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RESEARCH ARTICLE

Development and Validation of HPLC, HPTLC and UV Methods for the Quantitative Analysis of Remogliflozin Etabonate

Siddhesh S. Kargutkar[1], Rukaiyya T. Girase[2], Swapnil Patil[2], Kapil Agrawal[1], Nitin Haswani[1], Rahul Pawara[1]

[1]*R. C. Patel Institute of Pharmacy, Shirpur, Dist. Dhule 425405, Maharashtra, India*

[2]*R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist. Dhule 425405, Maharashtra, India*

Corresponding author. R. C. Patel Institute of Pharmacy, Shirpur, Dist. Dhule 425405, Maharashtra, India. E-mail: drrahulpawara2023@gmail.com (R. Pawara).

Abstract

Remogliflozin Etabonate (RE), a sodium-glucose co-transporter-2 (SGLT2) inhibitor, is widely used in the treatment of type 2 diabetes mellitus. The present study aimed to develop and validate simple, rapid, accurate, and economical RP-HPLC, RP-HPTLC, and UV-AUC spectroscopic methods for the quantitative estimation of RE in bulk drug and tablet dosage forms. RP-HPLC analysis was performed on a Qualisil BDS-C18 column (250 × 4.6 mm, 5 µm) using methanol: water (95:05, v/v) as the mobile phase at a flow rate of 1.0 mL/min, with detection at 227 nm. The RP-HPTLC method employed TLC Silica Gel 60 RP-18 F254S plates and a mobile phase comprising chloroform: ethyl acetate: ammonia (2.5:2:0.5, v/v/v), with densitometric scanning at 227 nm. The UV-AUC method utilized methanol as the solvent, and quantification was carried out over the wavelength range of 227–229 nm. The developed methods were validated in accordance with ICH Q2(R1) guidelines for linearity, accuracy, precision, sensitivity, robustness, and specificity. The RP-HPLC, RP-HPTLC, and UV-AUC methods exhibited excellent linearity over concentration ranges of 2–10 µg/mL, 200–1200 ng/band, and 5–30 µg/mL, respectively, with correlation coefficients greater than 0.997. Recovery studies demonstrated satisfactory accuracy, while precision studies showed low %RSD values, confirming the reproducibility of the methods. The developed methods were successfully applied to the assay of marketed tablet formulations, producing results within acceptable limits. The proposed analytical methods are simple, reliable, precise, and cost-effective, making them suitable for routine quality control and quantitative analysis of Remogliflozin Etabonate in pharmaceutical dosage forms.

Keywords: Remogliflozin Etabonate; HPTLC; HPLC; UV-AUC

1. Introduction

Type 2 diabetes mellitus (T2DM) is characterized by abnormalities of glucose and lipid homeostasis, which drive secondary micro- and macrovascular complications. Clinical evidence indicates that maintaining glycemic control and reducing postprandial glucose excursions can lower the risk of diabetic complications, e.g. reduce the risk of myocardial infarction, renal disease and retinopathy [1]. Remogliflozin etabonate is the orally active prodrug of remogliflozin, a selective sodium–glucose co-transporter 2 (SGLT2) inhibitor developed for the treatment of type 2 diabetes mellitus (T2DM). It selectively inhibits SGLT2 in the kidneys, reducing renal glucose reabsorption and increasing urinary glucose excretion. This insulin-independent mechanism lowers blood glucose levels and provides an effective therapeutic approach for the management of T2DM [2]. Remogliflozin etabonate has a fast oral pharmacokinetic profile. After administration, it is extensively absorbed from the gastrointestinal tract, with studies reporting absorption of more than 93%, and it is rapidly converted by nonspecific esterases into the active drug, remogliflozin. The active moiety then undergoes further metabolism, mainly through CYP3A4 with a smaller contribution from CYP2C19, followed by glucuronidation to inactive metabolites. Its elimination is also relatively quick. In the systemic circulation, remogliflozin is extensively metabolized, leading to N-dealkylation, O-dealkylation, oxidation, loss of glucose, and glucuronidation. In vitro studies have demonstrated that the primary enzyme involved in the CYP-based metabolism of remogliflozin is CYP34A, with a minor contribution from CYP2C19.The molecular weight of this drug is 522.59 g/mol, and its formula is C26H38N2O9. It is chemically known as 5-Methyl-4-(4-(1-methylethoxy) benzyl)-1-(1-methylethyl)-1H-pyrazol-3-yl 6-O-(ethoxy carbonyl)-β-D-glucopyranoside (Figure no. 1) [3].

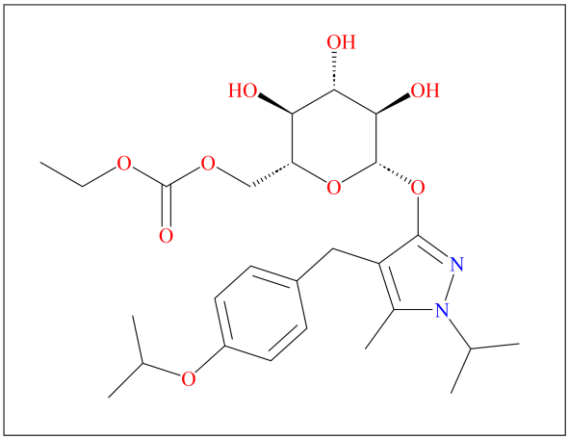

Figure 1. Chemical structure of Remogliflozin Etabonate.

The literature review revealed several analytical methods for quantitatively estimating Remogliflozin Etabonate in bulk drug and pharmaceutical dosage forms. A simple, precise, and sensitive UV spectrophotometric method was developed and validated for the determination of Remogliflozin Etabonate in bulk and tablet dosage forms in accordance with ICH guidelines [4]. A validated UV

spectrophotometric method with satisfactory linearity, precision, accuracy, and recovery was reported for routine quantitative analysis of Remogliflozin Etabonate [5]. Simultaneous development and validation of RP-HPLC and UV spectroscopic methods for the quantitative estimation of Remogliflozin Etabonate in bulk and pharmaceutical dosage forms have also been reported [6].

RP-HPLC methods for the determination of Remogliflozin Etabonate in bulk drug and pharmaceutical formulations have been developed and validated according to ICH guidelines [7]. A simple, selective, accurate, and robust RP-HPLC method for the routine quantitative estimation of Remogliflozin Etabonate has also been reported [8]. Selective RP-HPLC and UV spectroscopic methods employing an internal standard for the quantitative estimation of Remogliflozin Etabonate have also been developed and validated [9]. A stability-indicating HPTLC method for the determination of Remogliflozin Etabonate in pharmaceutical dosage forms has been developed and validated [10]. Development and validation of a simple, accurate, precise, and stability-indicating HPTLC method for the estimation of Remogliflozin Etabonate in bulk drug and tablet dosage forms have also been reported [11]. Remogliflozin Etabonate is soluble in methanol and acetonitrile and slightly soluble in other organic solvents, making these solvents suitable for its analytical estimation. Reliable analytical methods are essential for the quantitative determination of Remogliflozin Etabonate in bulk drug and pharmaceutical dosage forms. Therefore, the present study was undertaken to develop and validate simple, rapid, accurate, and economical RP-HPLC, RP-HPTLC, and UV spectrophotometric methods for the quantitative estimation of Remogliflozin Etabonate in accordance with the International Council for Harmonisation (ICH) Q2(R2) guidelines [12].

2. Experimental

2.1 Drug and chemicals

A gift sample of Remogliflozin Etabonate API was obtained from Glenmark Pharmaceuticals Pvt. Ltd., India. HPLC-grade acetonitrile and methanol used during the study were procured from Merck Life Science Pvt. Ltd., Mumbai, India. Analytical-grade acetic acid, ethyl acetate, and chloroform were purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Double-distilled water used throughout the study. Chromatographic analysis by HPLC was performed using a Qualisil 5 BDS-C18 analytical column (250 mm × 4.6 mm, 5 µm particle size) procured from Chromatography World Pvt. Ltd., Mumbai, India. HPTLC analysis was carried out on aluminum-backed TLC plates pre-coated with silica gel 60 F254 (0.2 mm thickness) and silica gel 60 RP-18 F254S plates, supplied by Rankem, RFCL Ltd., New Delhi, India.

2.2 Equipment and experimental conditions

2.2.1 HPLC analysis

The quantitative analysis of Remogliflozin Etabonate was carried out using a Shimadzu UFLC LC-20AD system equipped with an SPD-M20A photodiode array (PDA) detector, and chromatographic data were processed using LC Solution software (version 1.25). Chromatographic separation was performed on a Qualisil BDS-C18 column (250 mm × 4.6 mm, 5 µm particle size) maintained at ambient temperature. During method development, the solubility of Remogliflozin Etabonate was evaluated in different solvents, and methanol was selected as the diluent owing to its excellent solubility. Reverse-phase high-performance liquid chromatography (RP-HPLC) was selected as the chromatographic mode based on the physicochemical properties of the drug, while the Qualisil BDS-C18 column was chosen to achieve efficient separation and satisfactory peak characteristics. The detection wavelength was optimized by scanning the drug solution in the UV region, and the maximum absorbance (λmax) was observed at 227 nm. Therefore, chromatographic detection was carried out at this wavelength. Various mobile phase compositions were investigated during method optimization, and the optimum chromatographic separation was achieved using methanol:water (95:05, v/v) as the mobile phase at a flow rate of 1.0 mL/min. A 20 µL injection volume was used throughout the analysis. The stock standard solution was prepared by accurately weighing 10 mg of Remogliflozin Etabonate and transferring it into a 100 mL volumetric flask. The drug was dissolved in methanol, and the volume was made up to the mark with the same solvent to obtain a final concentration of 100 µg/mL. The prepared stock solution was used for the preparation of working standard solutions required for method development and validation studies.

2.2.2 HPTLC analysis

The RP-HPTLC analysis of Remogliflozin Etabonate was performed using a Camag HPTLC system (Muttenz, Switzerland) comprising a Linomat 5 sample applicator, Camag TLC Scanner 3, and WinCATs software (version 1.4.10) for data acquisition and processing. Chromatographic separation was carried out on aluminium-backed TLC Silica Gel 60 RP-18 F254S plates (10 cm × 10 cm, 0.2 mm layer thickness). Prior to method development, the solubility of Remogliflozin Etabonate was evaluated in different solvents, and methanol was selected as the most suitable solvent because of its excellent solubility. Reverse-phase high-performance thin-layer chromatography (RP-HPTLC) was employed for the separation of the drug.The detection wavelength was optimized by recording the UV absorption spectrum of Remogliflozin Etabonate, which exhibited maximum absorbance (λmax) at 227 nm. Accordingly, densitometric scanning was performed at this wavelength. A stock standard

solution was prepared by accurately weighing 10 mg of Remogliflozin Etabonate, transferring it into a 10 mL volumetric flask, dissolving it in methanol, and making up the volume with the same solvent to obtain a concentration of 1000 µg/mL.Several mobile phase compositions containing polar and non-polar solvents were evaluated to achieve optimum chromatographic separation. Among the investigated solvent systems, chloroform: ethyl acetate: ammonia (2.5:2:0.5, v/v/v) produced compact, well-resolved, and symmetrical bands with an Rf value of 0.55 ± 0.04 for Remogliflozin Etabonate. The development chamber was saturated with the mobile phase for 25 min before plate development. Chromatography was carried out to a development distance of 80 mm, after which the plates were air-dried for 5 min. Densitometric analysis was performed using the Camag TLC Scanner 3 controlled by WinCATs software (version 1.4.10) at 227 nm. The optimized chromatographic conditions provided satisfactory resolution and reproducible results, making the method suitable for the quantitative estimation of Remogliflozin Etabonate.

2.2.3 UV-AUC analysis

All spectrophotometric measurements were carried out using a Shimadzu UV-2450 UV–Visible spectrophotometer equipped with UV Probe software (version 2.21) and matched 10 mm quartz cells with a spectral bandwidth of 1 nm. The solubility of Remogliflozin Etabonate was evaluated in methanol, ethanol, acetonitrile, and dimethyl sulfoxide (DMSO), and methanol was selected as the solvent due to its superior solubility. A stock standard solution (100 µg/mL) was prepared by dissolving 10 mg of Remogliflozin Etabonate in 100 mL of methanol. The drug solution was scanned over the wavelength range of 200–400 nm, and the maximum absorbance (λmax) was observed at 227 nm. The area under the curve (AUC) in the wavelength range of 227–229 nm was selected for quantitative estimation. The developed UV spectrophotometric method was validated according to ICH Q2(R1) guidelines and successfully applied for the quantitative estimation of Remogliflozin Etabonate in bulk drug and tablet dosage forms.

2.3 Preparation of stock standard

2.3.1 HPLC analysis

A Shimadzu AUX-220 analytical balance was used to accurately weigh 10 mg of Remogliflozin Etabonate, which was transferred into a 100 mL volumetric flask and dissolved in methanol to obtain a stock standard solution of 100 µg/mL. Subsequently, 1 mL of the stock solution was transferred into a 10 mL calibrated flask and diluted to volume with methanol to obtain a working standard solution of 10 µg/mL, which was used for subsequent HPLC analysis.

2.3.2 HPTLC analysis

A Shimadzu AUX-220 analytical balance was used to accurately weigh 10 mg of Remogliflozin Etabonate, which was transferred into a 10 mL volumetric flask. The drug was dissolved in methanol (MeOH), and the volume was made up to the mark with the same solvent to obtain a stock standard solution of 1000 µg/mL.

2.3.3 UV-AUC analysis

Remogliflozin Etabonate exhibited good solubility in methanol; therefore, methanol (MeOH) was selected as the solvent for all UV spectrophotometric measurements. A 10 mg quantity of Remogliflozin Etabonate was accurately weighed using an analytical balance and transferred into a 100 mL volumetric flask containing approximately 60 mL of methanol. The solution was sonicated for 10 min, and the volume was made up to the mark with methanol to obtain a stock standard solution of 100 µg/mL. An aliquot of 1 mL of the stock solution was further diluted to 10 mL with methanol to obtain a working standard solution of 10 µg/mL. The solution was scanned over the wavelength range of 200–400 nm using methanol as the blank. The maximum absorbance (λmax) of Remogliflozin Etabonate was observed at 227 nm, and the area under the curve (AUC) in the wavelength range of 227–229 nm was selected for quantitative estimation.

2.3.4 Sample preparation

Ten Remogliflozin Etabonate tablets (100 mg) were accurately weighed, and the average weight was determined. The tablets were finely powdered using a glass mortar and pestle.

For RP-HPLC analysis, a quantity of powder equivalent to 10 mg of Remogliflozin Etabonate was transferred into a 100 mL volumetric flask containing 50 mL of methanol. The solution was sonicated for 20 min, diluted to volume with the same solvent, and filtered through a 0.45 µm membrane filter (Millifilter, Milford, MA, USA). An appropriate volume of the filtrate was further diluted with the mobile phase to obtain a final concentration of 6 µg/mL, and 20 µL of the solution was injected into the HPLC system for analysis.

For RP-HPTLC analysis, a quantity of tablet powder equivalent to 10 mg of Remogliflozin Etabonate was transferred into a 10 mL volumetric flask containing 8 mL of methanol, manually shaken for 15 min, and diluted to volume with the same solvent. The solution was filtered through Whatman filter paper, and 600 ng/band of the filtrate was applied onto RP-HPTLC plates. The plates were developed and scanned under the optimized chromatographic conditions, and the drug content was calculated using the calibration curve.

For UV-AUC analysis, tablet powder equivalent to 10 mg of Remogliflozin Etabonate was transferred into a 100 mL volumetric flask containing 50 mL of methanol. The solution was sonicated for 20 min, filtered, and diluted to volume with methanol to obtain a concentration of 100 µg/mL. An aliquot of 1.5 mL was further diluted to 10 mL with methanol to

obtain a final concentration of 15 µg/mL. The prepared solution was analyzed using the proposed UV-AUC method, and the drug content was determined from the corresponding calibration curve.

3. Results and discussion

Following the established protocols, the optimized RP-HPLC, RP-HPTLC, and UV-spectroscopic area under the curve (UV-AUC) methods were validated for linearity, accuracy, precision, specificity, selectivity, robustness, ruggedness, and sensitivity in accordance with the ICH Q2(R1) guidelines.

3.1 Development of optimum mobile phase

3.1.1 RP-HPLC

Different mobile phase compositions containing methanol, water, and acetonitrile were investigated to obtain optimum chromatographic separation of Remogliflozin Etabonate. Initial trials using methanol alone and various solvent combinations resulted in poor peak symmetry, increased tailing, and inadequate chromatographic resolution. Among the tested conditions, methanol (95:05, v/v) at a flow rate of 1.0 mL/min on a Qualisil BDS-C18 (250 mm × 4.6 mm, 5 µm) column provided the best chromatographic performance. Detection was carried out at 227 nm, producing a sharp, symmetrical peak with a retention time (Rt) of 4.09 ± 0.03 min.

3.1.2 RP-HPTLC

Various mobile phase compositions consisting of polar and non-polar solvents were evaluated to achieve satisfactory chromatographic separation of Remogliflozin Etabonate. Among the investigated solvent systems, chloroform acetate (2.5:2:0.5, v/v/v) produced the best resolution, yielding compact, sharp, and symmetrical bands with an Rf value of 0.55 ± 0.04. The optimum chromatographic performance was obtained after 25 min of chamber saturation at room temperature, and densitometric analysis was carried out at 227 nm.

3.2 Linearity and calibration curve

The linearity of the developed RP-HPLC method was evaluated using standard solutions of Remogliflozin Etabonate in the concentration range of 2–10 µg/mL. Appropriate aliquots of the stock standard solution were diluted with the mobile phase, and 20 µL of each solution was injected under the optimized chromatographic conditions. A calibration curve was constructed by plotting the peak area against drug concentration. The linear regression equation was found to be y = 54812x + 11835, with a correlation coefficient of R^2 = 0.9991, indicating an excellent linear relationship between peak area and concentration over the selected concentration range. The results demonstrate that the proposed RP-HPLC method is suitable for the quantitative estimation of Remogliflozin Etabonate. The calibration curve is shown in Figure 2.

The linearity of the developed RP-HPTLC method was evaluated using standard solutions of Remogliflozin Etabonate in the concentration range of 200–1200 ng/band. Aliquots of 0.2–1.2 µL of the stock standard solution were applied onto RP-HPTLC plates under the optimized chromatographic conditions. A calibration curve was constructed by plotting the peak area against drug concentration. The linear regression equation was found to be y = 8.2315x + 1238.6, with a correlation coefficient of R^2 = 0.9978, indicating an excellent linear relationship between peak area and concentration over the selected concentration range. The developed RP-HPTLC method was found to be suitable for the quantitative estimation of Remogliflozin Etabonate. The calibration curve is shown in Figure 3.

The linearity of the proposed UV spectrophotometric methods was evaluated using standard solutions of Remogliflozin Etabonate in the concentration range of 5–30 µg/mL. For Method A (Zero-order Absorbance), the calibration curve was constructed by plotting absorbance against concentration at 227 nm. The linear regression equation was found to be y = 0.0322x + 0.0252, with a correlation coefficient of R^2 = 0.9993, indicating excellent linearity over the selected concentration range. Similarly, for Method B (Zero-order AUC), the calibration curve was constructed by plotting the area under the curve (227–229 nm) against concentration. The linear regression equation was found to be y = 0.0188x − 0.0018, with a correlation coefficient of R^2 = 0.9995, demonstrating an excellent linear relationship between AUC and concentration. The results confirmed that both UV spectrophotometric methods are suitable for the quantitative estimation of Remogliflozin Etabonate. The calibration curve for Method A (Zero-order Absorbance) is shown in Figure 4, and the calibration curve for Method B (Zero-order AUC) is shown in Figure 5.

3.3 Precision

The precision of the developed RP-HPLC, RP-HPTLC, UV spectrophotometric (Method A), and UV-AUC (Method B) methods was evaluated in terms of repeatability, intra-day precision, and inter-day precision. Precision was expressed as the percentage relative standard deviation (%RSD). For RP-HPLC, Remogliflozin Etabonate (RE) was analyzed at 4, 6, and 8 µg/mL; for RP-HPTLC at 600, 800, and 1000 ng/band; and for UV Methods A and B at 10, 15, and 20 µg/mL. The obtained %RSD values were within the acceptable limits, demonstrating excellent precision and reproducibility of all the developed analytical methods, as indicated in Table No. 1.

Table 1. Precision studies of Remogliflozin Etabonate by RP-HPLC, RP-HPTLC and UV spectrophotometric methods.

Analysis	Concentration (ng/band)	Intraday: amount found (ng/band)	Intraday %RSD	Inter-day: amount found (ng/band)	Inter-day %RSD
RP-HPLC	4 µg/mL	4.03	0.43	4.02	0.72

Analysis	Concentration (ng/band)	Intraday: amount found (ng/band)	Intraday %RSD	Inter-day: amount found (ng/band)	Inter-day %RSD
	6 µg/mL	6.05	0.28	6.02	0.17
	8 µg/mL	7.94	0.76	7.89	0.36
RP-HPTLC	600 ng/band	611.53	0.11	611.20	0.12
	800 ng/band	814.94	0.19	805.15	0.32
	1000 ng/band	1003.71	0.64	1007.38	0.44
UV-AUC (Method A)	10 µg/mL	10.14	0.17	9.92	0.30
	15 µg/mL	14.94	1.00	14.98	0.77
	20 µg/mL	19.90	0.32	19.89	1.34
UV-AUC (Method B)	10 µg/mL	9.99	0.81	10.02	0.61
	15 µg/mL	14.95	0.94	14.97	1.24
	20 µg/mL	20.11	0.79	20.09	0.55

3.4 Limits of detection and quantification

The detection limit (LOD) and quantification limit (LOQ) of the developed RP-HPLC, RP-HPTLC, UV spectrophotometric (Method A), and UV-AUC (Method B) methods were determined from the standard deviation of the responses and the slopes of the corresponding calibration curves (n = 3) in accordance with the International Council for Harmonisation (ICH) Q2(R1) guidelines. For RP-HPLC, the lower concentration levels of 2.0–4.0 µg/mL were used for the determination of LOD and LOQ. During RP-HPTLC method validation, RE solutions in the concentration range of 200–400 ng/band were applied to the plates. For the UV methods, the lower concentration range of 5–10 µg/mL was employed. The LOD and LOQ values were calculated using the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the response (n = 3) and B is the slope of the corresponding calibration curve. The LOD and LOQ values were found to be 0.19 and 0.57 µg/mL for RP-HPLC, 4.84 and 14.69 ng/band for RP-HPTLC, 0.022 and 0.067 µg/mL for UV spectrophotometric Method A, and 0.030 and 0.091 µg/mL for UV-AUC Method B, respectively. These results demonstrate the high sensitivity of the developed analytical methods for the quantitative estimation of Remogliflozin Etabonate.

3.5 Robustness (RP-HPLC and RP-HPTLC)

For robustness evaluation, the developed RP-HPLC and RP-HPTLC methods were assessed by introducing deliberate minor changes in the optimized analytical conditions. In the RP-HPLC method, the study was performed at a concentration of 6 µg/mL, where the effects of slight variations in flow rate (0.8 and 1.2 mL/min) and detection wavelength (226 and 228 nm) were investigated. For the RP-HPTLC method, six replicate analyses were carried out at a concentration of 600 ng/band. The influence of minor variations in mobile phase composition [chloroform: ethyl acetate: ammonia (2.5:2:0.5 and 3:2:0.5, v/v/v)], mobile phase volume (4 and 6 mL), chamber saturation time (20 and 30 min), development distance (7.5 and 8.5 cm), and the time intervals between sample application, plate development, and scanning was evaluated. In both methods, the deliberate variations did not produce any significant changes in the chromatographic response, and the %RSD values remained below 2.0%, demonstrating that the developed RP-HPLC and RP-HPTLC methods are robust and reliable for the quantitative analysis of Remogliflozin Etabonate.

Table 2. Robustness studies for RP-HPLC and RP-HPTLC methods for Remogliflozin Etabonate (n = 3).

Method	Parameter	Condition	%RSD
RP-HPLC	Flow rate (mL/min)	0.8	1.70
		1.2	1.01
	Detection wavelength (nm)	226	0.08
		228	0.02
RP-HPTLC	Mobile phase composition	Chloroform: Ethyl acetate: Ammonia (2.5:2:0.5, v/v/v)	0.46
		Chloroform: Ethyl acetate: Ammonia (3:2:0.5, v/v/v)	0.68
	Mobile phase volume (mL)	4	0.66
		6	0.55
	Development distance (cm)	7.5	0.27
		8.5	0.90
	Duration of saturation (min)	20	0.41

Method	Parameter	Condition	%RSD
		30	0.28

3.6 Accuracy

The accuracy of the developed RP-HPLC, RP-HPTLC, and UV spectrophotometric methods was evaluated by recovery studies at three concentration levels (80%, 100%, and 120%) using the standard addition technique. A known amount of Remogliflozin Etabonate standard was added to the pre-analyzed tablet solution, and the samples were reanalysed using the respective validated methods. The percentage recovery ranged from 100.45% to 101.53% for the RP-HPLC method, 99.05% to 100.03% for the RP-HPTLC method, 100.30% to 101.99% for UV Method A (Zero-order absorbance), and 98.74% to 101.31% for UV Method B (Area Under Curve). The obtained recoveries and low %RSD values demonstrated the excellent accuracy of all the developed analytical methods for the quantitative estimation of Remogliflozin Etabonate.

Table 3. Recovery studies for the developed RP-HPLC, RP-HPTLC and UV spectrophotometric methods.

Method	Initial amount applied	Amount added	% Recovery	%RSD
RP-HPLC	6 µg/mL	4.8 µg/mL	100.58	0.25
		6.0 µg/mL	100.45	0.18
		7.2 µg/mL	101.53	0.09
RP-HPTLC	600 ng/band	480 ng/band	100.03	1.36
		600 ng/band	99.05	0.37
		720 ng/band	99.87	1.74
UV Method A	15 µg/mL	12 µg/mL	100.88	0.51
		15 µg/mL	100.30	1.46
		18 µg/mL	101.99	0.83
UV Method B (AUC)	15 µg/mL	12 µg/mL	98.74	0.30
		15 µg/mL	99.32	0.64
		18 µg/mL	101.31	0.40

3.7 Ruggedness

The ruggedness of the developed UV spectrophotometric methods was evaluated at a concentration of 15 µg/mL by two independent analysts under identical operational and environmental conditions. The percentage amount recovered and %RSD values obtained by both analysts were within the acceptable limits (%RSD < 2.0%), confirming that the proposed Zero-order (Method A) and AUC (Method B) methods are rugged and suitable for routine quantitative analysis of Remogliflozin Etabonate.

3.8 Method robustness summary

The robustness of the developed RP-HPLC and RP-HPTLC methods was evaluated by introducing deliberate minor variations in the chromatographic conditions. For RP-HPLC, the effect of changes in flow rate (0.8 and 1.2 mL/min) and detection wavelength (226 and 228 nm) was studied at a concentration of 6 µg/mL. For RP-HPTLC, six replicate analyses were performed at 600 ng/band by varying the mobile phase composition, mobile phase volume, chamber saturation time, development distance, and the time interval between sample application, plate development, and scanning. In both methods, the %RSD values remained within the acceptable limits, confirming the robustness of the developed methods.

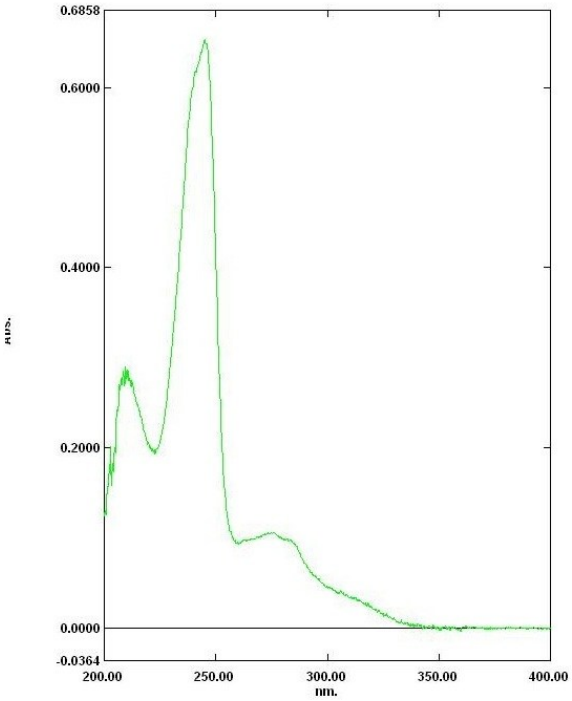

Figure 2. Zero-order UV spectrum of Remogliflozin Etabonate.

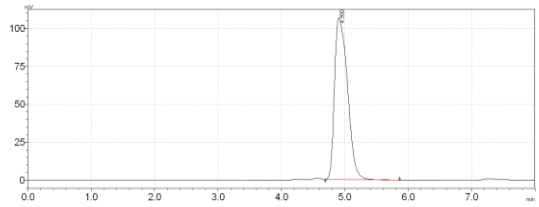

Figure 3. Optimized chromatogram for Remogliflozin Etabonate in a mobile phase of methanol and water.

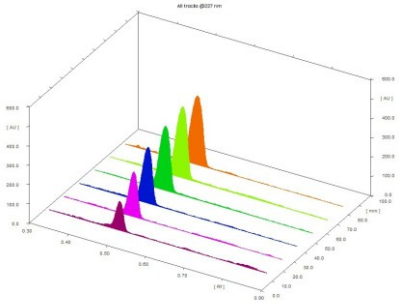

Figure 4. Three-dimensional linearity plot for RP-HPTLC.

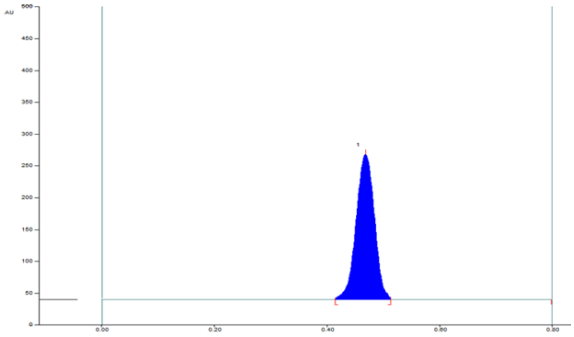

Figure 5. Typical densitogram for RP-HPTLC (Rf = 0.55 ± 0.04).

4. Conclusion

Novel, simple, rapid, accurate, and economical RP-HPLC, RP-HPTLC, and UV-AUC spectroscopic methods were successfully developed and validated for the quantitative estimation of Remogliflozin Etabonate in bulk drug and tablet dosage forms. The methods were validated according to ICH Q2 (R1) guidelines and demonstrated excellent linearity, precision, accuracy, sensitivity, robustness, and specificity within the selected analytical ranges. The developed methods were successfully applied to the assay of marketed tablet formulations, with satisfactory recovery and reproducible results. Therefore, these methods are suitable for routine quality control analysis of Remogliflozin Etabonate in pharmaceutical formulations.

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Statements and declarations

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Ethics declaration:

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References

1. Kapur A, O'Connor-Semmes R, Hussey EK, Dobbins RL, Tao W, Hompesch M, et al. First human dose-escalation study with remogliflozin etabonate, a selective inhibitor of the sodium-glucose transporter 2 (SGLT2), in healthy subjects and in subjects with type 2 diabetes mellitus. BMC Pharmacol Toxicol. 2013;14:26. https://doi.org/10.1186/2050-6511-14-26
2. Markham A. Remogliflozin etabonate: first global approval. Drugs. 2019;79(10):1157-1161. https://doi.org/10.1007/s40265-019-01148-3
3. Mohan V, Mithal A, Joshi SR, Aravind SR, Chowdhury S. Remogliflozin etabonate in the treatment of type 2 diabetes: design, development, and place in therapy. Drug Des Devel Ther. 2020;14:2487-2501. https://doi.org/10.2147/DDDT.S258771
4. Vidhi D, Patel P. Method development and validation of UV spectrophotometric estimation of remogliflozin etabonate in bulk and its tablet dosage form. Res J Pharm Technol. 2021;14(4):2042-2044. https://doi.org/10.52711/0974-360X.2021.00361
5. Itigimatha N, Chadchan KS, Yallur BC, Hadagali D. Simple and sensitive RP-HPLC and UV spectroscopic methods for the determination of remogliflozin etabonate in pure and pharmaceutical formulations. Turk J Pharm Sci. 2022;19(2):213-218. https://doi.org/10.4274/tjps.galenos.2021.57948
6. Surywanshi KP, Jadhav SP, Surana KR, Pathade PA, Mahajan SK. Development and validation of a robust RP-HPLC method for the estimation of remogliflozin etabonate in bulk and tablet dosage forms. Biosci Biotechnol Res Asia. 2025;22(3):1136-1146. https://doi.org/10.13005/bbra/3429
7. Killo SR, Galla R, Narikimalli A. Remogliflozin etabonate and teneligliptin simultaneous estimation in pharmaceutical dosage form using a stability indicating HPLC-DAD procedure. J Res Pharm. 2024;28(5):1758-1767. https://doi.org/10.29228/jrp.899
8. Attimarad M, Elgorashe RE, Subramaniam R, Islam MM, Venugopala KN, Nagaraja S, et al. Development and validation of rapid RP-HPLC and green second-derivative UV spectroscopic methods for simultaneous quantification of metformin and remogliflozin in formulation using experimental design. Separations. 2020;7(4):59. https://doi.org/10.3390/separations7040059
9. Joshi P, Kotadiya R. Progress in analytical techniques for remogliflozin etabonate, vildagliptin and metformin hydrochloride: a recently approved FDC. Curr Pharm Anal. 2023;19(2):136-162. https://doi.org/10.2174/1573412919666221226122606
10. Shah DA, Gondalia II, Patel VB, Mahajan A, Chhalotiya U, Nagda DC. Stability indicating thin-layer chromatographic method for estimation of antidiabetic drug remogliflozin etabonate. Future J Pharm Sci. 2021;7(1):83. https://doi.org/10.1186/s43094-021-00234-y
11. Muruganathan G, Thangavel M, Kochupapy RT. Stability-indicating HPTLC method for determination of remogliflozin etabonate and vildagliptin in tablets. J Chromatogr Sci. 2024;62(1):27-34. https://doi.org/10.1093/chromsci/bmad030

12. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Validation of analytical procedures: text and methodology Q2(R1). Geneva: International Council for Harmonisation; 2005.