The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
21(2): S.I (2), 308-315, 2026
www.thebioscan.com

STABILITY-INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF REMOGLIFLOZIN ETABONATE, TENELIGLIPTIN, AND METFORMIN HYDROCHLORIDE IN SYNTHETIC MIXTURE

Farid Koilawala, Sonal H Vaishnav[*1]

[1]Department of Pharmaceutical Quality Assurance, School of Pharmacy, Rai University, Saroda, Dholka, Ahmedabad, Gujarat – 382260, India

***Corresponding Author:** Ms. Sonal H Vaishnav, Assistant Professor, Department of Pharmaceutical Chemistry and Quality Assurance, School of Pharmacy, Rai University, Ahmedabad, Gujarat, India.

Email: sonal.vaishnav@raiuniversity.edu.in

DOI: https://doi.org/10.63001/tbs.2026.v21.i02.S.I(2).pp308-315

Keywords:
RP-HPLC;
Remogliflozin Etabonate;
Teneligliptin;
Metformin Hydrochloride;
Stability-Indicating Method;
ICH Q2(R1);
Forced Degradation;
Method Validation;
Type 2 Diabetes Mellitus

Received on: 23-03-2026

Accepted on: 10-04-2026

Published on: 20-04-2026

ABSTRACT

A simple, accurate, precise, specific, robust, and stability-indicating Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous determination of remogliflozin etabonate (REM), teneligliptin (TEN), and metformin hydrochloride (MET) in a synthetic mixture. Chromatographic separation was achieved on a Phenomenex Luna C18 column (250 mm × 4.6 mm, 5 µm) using an isocratic mobile phase consisting of phosphate buffer (pH 4.0) and acetonitrile (60:40 v/v) at a flow rate of 1.0 mL/min, with UV detection at 226 nm. Under optimized conditions, baseline-resolved peaks were obtained at retention times of 4.23 min (REM), 7.89 min (TEN), and 11.45 min (MET). The method was validated according to ICH Q2(R1) guidelines for system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, and solution stability. Excellent linearity was observed over the ranges 2–12 µg/mL (REM), 2–12 µg/mL (TEN), and 10–60 µg/mL (MET), with correlation coefficients (r^2) ≥ 0.9997. Mean percentage recoveries were 100.05 ± 0.49%, 99.96 ± 0.53%, and 100.13 ± 0.48% for REM, TEN, and MET respectively. Percentage relative standard deviation (%RSD) for both intraday and interday precision was below 0.6%. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions confirmed the stability-indicating capability of the method, with all degradation products completely resolved from parent drug peaks. Mass balance ranged from 97.8% to 99.8% across all stress conditions. Assay of the synthetic mixture yielded results within 98–102% of label claim. The validated method is suitable for routine quality control, stability studies, and regulatory submissions for this clinically important triple antidiabetic combination.

1. INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a progressive metabolic disorder characterized by insulin resistance, declining β-cell function, and impaired insulin secretion, affecting approximately 537 million adults globally as of 2021 with projections reaching 783 million by 2045 [1]. Chronic hyperglycemia precipitates severe microvascular (retinopathy, nephropathy, neuropathy) and macrovascular complications (cardiovascular disease, stroke), imposing enormous morbidity, mortality, and economic burden, particularly in India—the diabetes capital of the world [2].

Monotherapy frequently proves inadequate in achieving durable glycemic control as the disease progresses due to declining β-cell function. Consequently, triple combination therapy employing drugs with complementary mechanisms of action has emerged as a cornerstone of modern diabetes management [3]. The combination of remogliflozin etabonate (SGLT2 inhibitor), teneligliptin (DPP-4 inhibitor), and metformin hydrochloride (biguanide) provides synergistic benefits by targeting multiple pathophysiological defects simultaneously: increased renal glucose excretion, enhanced incretin-mediated insulin secretion, and suppression of hepatic gluconeogenesis [4,5].

Remogliflozin etabonate, an orally active prodrug approved in India in 2019, is hydrolyzed by intestinal esterases to its active metabolite remogliflozin, which selectively inhibits SGLT2 transporters responsible for approximately 90% of renal glucose reabsorption [6]. Teneligliptin, a third-generation DPP-4 inhibitor, features a unique J-shaped five-ring structure providing exceptionally strong and prolonged enzyme binding, enabling once-daily dosing with minimal drug interactions [7,8]. Metformin hydrochloride, the first-line pharmacological agent for T2DM as per all major guidelines, primarily suppresses hepatic glucose production and enhances peripheral insulin sensitivity [9].

Despite the growing clinical utilization of this triple combination, a thorough review of the literature reveals

that most published analytical methods address only binary combinations (remogliflozin–metformin or teneligliptin–metformin) [10–13], with very few methods reported for the simultaneous estimation of all three drugs [14]. The development of a validated stability-indicating method for this triple combination is essential to meet regulatory requirements for quality control, stability studies, and generic drug submissions, as mandated by ICH Q2(R1) [15] and ICH Q1A(R2) [16] guidelines.

The present study describes the development and comprehensive validation of a stability-indicating RP-HPLC method for the simultaneous determination of remogliflozin etabonate, teneligliptin, and metformin hydrochloride in a synthetic mixture, along with forced degradation studies to confirm its stability-indicating capability.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Remogliflozin etabonate (≥99.5% purity), teneligliptin (≥99.0% purity), and metformin hydrochloride (≥99.5% purity) working standards were procured. HPLC-grade acetonitrile (≥99.9%) and methanol (≥99.9%), Milli-Q water (18.2 MΩ·cm), potassium dihydrogen phosphate (KH_2PO_4, AR grade), orthophosphoric acid (AR grade, 85% w/v), hydrochloric acid (AR grade, 37% w/v), sodium hydroxide (AR grade), and hydrogen peroxide (AR grade, 30% w/v) were used. All reagents were of analytical or HPLC grade.

2.2 Instrumentation

An HPLC system equipped with a quaternary pump, autosampler (100 µL injection loop), column oven, and photodiode array (PDA) detector was used. Chromatographic data were processed using a validated data acquisition system. Additional equipment included a UV-Visible spectrophotometer (double beam, 1 cm matched quartz cells), analytical balance (readability 0.01 mg), calibrated pH meter, ultrasonicator (40 kHz), Milli-Q water purification system, hot air oven, UV chamber (254 nm/366 nm), Class A volumetric glassware, and 0.45 µm PVDF membrane and syringe filters.

2.3 Preparation of Mobile Phase and Standard Solutions

Phosphate buffer (pH 4.0) was prepared by dissolving 6.805 g of KH_2PO_4 in approximately 900 mL of HPLC-grade water, adjusting pH to 4.0 with orthophosphoric acid, and making up to 1000 mL. The buffer was filtered through a 0.45 µm membrane filter and degassed by ultrasonication for 15 minutes. The mobile phase consisted of phosphate buffer (pH 4.0) and acetonitrile in a ratio of 60:40 v/v, prepared fresh daily.

Individual standard stock solutions (1000 µg/mL) of each drug were prepared by accurately weighing 100 mg of each working standard, transferring to separate 100 mL volumetric flasks, dissolving in mobile phase by sonication for 10 minutes, and diluting to volume. Working standard solutions were prepared by appropriate dilution over concentration ranges of 2–12 µg/mL (REM), 2–12 µg/mL (TEN), and 10–60 µg/mL (MET).

2.4 Optimized Chromatographic Conditions

Table 1. Optimized RP-HPLC chromatographic conditions.

Parameter	Specification
Column	Phenomenex Luna C18 (250 mm × 4.6 mm, 5 µm)
Mobile Phase	Phosphate Buffer pH 4.0 : Acetonitrile (60:40 v/v)
Flow Rate	1.0 mL/min
Column Temperature	30°C
Detection Wavelength	226 nm
Injection Volume	20 µL
Run Time	15 minutes
Elution Mode	Isocratic

2.5 Method Validation

System Suitability. Six replicate injections of the mixed standard solution were analyzed. Theoretical plates (N), tailing factor (T), resolution (Rs), retention factor (k'), and %RSD of peak areas and retention times were evaluated.

Specificity. Analyzed by comparing chromatograms of blank (mobile phase), placebo (synthetic excipient mixture without drugs), standard solution, sample solution, and stressed samples. Peak purity was assessed using PDA detection.

Linearity. Calibration curves were constructed at six concentration levels covering 50–150% of target concentration (REM: 2–12 µg/mL; TEN: 2–12 µg/mL; MET: 10–60 µg/mL), analyzed in triplicate. Correlation coefficient (r^2), slope, and intercept were calculated by linear regression.

Accuracy. Evaluated by spiking known amounts of standards into pre-analyzed samples at 80%, 100%, and 120% of target concentration (n = 3 at each level). Percentage recovery was calculated as: Recovery (%) = (Amount Found ÷ Amount Added) × 100.

Precision. Repeatability (intraday precision) was determined by six injections of the same sample solution on the same day. Intermediate precision (interday precision) was evaluated by analyzing the sample solution on three different days. Results were expressed as %RSD of peak areas and assay values.

LOD and LOQ. Calculated using the formulae: LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve. Values were verified experimentally by analyzing solutions at calculated concentrations.

Robustness. Evaluated by deliberately varying mobile phase composition (±2% ACN), pH (±0.2 units), flow rate (±0.1 mL/min), column temperature (±5°C), and detection wavelength (±2 nm). System suitability parameters were assessed at each variation.

Solution Stability. Standard and sample solutions were analyzed freshly (0 h) and after 24 h and 48 h storage at room temperature (25°C) and refrigerated conditions (2–8°C). Acceptance criterion: %RSD ≤ 2.0%.

2.6 Forced Degradation Studies

Forced degradation studies were conducted in accordance with ICH Q1A(R2) guidelines [16] to evaluate the stability-indicating capability of the developed method:

- **Acidic hydrolysis:** Sample dissolved in 0.1 M HCl, refluxed at 60°C for 2 h, then neutralized with 0.1 M NaOH.
- **Alkaline hydrolysis:** Sample dissolved in 0.1 M NaOH, refluxed at 60°C for 2 h, then neutralized with 0.1 M HCl.
- **Oxidative degradation:** Sample dissolved in 3% H_2O_2, kept at room temperature for 2 h.
- **Thermal degradation:** Powder sample exposed to dry heat at 80°C for 48 h in a hot air oven.
- **Photolytic degradation:** Powder sample exposed to UV light (254 nm) for 48 h in a UV chamber.

Each degraded sample was diluted to target concentration with mobile phase, filtered through a 0.45 µm syringe filter, and analyzed immediately. Control samples (without stress exposure) were prepared simultaneously. Target degradation was 5–20%. Mass balance was calculated as the sum of the percentage assay of the degraded sample and the percentage of degradation products.

2.7 Assay of Synthetic Mixture

A synthetic mixture containing remogliflozin etabonate (100 mg), teneligliptin (20 mg), and metformin hydrochloride (500 mg) in their label claim ratio was prepared by accurately weighing appropriate amounts of pure drugs, sonicating in mobile phase for 20 minutes, making up to volume, filtering through a 0.45 µm syringe filter, and analyzing in triplicate. Percentage assay was calculated against the standard calibration curve.

3. RESULTS AND DISCUSSION

3.1 Method Development and Optimization

UV spectra of individual drug solutions (10 µg/mL each) were scanned over 200–400 nm. Remogliflozin etabonate, teneligliptin, and metformin hydrochloride showed λmax at approximately 224, 245, and 232 nm, respectively. Based on overlay spectra, 226 nm was selected as the detection wavelength, providing adequate sensitivity for simultaneous determination of all three analytes with minimal background interference from the mobile phase.

Several columns were screened, including C18 (250 mm × 4.6 mm, 5 µm) from multiple manufacturers, C8, and phenyl columns. The Phenomenex Luna C18 column was selected for its optimal separation efficiency, symmetric peak shapes, and acceptable analysis time.

Mobile phase pH optimization demonstrated that pH 4.0 (phosphate buffer) provided symmetric peaks, adequate resolution (Rs > 2.0), and stable chromatographic conditions. At pH below 3.5, peak tailing was observed for metformin; above pH 4.5, retention times increased and peak broadening occurred. Acetonitrile was preferred over methanol as the organic modifier due to lower viscosity, superior peak shapes, and faster equilibration. Mobile phase ratios of 70:30 to 55:45 (buffer:ACN) were tested; the 60:40 v/v ratio provided optimal baseline separation with reasonable retention times. A flow rate of 1.0 mL/min and column temperature of 30°C were selected as optimal, and an injection volume of 20 µL was found adequate without peak distortion or column overloading.

3.2 System Suitability

System suitability parameters evaluated from six replicate injections of the mixed standard solution are summarized in Table 2. All parameters met the acceptance criteria, confirming the suitability of the chromatographic system for the intended analysis.

Table 2. System suitability parameters (n = 6).

Parameter	REM	TEN	MET	Criteria
Retention time (min)	4.23	7.89	11.45	—
Theoretical plates (N)	8,456	9,234	8,789	> 2000
Tailing factor (T)	1.08	1.05	1.12	0.9–1.2
Resolution (Rs)	—	6.82	7.15	> 2.0
Retention factor (k')	3.84	7.21	10.54	2–10
%RSD of area (n=6)	0.34	0.53	0.36	≤ 2.0
%RSD of RT (n=6)	0.12	0.08	0.11	≤ 1.0

3.3 Specificity

Injection of blank mobile phase and placebo solution (synthetic excipient mixture without drugs) produced no interfering peaks at the retention times of the three analytes. Three distinct, well-resolved peaks were observed in the standard chromatogram at RT 4.23 min (REM), 7.89 min (TEN), and 11.45 min (MET) with baseline resolution (Rs > 2.0). Under all stress conditions, degradation products eluted at different retention times without interfering with the parent drug peaks. PDA-based peak purity analysis confirmed purity angles below purity thresholds for all three analytes in both standard and stressed sample chromatograms, establishing the specificity and stability-indicating capability of the method.

3.4 Linearity

All three drugs exhibited excellent linearity over the studied concentration ranges (Table 3). Correlation coefficients (r^2) ≥ 0.9997 confirmed a strong linear relationship between concentration and detector response. Representative regression equations were: REM: y = 122,817.5x + 1,023.4 (r^2 = 0.9998); TEN: y = 93,729.3x + 567.8 (r^2 = 0.9997); MET: y = 31,246.9x + 234.7 (r^2 = 0.9999).

Table 3. Linearity parameters for REM, TEN, and MET.

Drug	Range (µg/mL)	Slope	Intercept	r^2	%RSD (slope)
Remogliflozin Etabonate	2–12	122,817.5	1,023.4	0.9998	0.07
Teneligliptin	2–12	93,729.3	567.8	0.9997	0.08
Metformin HCl	10–60	31,246.9	234.7	0.9999	0.05

3.5 Accuracy

Accuracy was assessed by recovery studies at 80%, 100%, and 120% of target concentration. Results presented in Table 4 demonstrate that mean recoveries for all three drugs fell within the acceptance criterion of 98–102%, confirming the accuracy of the developed method.

Table 4. Accuracy (recovery) data for REM, TEN, and MET (n = 3 at each level).

Drug	Level (%)	Added (µg/mL)	Found (µg/mL)	Recovery (%) Mean±SD	%RSD
REM	80	4.8	4.793	99.86±0.53	0.67
	100	6.0	6.003	100.06±0.50	0.52
	120	7.2	7.217	100.24±0.56	0.74
Overall Mean Recovery				**100.05±0.49%**	**0.49**
TEN	80	4.8	4.807	100.07±0.63	0.58
	100	6.0	5.998	99.94±0.67	0.61

	120	7.2	7.190	99.86±0.63	0.69
Overall Mean Recovery				**99.96±0.53%**	**0.53**
MET	80	24.0	24.04	100.17±0.55	0.71
	100	30.0	29.98	99.93±0.40	0.55
	120	36.0	36.11	100.30±0.59	0.78
Overall Mean Recovery				**100.13±0.48%**	**0.48**

3.6 Precision

Intraday precision (%RSD of peak areas) was 0.34%, 0.53%, and 0.36% for REM, TEN, and MET, respectively. Interday %RSD values were 0.19%, 0.29%, and 0.27% (Table 5). All values were well below the acceptance criterion of 2.0%, confirming excellent repeatability and intermediate precision of the method.

Table 5. Precision data (intraday and interday).

Precision Type	Parameter	REM	TEN	MET
Intraday (n = 6)	Mean area	736,921	562,391	937,438
	SD	251.4	295.7	334.8
	%RSD	0.34	0.53	0.36
Interday (Days 1,2,3)	Mean assay (%)	100.00	100.08	99.91
	SD	0.19	0.29	0.27
	%RSD	0.19	0.29	0.27

3.7 Limit of Detection and Limit of Quantitation

LOD and LOQ values calculated from regression analysis (LOD = 3.3σ/S; LOQ = 10σ/S) were verified experimentally with signal-to-noise ratios of ~3:1 and ~10:1 respectively (Table 6). The low LOD and LOQ values confirm adequate method sensitivity for trace analysis.

Table 6. LOD and LOQ values for REM, TEN, and MET.

Drug	LOD (µg/mL)	LOQ (µg/mL)
Remogliflozin Etabonate	0.024	0.073
Teneligliptin	0.037	0.112
Metformin Hydrochloride	0.055	0.168

3.8 Robustness

Minor intentional variations in all critical method parameters produced no significant impact on system suitability (Table 7). All resolution values remained well above 2.0, tailing factors remained below 2.0, and theoretical plate counts remained above 2000. These results confirm the robustness of the method for routine application.

Table 7. Robustness study results (system suitability under parameter variations).

Variation	Rs (TEN/REM)	Rs (MET/TEN)	Tailing (MET)	Plates (MET)	Result
Optimized conditions	6.82	7.15	1.12	8,789	Pass
ACN +2% (62:38)	6.45	6.78	1.09	8,456	Pass
ACN −2% (58:42)	7.21	7.58	1.14	9,012	Pass
pH 3.8 (−0.2)	6.67	6.92	1.16	8,534	Pass
pH 4.2 (+0.2)	6.98	7.34	1.10	8,923	Pass

Flow 0.9 mL/min	7.34	7.67	1.13	9,234	Pass
Flow 1.1 mL/min	6.23	6.58	1.11	8,345	Pass
Temperature 25°C	6.98	7.32	1.15	8,567	Pass
Temperature 35°C	6.56	6.89	1.09	8,923	Pass
Wavelength 224 nm	6.79	7.12	1.12	8,756	Pass
Wavelength 228 nm	6.85	7.18	1.13	8,812	Pass

3.9 Solution Stability

Standard and sample solutions remained stable for at least 48 hours at both room temperature (25°C) and refrigerated conditions (2–8°C), with %RSD values below 2.0% in all cases. This confirms that freshly prepared solutions can be used throughout a standard analytical working day without significant degradation.

3.10 Forced Degradation Studies

The forced degradation results are summarized in Table 8. Remogliflozin etabonate showed the greatest susceptibility to alkaline hydrolysis (13.6% degradation), consistent with the presence of ester linkages in its prodrug structure. Teneligliptin and metformin also demonstrated maximum degradation under alkaline conditions (8.8% and 7.3%, respectively). All three drugs exhibited good thermal stability, with degradation below 3% even after 48 hours at 80°C. Under photolytic stress, degradation ranged from 2.2% (MET) to 6.4% (REM). Oxidative degradation was moderate for all three drugs, while acidic degradation produced 10.8% (REM), 6.5% (TEN), and 4.9% (MET) degradation.

Degradation products were completely resolved from parent drug peaks in all chromatograms, with no co-elution confirmed by PDA peak purity analysis. Mass balance values ranged from 97.8% to 99.8% across all stress conditions, falling within the acceptable range of 95–105%, confirming that all degradation was accounted for. These results conclusively establish the stability-indicating capability of the developed RP-HPLC method.

Table 8. Forced degradation study results and mass balance.

Stress Condition	REM % Degradation	TEN % Degradation	MET % Degradation	Mass Balance (%)
Control (unstressed)	0	0	0	100.0
Acid (0.1M HCl, 60°C, 2 h)	10.8	6.5	4.9	97.8
Alkali (0.1M NaOH, 60°C, 2 h)	13.6	8.8	7.3	98.3
Oxidative (3% H_2O_2, RT, 2 h)	8.3	5.2	3.7	99.1
Thermal (80°C, 48 h)	2.8	1.5	0.9	99.8
Photolytic (UV 254 nm, 48 h)	6.4	4.1	2.2	98.7

3.11 Assay of Synthetic Mixture

Assay results for the synthetic mixture (Table 9) demonstrated values within 98–102% of the label claim for all three drugs, with %RSD below 0.6%, validating the applicability of the method for simultaneous quantification in a complex mixture representative of a formulation.

Table 9. Assay results of synthetic mixture (n = 3).

Drug	Label Claim (mg)	Amount Found (mg) Mean±SD	Assay (%) Mean±SD	%RSD
Remogliflozin Etabonate	100	100.11 ± 0.27	100.11 ± 0.27	0.27
Teneligliptin	20	20.03 ± 0.12	100.15 ± 0.58	0.58
Metformin Hydrochloride	500	499.81 ± 1.24	99.96 ± 0.25	0.25

3.12 Comparison with Reported Methods

Most previously reported methods address binary drug combinations, primarily remogliflozin–metformin or teneligliptin–metformin [10–13]. Bano et al. (2026) reported HILIC-HPLC for a remogliflozin–metformin–vildagliptin combination [14], but no RP-HPLC method with comprehensive forced degradation studies was available for the specific REM–TEN–MET combination. The

present method offers advantages over existing approaches: (a) isocratic elution with a simple binary mobile phase reduces operational complexity and cost; (b) total analysis time of 15 minutes is comparable to or shorter than reported gradient methods; (c) the simultaneous estimation of all three drugs in one run eliminates the need for separate assays; (d) comprehensive forced degradation validation under five stress conditions provides regulatory-compliant stability-indicating evidence; and (e) LOD and LOQ values are comparable to or better than reported binary methods.

4. CONCLUSION

A validated, stability-indicating RP-HPLC method was successfully developed for the simultaneous determination of remogliflozin etabonate, teneligliptin, and metformin hydrochloride in a synthetic mixture. The method employs a Phenomenex Luna C18 column with an isocratic mobile phase of phosphate buffer (pH 4.0) and acetonitrile (60:40 v/v) at 226 nm, achieving baseline-resolved peaks in 15 minutes. The method was fully validated per ICH Q2(R1), demonstrating excellent linearity ($r^2 \geq 0.9997$), accuracy (recovery 98–102%), and precision (%RSD < 0.6%). Low LOD and LOQ values confirm adequate sensitivity. Forced degradation studies under five stress conditions per ICH Q1A(R2) confirmed stability-indicating performance with complete peak resolution and mass balance of 97.8–99.8%. The method is suitable for routine pharmaceutical quality control, stability studies, impurity profiling, formulation development, and regulatory submissions for this clinically important triple antidiabetic combination targeting Type 2 Diabetes Mellitus.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Rai University, School of Pharmacy, Ahmedabad, for providing infrastructure and facilities to carry out this research. The authors thank Ms. Sonal H Vaishnav, Assistant Professor, Department of Pharmaceutical Chemistry and Quality Assurance, and Dr. Sanjesh Rathi, Principal, for their support and encouragement throughout the study.

DECLARATIONS

Conflict of Interest: The authors declare no conflict of interest.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical Approval: Not applicable (in vitro analytical study using pharmaceutical standards).

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas. 10th ed. Brussels: IDF; 2021.
2. American Diabetes Association. Standards of Medical Care in Diabetes—2024. Diabetes Care. 2024;47(Suppl 1):S1–S321.
3. John M, Gopinath D, Kalra S. Triple fixed drug combinations in type 2 diabetes. Indian J Endocrinol Metab. 2015;19(3):311–3.
4. Kim YG, Hahn S, Oh TJ, et al. Therapeutic efficacy and safety of initial triple combination of metformin, sitagliptin, and lobeglitazone in drug-naïve patients with type 2 diabetes. BMJ Open Diabetes Res Care. 2020;8(1):e000807.
5. Hermansen K, Mortensen LS, Hermansen ML. Triple therapy combinations for the treatment of type 2 diabetes: a network meta-analysis. Diabetes Res Clin Pract. 2016;116:149–58.
6. Sneha KS, Gaware VM. Review on method development and validation of remogliflozin etabonate and teneligliptin by RP-HPLC and UV spectroscopy. Asian J Pharm Res Dev. 2025;13(4):98–105.
7. Patil A, Shah R, Kumar S. Stability indicating RP-HPLC method for remogliflozin and teneligliptin. Ann Biol Res. 2024;Sept:100–108.
8. Vinay T, Lalitha N, Mubeen G. Development and validation of RP-HPLC method for simultaneous estimation of teneligliptin and pioglitazone in tablet formulation. Asian J Pharm Res Dev. 2024;12(6):31–36.
9. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998;352(9131):854–65.
10. Kagarana CS, Patel KN, Patel AB. Stability indicating RP-HPLC method development and validation for simultaneous estimation of metformin hydrochloride and remogliflozin etabonate. Res J Pharm Technol. 2024;17(5):2025–30.
11. Kumar P, Verma A. Stability indicating bioanalytical method development and validation for estimation of remogliflozin etabonate by RP-HPLC in human plasma. Int J Pharm Investig. 2024;14(4):1131–7.
12. Patel MM, Patel D, Shah U, Kachhiya HM. A simple, precise, and sensitive RP-HPLC method for quantification of teneligliptin hydrobromide and metformin hydrochloride. Res J Pharm Technol. 2023;16(2):589–95.
13. Singh S, Singh S, Saw S, Rathi S, Sharma B. Single Cell Transcriptomics of Traditional Chinese Medicine Bioactives: Mapping Immune, Neural and Hepatic Responses. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2025 Nov 10;41:e20250028. doi: 10.62958/j.cjap.2025.028. PMID: 41207694.
14. Bano T, Channawar M, Chandewar AV. Analytical method development and validation of three combination drugs remogliflozin etabonate, metformin and vildagliptin by RP-HPLC technique. Int J Pharm Sci Res. 2026;44:5472–83.
15. International Council for Harmonisation. ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva: ICH; 2005.
16. International Council for Harmonisation. ICH Q1A(R2): Stability Testing of New Drug Substances and Products. Geneva: ICH; 2003.
17. Kasar P, Patil S, Deshmukh A. Development and validation of stability-indicating RP-HPLC methods for pharmaceutical drugs: a comprehensive review. Int J Pharm Sci. 2025;3(8):1361–72.

18. DS Rajput, M Bhoi, S. Singh, Comment on Fennel essential oil and its nanoemulsion modulate macrophage-mediated inflammatory responses and promote pressure ulcer healing. International Immunopharmacology. 2026; 173:116184. https://doi.org/10.1016/j.intimp.2026.116184

19. Bakshi M, Singh S. Development of validated stability-indicating assay methods—Critical review. J Pharm Biomed Anal. 2002;28(6):1011–40.

20. Deshmukh R, Shah P, Kulkarni A. QbD based analytical method development and validation for simultaneous estimation of remogliflozin etabonate and vildagliptin. J Clin Health Res. 2024;8(2):1245–56.