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Original Article

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC) METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF REMOGLIFLOZIN ETABONATE AND VILDAGLIPTIN IN BULK AND ITS TABLET FORMULATION

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ABSTRACT

Objective: Within the scope of this investigation, an HPTLC technique for measuring the concentrations of Remogliflozin etabonate (REMO) and Vildagliptin (VIL) in a commercial product named REMO-V, which contains 100 mg of REMO and 50 mg of Vildagliptin (VIL), was developed.

Methods: It was necessary to evaluate the new approach for linearity, precision, specificity, and robustness in order to ensure that it operated properly. The chromatograms were created using a mobile phase containing Chloroform as follows: The concentrations of toluene, methanol, and n-butanol (4.5:4:1:0.5, v/v) were measured on a pre-coated TLC aluminum pre-coated plate (60F 254), and the absorbance at 233 nm was used to determine the amount of each component present. It was necessary to conduct forced degradation testing on bulk medicinal material in order to demonstrate the new method's capacity to demonstrate how stable and specific it is.

Results: The Rf values for Remogliflozin etabonate (0.63), Vildagliptin (0.75), and Remogliflozin etabonate (0.63), respectively, were 0.63 and 0.75. On the REMO side, the linearity of the technique was found to be between 20 and 60 g/band; on the VIL side, it was found to be between 10 and 30 g/band. Having R2 values of 0.9939 for both REMO and VIL, it is clear that there is significant linearity in the way they interact with one another. These are the lower limits of detection and quantification for REMO, which were 0.09 for VIL and 0.38 for REMO, respectively, when compared to the upper limits of detection and quantification for VIL. The lower detection and quantification limits for VIL are also the same as for other pathogens. RSD was less than 2 percent in this study. Thus, the approach was shown to be accurate and exact for both interday and intraday accuracy, indicating that it is reliable. The amount of REMO or VIL that might be recovered is as follows: 98.7 percent to 101.27 percent, and 97.37 percent to 100.83 percent were the results.

Conclusion: It was discovered that the method for determining Remogliflozin and Vildagliptin was easy, accurate, and stable in both its pure form and its tablet dose form and that it could be used to both (REMO-V, Glenmark, Ltd)

Keywords: Remogliflozin etabonate, Vildagliptin, High-performance thin-layer chromatography, Validation, Forced degradation

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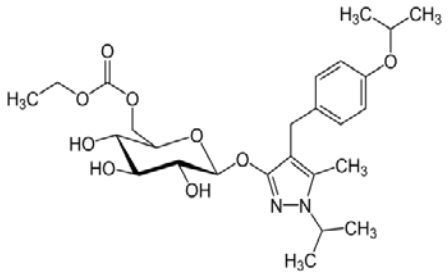
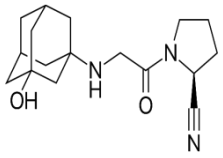
INTRODUCTION

REMO is the chemical name for Remogliflozin etabonate, which is ethyl [(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[5-methyl-1-]] [(2R,3S,4S, 5R,6S)-3,4,5-trihydroxy-6-[5-methyl-1-]] [(2R,3S,4S,5R,6S)-3,4,5 (propan-2-yl)-4-[4-(propan-2-yloxy) phenyl] is a-4-[4-(propan-2-yloxy) phenyl] compound. methyl} Between the elements oxy and oxan-2, a chemical reaction occurs. Methyl carbonate is primarily used in the treatment of diabetes [1-3]. Vildagliptin is the chemical name for this medication (2S) (1-2-[(3-hydroxyadamantan1yl) amino]-1-2-[(3-hydroxyadamantan1yl) amino] Acetyl pyrrolidine-2-carbonitrile is a novel oral anti-hyperglycemic medication that belongs to a new family of medications that inhibit dipeptidyl peptidase activity [4-7].



Structure of remogliflozin etabonate



Structure of vildagliptin

Several analytical methodologies for quantitative evaluation of REMO and VIL pharmaceutical preparations have been recorded in the literature, according to a study of the literature. HPTLC for VIL [8] and UV spectroscopy, as well as the RP-HPLC and HPTLC for REM estimation in bulk and tablet dosage form, are examples of these approaches. At this time, REM is not officially recognised by any pharmacopeia, and there is no published HPTLC approach for assessing REMO and VIL combinations in pharmaceutical dosage forms at this time. The intended study includes HPTLC testing of REMO and VIL in bulk and tablet dose forms, as well as other methods.

MATERIALS AND METHODS

Chemicals and regents

Glenmark Pharmaceutical Sikkim, India, provided a free sample of REMO and VIL as a thank you, and the branded formulation (Remo-V tablet) was obtained from a local market in Pimpri Chinchwad, Pune. All chemicals, including chloroform, toluene, methanol, and n-butanol, were purchased in analytical grade from LOBA CHEMIE Pvt Ltd. in Mumbai, India.

Chromatographic variables and instruments

The medication was separated using a CAMAG Linomat 5 sample applicator on a precoated silica gel aluminium plate 60 F254 (10 10) with a thickness of 250 m and a precoated silica gel aluminium plate 60 F254 (MERCK, Darmstadt, Germany). Broad bands of samples were applied to the plate using a Camag 100 L sample syringe (Hamilton, Switzerland) using a 6 mm wide band of samples. With the help of the mobile phase, linear ascending development was carried out in a 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland). Chloroform: Toluene: Methanol: n-Butanol (4.5:4:1:0.5, in parts per thousand); the mobile phase in the chamber was soaked for 15 min before being removed. Following development, the TLC plates were dried using a hair dryer and an air current to ensure that they were completely dry. All developments were scanned densitometrically at 244 nm using a CAMAG thin layer chromatographic scanner and WINCATS software version 1.4.2, which was used in conjunction with a CAMAG thin layer chromatographic scanner. A deuterium lamp providing a continuous UV spectrum between 200 and 400 nm was employed as the radiation source in this experiment.

Preparation of standard stock solution

It was shown that dissolving 100 mg of REMO or VIL in 10 ml methanol produced standard stock solutions with concentrations of 10 mg L-1 and 10 mg L-1, respectively [9], which could be used to make standard stock solutions with higher concentrations [10, 11]. The standard working solution of VIL was prepared by dissolving 50 mg of the medication in 10 ml of methanol to achieve a concentration of 5 mg ml-1, which was then used as a concentration reference.

Preparation of sample solution

The average weight of the tablets was computed after they were weighed a total of 20 times. A powder containing 100 mg of REMO and 50 mg of VIL was transferred to a 10 ml volumetric flask, and 10 ml of methanol was added to dilute the mixture. Prior to injection, this solution was filtered using a 0.45 mm membrane filter to remove impurities.

Method validation

Guidelines Q2 (R1) for the validation of analytical techniques were published by the International Conference on Harmonization (ICH) [10]. It was determined if the created technique was linear, accurate, precise, particular and resilient in terms of the metrics used to evaluate it.

Linearity

The linearity of the data was calculated using a conventional approach. The calibration graphs for REMO (10-60 g/band; n=6) and VIL (10 to 30 g/band; n=6) were drawn for different concentrations of REMO (10-60 g/band; n=6) and VIL (10 to 30 g/band; n=6).

Accuracy/recovery study

Drug recovery studies were carried out in accordance with conventional practise in order to determine the accuracy parameters. Three distinct percentages were used: 80 percent, 100 percent, and 120 percent of the overall population. In order to determine the percentage of recovery, the findings were entered into a table and shown within.

Specificity

The specificity of the approach was determined by comparing the Rf values and spectra of the standard with the sample in question. In order to establish the purity of the drug peaks, we examined the spectra at the peak start, maximum position, and peak endpoints. The peak purity was calculated with the help of the Win CATS software.

Precision

The precision and repeatability of the system were utilised to demonstrate the accuracy of the procedure. Six System precision copies were generated for each of the two drugs, and the RSD was calculated for each of the six replicates. Both intra-day and inter-day fluctuations exhibited a high degree of repetition. Within-day investigations comprised collecting three measurements three times in a single day to compute the percent RSD of the response, which was calculated in the overall study. In the interday variation investigation, the percentage RSD of the response was estimated by collecting three measurements on three consecutive days throughout the course of three days.

Limit of detection (LOD) and limit of quantification (LOQ)

As a result of 3.3a/s and 10a/s occurrences, the novel method's detection and quantification limitations were developed.

Robustness

After testing the strategy's resilience by modifying the best method parameters in modest but significant ways, it was discovered that the methodology was resilient. In order to evaluate if changes in mobile phase composition (1 ml), volume (1.0 ml), and time (5 percent) during chamber saturation with mobile phase influenced medication Rf value, experiments were carried out.

Forced degradation

It was necessary to conduct forced degradation studies on bulk drug material in order to determine if the suggested method's selectivity and stability-indicating characteristics were valid. The degradation was carried out under a variety of stress conditions, including acid, base, hydrolytic, oxidative, thermolytic, and photolytic.

RESULTS AND DISCUSSION

Optimization of the HPTLC method

It has been carefully evaluated for linearity and accuracy in terms of detection and quantification limits, as well as for sturdiness when it comes to this strategy. The United States Pharmacopeia (USP) has produced validation standards for pharmaceutical analytical processes that are used in the pharmaceutical industry. Some of the most frequently used validation parameters have been examined in detail in this section [11].

When measuring REMO and VIL in pharmaceutical formulations, it was required to employ a solvent solution that generated dense and compact spots with high Rf values, which was only possible with a high Rf solvent solution. Rf values of 0.63 and 0.75, respectively, for REMO and VIL were obtained in the Chloroform mobile phase, which consisted of the following components: toluene: methanol: n-butanol (4.5:4:1:0.5, v/v) (fig. 2). We developed a simple, precise, and accurate HPTLC method for estimating the levels of the drugs remogliflozin etabonate and vildagliptin in blood samples (at 233 nm).

Linearity

The linear regression data from the calibration plots revealed a clear linear relationship over concentration for REMO 20-60 g/band; n=6 and VIL 10 to 30 g/band; n=6 for both REMO and VIL (correlation coefficient, r2, REMO 0.993 and VIL 0.995). (fig. 3 and fig. 4). Fig. 3, 4, and table 1 represent the results of the experiment.

Specificity

It was necessary to identify the existence of an interfering peak within the retention period of the analyte peak in order to assess specificity. It was discovered that there was no peak in the chromatogram for the blank sample during the vildagliptin retention period, indicating that the excipient did not interfere with the analysis.

Limit of detection (LOD) and limit of quantification (LOQ)

In the case of REMO, the 'Limit of Detection' was determined and recorded as 0.023 g/band, whereas in the case of VIL, it was 0.109 g/band. The 'Limit of Quantification' for REMO was found to be 0.038 g/band and for VIL it was found to be 0.052 g/band, respectively. In table 1, you can see the outcomes of the study.

Precision

The accuracy of the inter-day and intra-day intervals was examined in accordance with conventional procedures. The percent RSD was determined to be less than 2 percent, suggesting that the technique is accurate and repeatable. The procedure is accurate and reproducible (table 1).

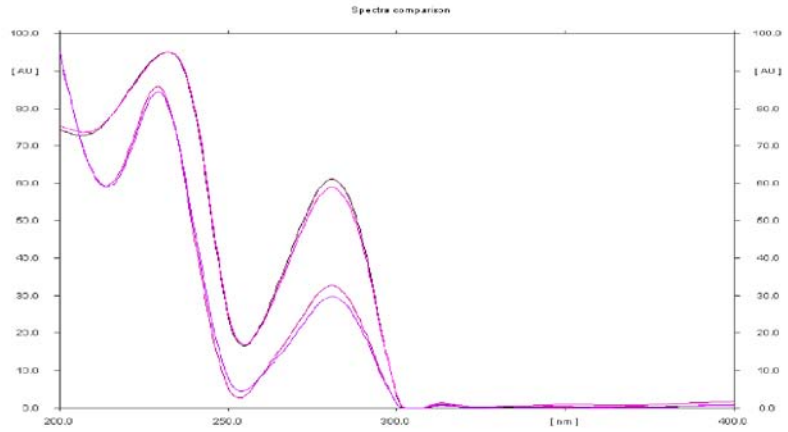


Fig. 1: Overlay absorption spectra of remogliflozin etabonate and vildagliptin in methanol

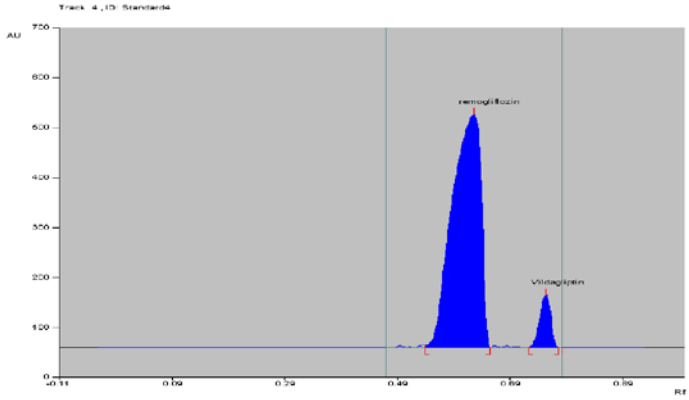


Fig. 2: Typical densitogram of remogliflozin etabonate and vildagliptin standard in mixture solution

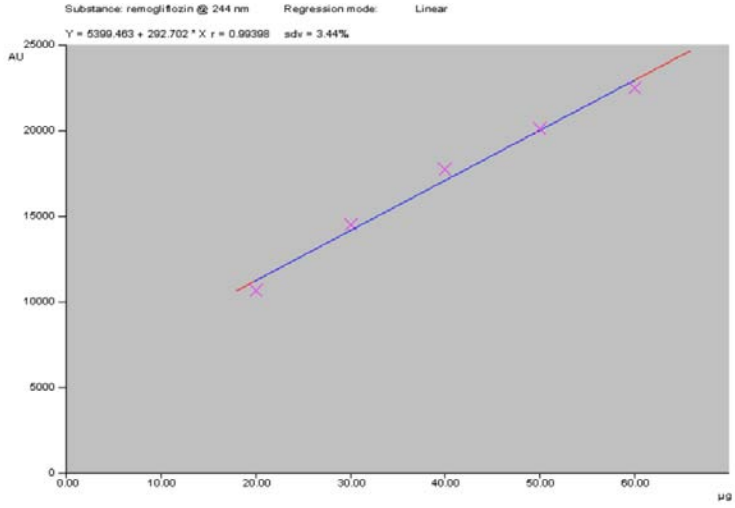


Fig. 3: Linearity graph of remogliflozin etabonate at 244 nm

Table 1: Summary of linearity, LOD, LOQ and precision

Parameters	For remogliflozin etabonate	For vildagliptin
Range (Beer's Law Limit, µg/band)	20-60 µg/band	10-30 µg/band
Correlation coefficient	0.993	0.995
Limit of detection (µg/band)	0.023	0.109
Limit of quantitation (µg/band)	0.038	0.052
Precision indicated by % RSD (intraday)	0.485	1.304
Precision indicated by % RSD (interday)	0.180	0.953

(n = 3)

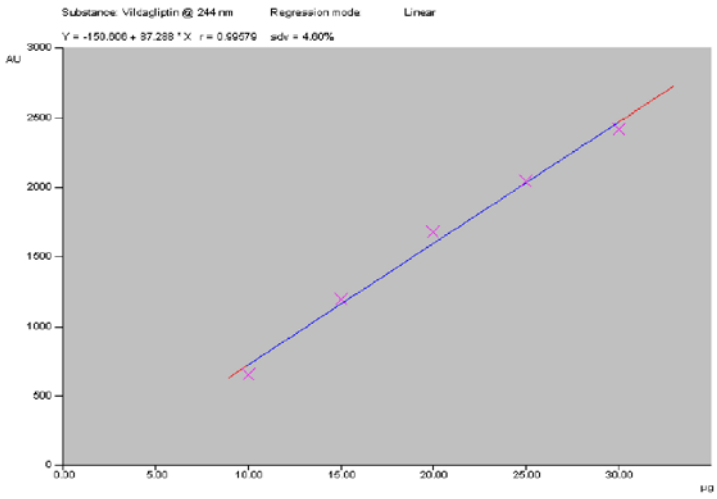


Fig. 4: Linearity graph of vindagliptin at 244 nm

Recovery study

The accuracy of the recommended approach's recovery was evaluated using the standard addition technique at three different concentrations (50 percent, 100 percent, and 150 percent) of the standard solution, and the results were encouraging. It was necessary to chromatograph the samples three times in order to calculate the percent recovery. The results of the accuracy tests are shown in table 2. Removing the excipients from the tablet formulation did not interfere with the analysis, as established by the % recovery of REMO and VIL, which was found to be in the ranges of 98 percent to 101.2 percent and 97.37 percent to 100.83 percent, respectively.

Table 2: Recovery studies of remogliflozin etabonate and vildagliptin

Level of % recovery	Amount spotted		Amount recovered		Mean % recovery	
	REMO	VIL	REMO	VIL	REMO	VIL
80%	180	90	176.4	87.63	98%	97.37%
100%	200	100	199.8	99.16	99.80%	99.16%
120%	220	110	222.64	110.91	101.2%	100.83%

(n = 3)

Robustness

On the other hand, the robustness of the suggested technique was observed. The impact of technique parameter modifications was not statistically significant since the Rf values for REMO and VIL were within 0.05 Rf units of each other, indicating that there was no statistically significant shift in peak area between the two methods. The conclusions of the robustness study are shown in table 3.

Table 3: Results of robustness study

Factor	Rf value remogliflozin etabonate	Rf value if vildagliptin	%RSD of remogliflozin etabonate	% RSD if vildagliptin
Mobile phase composition (±0.1 ml)	0.56	0.72	0.468	0.960
Amount of Mobile Phase (±1.0 ml)	0.55	0.72	0.814	1.623
Duration for chamber saturation (±5%)	0.57	0.73	0.353	1.31

Analysis of tablet formulation

The proposed approach was also put through its paces using the REMO and VIL Tablet tests. On the appropriately weighed drug amounts, six duplicate determinations were done using the same procedure. The percentage of drug content in REMO was judged to be 99.80 percent, whereas the percentage of drug content in VIL was determined to be 99.16 percent (table 4).

Table 4: Results from analysis of pharmaceutical formulation (n = 3)

Drug	Labeled amount mg/tablet	Amount found, mg/tablet	Amount found %
Remogliflozin etabonate	100	99.8	99.8
Vildagliptin	50	49.58	99.16

Forced degradation studies

According to ICH recommendations [12], forced degradation tests for a variety of parameters were carried out. According to the standards, the deterioration should be noticed at a rate ranging from 5 to 20% (table 4). Following the completion of acidic, basic, oxidative, and thermal degradation tests in accordance with International Conference on Harmonization (ICH) criteria [13], the following methods were implemented for the research.

Acid degradation

Added peaks were seen at Rf values of 0.41 and 0.75 for REMO and VIL, respectively, as shown in fig. 4. Acid degradation of materials was shown in fig. 4. In acid medium, REMO was found to be 9.94 percent destroyed, while VIL was found to be 5.49 percent degraded, according to the findings. There was a significant separation between the peaks of REMO and VIL degradation products and those of the REMO and VIL drug peaks. (fig. 5 and table 5)

Alkali hydrolysis

The results of alkali hydrolysis of the samples are shown in fig. 5. Both REMO and VIL medications have Rf values of (0.43 and 0.01), respectively, for REMO and VIL. In alkali media, REMO was found to be 9.75 percent degraded, while VIL was found to be 7.12 percent degraded, according to the findings. The breakdown products of both pharmaceutical companies had distinct peaks that were well separated from the peaks of the REMO and VIL drugs (fig. 6 and table 6).

Oxidation-induced degradation study

The oxidative stress degradation densitogram of REMO and VIL in combination is shown in fig. 6. It was revealed that the medications were being oxidatively destroyed to some extent. Rf values of 0.55 and 0.80 were found to be associated with additional peaks in the data for REMO and VIL, respectively. Under oxidative conditions, REMO was found to be 10.53 percent damaged, while VIL was found to be 5.12 percent degraded, according to the findings (fig. 7 and table 5).

Neutral hydrolysis

The results of Neutral hydrolysis for the samples are shown in fig. 7. It was determined that the drugs had been slightly deteriorated. The Rf values of 0.58 and 0.73 were shown to be significant in identifying additional peaks for REMO and VIL, respectively. The results of the study revealed that REMO had deteriorated by 11.25 percent in neutral conditions, while VIL had deteriorated by 6.25 percent (fig. 8 and table 5).

Thermal degradation studies

Thermal degradation study was performed on samples that had been dried at 60 °C for 30 min in a dry heat oven. REMO and VIL were found to be labile according to the thermal degradation study (fig. 8), with additional peaks emerging at 0.61 and 0.83 (Rf values), respectively, in the REMO and VIL samples. The results revealed that REMO and VIL had worsened by 9.31 percent and 9.22 percent, respectively (fig. 9 and table 5).

Photodegradation study

Fig. 10 shows the chromatograms of REMO and VIL after exposure to ultraviolet light (254 nm). It was observed that the drugs were degrading as a result of photodegradation. Further peaks were seen at Rf values of 0.61 and 0.75 for REMO and VIL, respectively, as a result of the res. According to the results of the photodegradation test, REMO was found to be 12.37 percent degraded, whereas VIL was found to be 5.274 percent degraded.

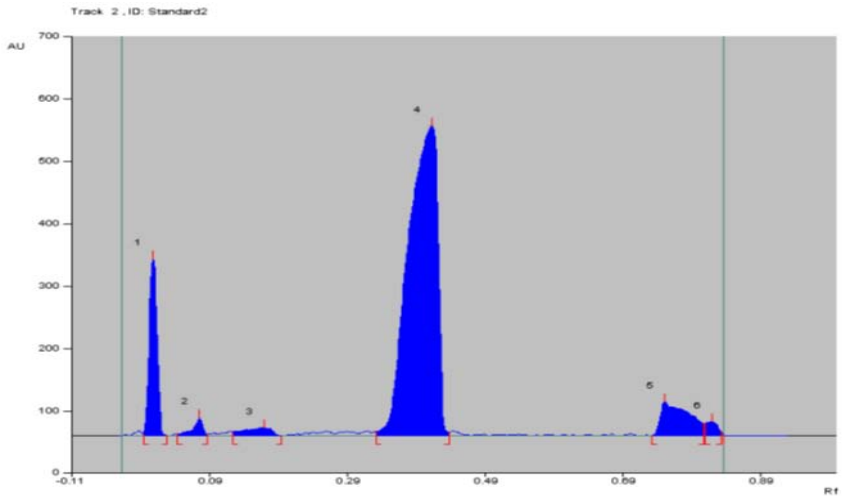


Fig. 5: Densitogram of acid (0.1 M HCl) treated sample

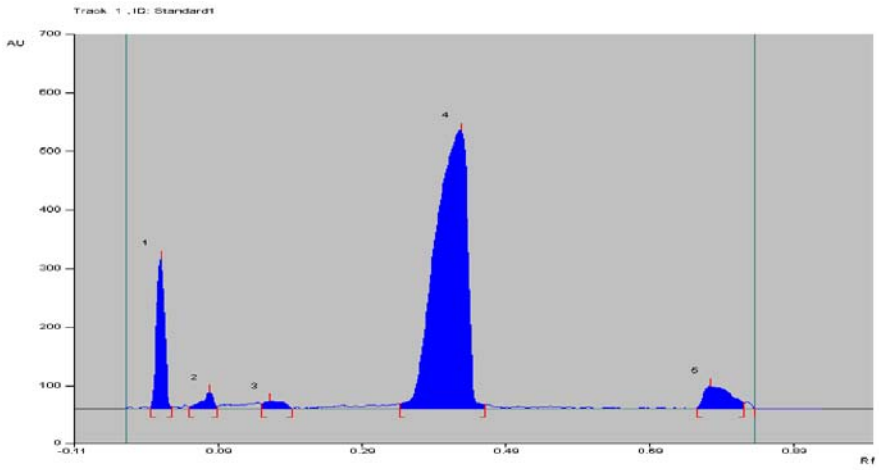


Fig. 6: Densitogram of Alkali (0.1 N NaOH) treated sample

Table 5: Forced degradation studies data of remogliflozin etabonate and vildagliptin by the developed HPTLC method

Stress condition	Temperature and time	Percent assay of active substance remogliflozin etabonate	Rf value of degraded product remogliflozin etabonate	Percent assay of active substance vildagliptin	Rf value of degraded product vildagliptin
Acid (0.1N HCl)	Room temp. 1 h.	90.06	0.41	94.51	0.75
Alkali (1N NaOH)	Room temp. 1 h.	90.25	0.43	92.88	0.01
Oxide (3.0% H2O2)	Room temp. 1 h.	89.27	0.55	94.88	0.80
Neutral	Room temp. 1 h.	89.83	0.58	94.79	0.74
Heat	At 600 30 min	90.69	0.61	90.78	0.83
UV-Exposure	UV 254 wavelength 24h	87.63	0.61	94.73	0.75

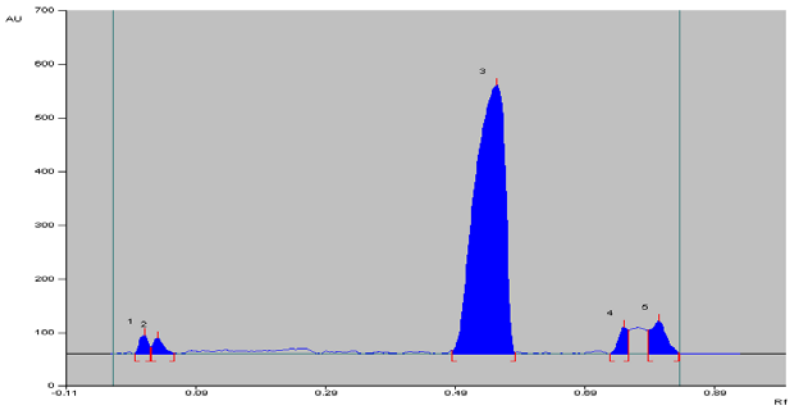


Fig. 7: Densitogram of oxide (3% H_2O_2) treated sample

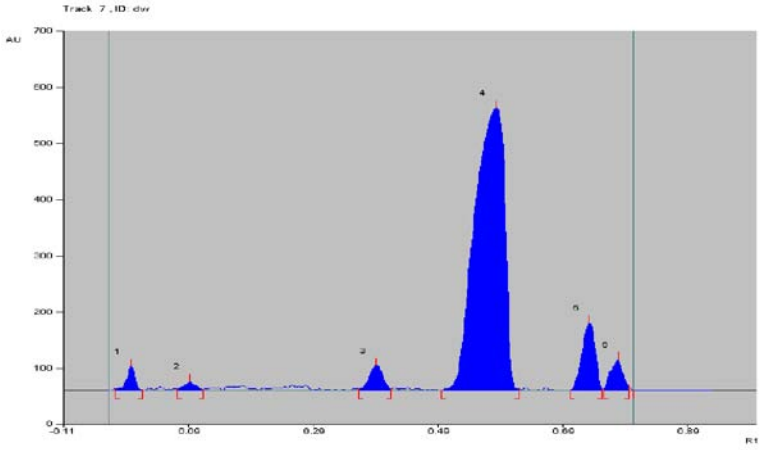


Fig. 8: Densitogram of the neutral treated sample

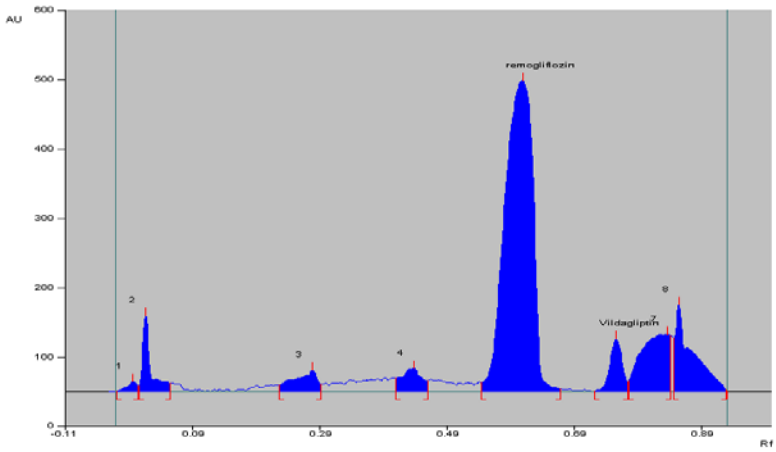


Fig. 9: Densitogram of dry heat-treated sample

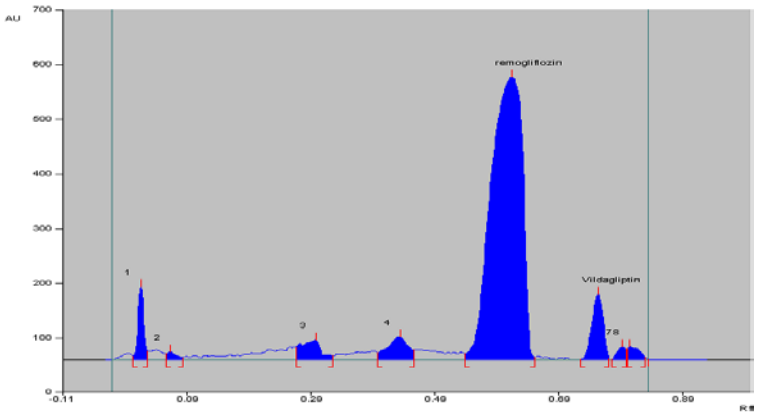


Fig. 10: Densitogram of UV light 254 treated sample

CONCLUSION

This work developed and validated an HPTLC technique for determining the presence of REMO and VIL in both pure and dosed form in pure form. In addition, the REMO and VIL tests were employed to assess the proposed technique. VIL had a 99.16 percent drug content, whereas REMO had a 99.80 percent drug content, according to the results of the tests. The stated technique for determining REMO and VIL in both pure and tablet form yielded results that were simple, precise, accurate, quick, and specific, and it did so in a straightforward manner. The formulation samples that were retrieved were consistent with the claims made on their respective labels, showing that formulation excipients had no impact on the estimate. It was necessary to conduct forced degradation studies on bulk drug material in order to determine if the suggested method's selectivity and stability-indicating characteristics were valid. It follows as a result that routine testing of REMO and VIL in both their purified and dosed forms is simplified and uncomplicated utilising this approach. In future more precise work will be required in forced degradation study of Remogliflozin Etabonate and Vildagliptin in its pure form.

ABBREVIATIONS

REMO-remogliflozin etabonate, VIL-vildagliptin, HPTLC-high performance thin layer chromatography, Rf-retention factor, LOD-limit of detection, LOQ-limit of quantification, µg-microgram, ml-milliliters, ICH-international conference on harmonization.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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