobile phase comprising 0.1% trifluoroacetic acid (TFA) in water and acetonitrile (66:33 %v/v). The stationary phase utilized was an Agilent Zorbax ODS C18 column of size 150 mm × 4.5 mm internal

diameter and 5 µm particle size. Flow rate was kept constant at 1.0 mL/min, and detection was done at a wavelength of 215 nm, the isosbestic point for Sitagliptin and Empagliflozin. Column temperature was kept at 25°C, and injection volume was 50 µL and total run time of 15 minutes. These conditions were optimized to give sharp, well-resolved peaks with minimal tailing and appropriate retention times for both analytes.

System Suitability

System suitability tests were performed prior to validation to ensure optimal performance of the HPLC system. Parameters such as retention time, tailing factor, theoretical plates, and %RSD of peak areas were within acceptable limits.

Table 3 System Suitability Results

Parameter	Sitagliptin	Empagliflozin	Acceptance Criteria
%RSD of Area	0.3	0.2	Not more than 2%
Tailing Factor	1.5	1.29	Not more than 2
Theoretical Plates	2793	3646	Not less than 2000
Retention Time (min)	3.47	10.66	-

All criteria were satisfied, confirming system suitability for validation

Specificity

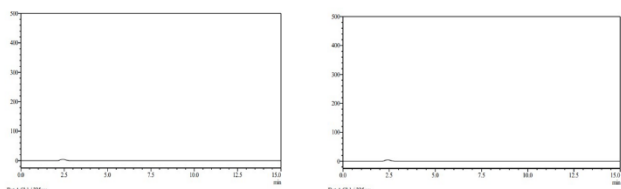


Figure 4 Chromatogram of Placebo and Diluent

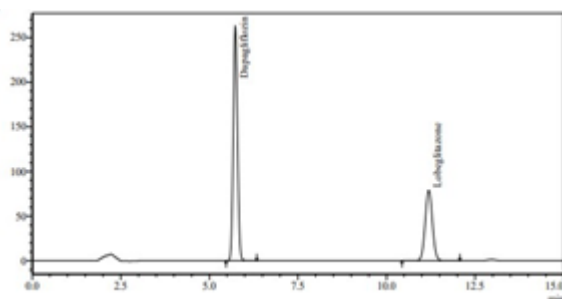


Figure 5 Chromatogram of standard

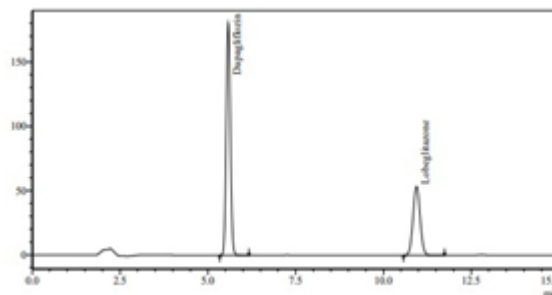


Figure 6 Chromatogram of Sample

Results: The results of specificity demonstrated that the analyte peaks were well-resolved and free from interferences at the baseline. It was confirmed that the method was specific and capable of accurately quantifying the analytes in the presence of excipients

Linearity

Linearity was established across 5 concentration levels (50% to 150%) for each analyte.

Table 4 Linearity Results of Dapagliflozin Propanediol monohydrate and Lobe-glitazone Sulphate

Sr. no.	Lobeglitazone		Dapagliflozin	
	Conc (ppm)	Area	Conc (ppm)	Area
1	0.5	605526	10	1205398
2	0.75	912748	15	1755776
3	1	1257076	20	2424500
4	1.25	1558740	25	3028983
5	1.5	1937753	30	3695915

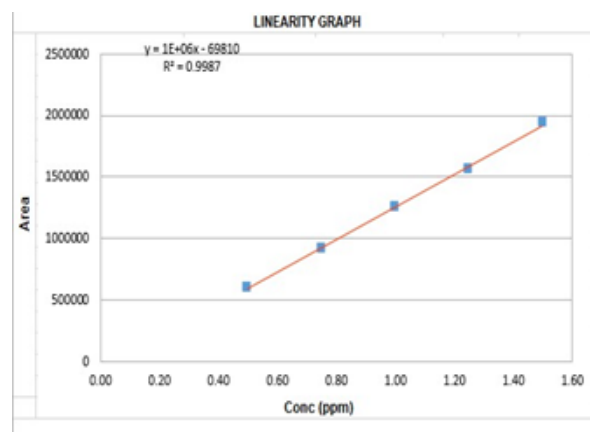


Figure 7 Linearity Results of Dapagliflozin Propanediol monohydrate

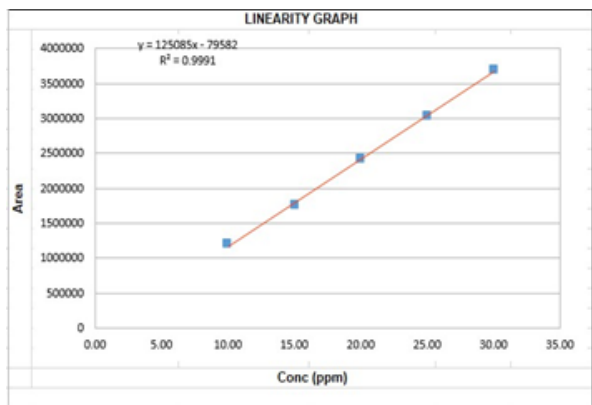


Figure 8 Linearity Results of Lobeglitazone Sulphate

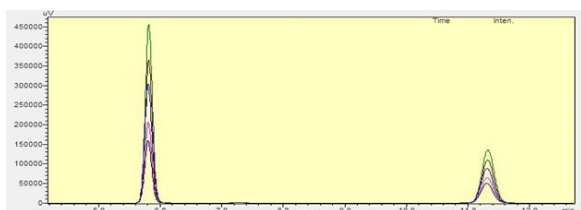


Figure 9 Linearity overlay of Dapagliflozin and Lobeglitazone

Accuracy

Accuracy was assessed via recovery studies at three concentration levels (50%, 100%, 150%).

Table 5 Accuracy results of lobeglitazone Sulphate

Recovery Conc. Level	Mg added	Mg found	% recovery	Mean Recovery	%RSD
50%	0.0050	0.0049	98.0	98.7	1.2
	0.0050	0.0050	100.0		
	0.0050	0.0049	98.0		
100%	0.0100	0.0102	102.0	101.3	0.6
	0.0100	0.0101	101.0		
	0.01	0.01	101.0		
150%	0.0150	0.0148	98.7	98.4	1.0
	0.0150	0.0149	99.3		
	0.0150	0.0146	97.3		

Table 6 Accuracy results of dapagliflozin

Recovery Conc. Level	Mg added	Mg found	% recovery	Mean Recovery	%RSD
50%	0.100	0.099	98.6	99.6	1.1
	0.100	0.099	99.3		
	0.100	0.101	100.8		
	0.100	0.101	100.8		
100%	0.200	0.203	101.5	101.6	0.2
	0.200	0.204	101.8		
	0.200	0.203	101.4		
	0.200	0.203	101.4		
150%	0.300	0.292	97.2	98.4	1.1
	0.300	0.296	98.8		
	0.300	0.298	99.2		

The acceptance criteria for accuracy are the recovery of analyte should be between 98-102% across the range of the assay. Recovery ranges were found to be 100.5-101.3% and 99.8-101.2 for Dapagliflozin and Lobeglitazone respectively.

Precision

Table 7 Intraday Precision

Drug	Average Area	%RSD
Lobeglitazone	1114993.33	0.13%
Dapagliflozin	2166998.66	0.21%

Table 8 Interday Precision

Drug	Concentration Range	%RSD
Lobeglitazone	50–150 µg/mL	0.33–
Dapagliflozin	50–150 µg/mL	0.46–

%RSD was <2% across all sets, confirming repeatability and intermediate precision

Robustness

Robustness was assessed by altering flow rate (± 0.1 mL/min), temperature ($\pm 5^\circ\text{C}$), and mobile phase composition ($\pm 2\%$).

Table 9 Robustness data

Parameter	%RS	Tailin	Theoretic	RT
Flow rate	0.0–	1.09–	>3500	5.88
Temperatu	0.1–	1.05–	>3500	6.40

LOD and LOQ

Table 10 LOD and LOQ Results

Drug	LOD (µg/mL)	LOQ (µg/mL)
Lobeglitazone	0.165	0.500
Dapagliflozin	2.785	8.441

Assay of Synthetic Mixture

Table 11 Assay results

Drug	Label	Found	%Assay
Lobeglitazone	0.5	99.95 ±	99.97 ±
Dapagliflozin	10	25.02 ±	100.07 ±

Assay results were within the acceptable limit (98–102%) confirming the method’s suitability for routine analysis.

DISCUSSION

The present study successfully developed and validated a robust, accurate, and precise RP-HPLC method for the simultaneous estimation of Dapagliflozin and Lobeglitazone in synthetic mixtures. The developed

method employed isocratic elution with Phosphate buffer pH 3.5:Methanol (35:65%v/v) on a InertSustain C18(250mm x 4.6mm x 5 μ) column, which provided excellent peak symmetry, resolution, and retention. confirming the method's specificity

Validation of the method was conducted as per ICH Q2(R2) guidelines. Linearity was demonstrated over a wide concentration range for both analytes with correlation coefficients (r^2) exceeding 0.999, indicating excellent proportionality between analyte concentration and detector response. The accuracy, evaluated through recovery studies at 50%, 100%, and 150% levels, showed mean recoveries within the 98–102% acceptance range, confirming the method's reliability

Precision studies, including intra-day and inter-day variations, yielded %RSD values below 2%, affirming the method's reproducibility. Furthermore, robustness testing revealed that small deliberate changes in chromatographic parameters did not significantly affect the performance, proving the method's stability under variable conditions. Low values of LOD and LOQ confirmed the method's high sensitivity for detecting and quantifying low concentrations of both drugs

The assay of synthetic mixtures demonstrated that the method consistently delivered results within acceptable limits, making it suitable for routine quality control analysis. When compared to previously reported UV spectrophotometric methods, this RP-HPLC method showed superior

sensitivity, specificity, and reliability. Moreover, the method fills a crucial analytical gap, especially considering the limited availability of pharmacopoeia methods for Lobjlitazone, making this work both relevant and novel.

CONCLUSION

A novel RP-HPLC method has been established for the simultaneous determination of Dapagliflozin and Lobjlitazone in a synthetic mixture. The method demonstrated excellent resolution between the two drugs, as well as with degradants during forced degradation studies. Validation of the method confirmed its specificity, linearity, precision, accuracy, and robustness. It proved to be stability-indicating, specific, simple, accurate, precise, and cost-effective. Therefore, the proposed RP-HPLC method is well-suited for routine analysis of Dapagliflozin and Lobjlitazone in synthetic mixture and can be applicable for pharmaceutical dosage form within quality control laboratories.

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