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# Development and validation of method for simultaneous estimation of Triple Drug Combination Employed for Type II Diabetes Mellitus and its validation using ICH Q2R1

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## ABSTRACT

This study reports the method development and validation for anti-diabetic drugs by UPLC. A new, simple, rapid, selective, precise and accurate ultra performance liquid chromatography assay has been developed for simultaneous estimation of Remogliflozin, Vildagliptin and Metformin. The separation was achieved by using ODS 3V column with dimensions 5 µm, 4.6 mm x 250 mm. The pH of mobile phase was adjusted to 4.5 with acetonitrile. The flow rate was 0.4 mL/min, and the separated drugs were detected using UPLC detector at the wavelength of 260 nm. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent. It was successfully applied for the analysis of these drugs in marketed formulations and could be effectively used for the routine analysis of formulations.

**Keywords:** Remogliflozin, Vildagliptin, Metformin, ICH guidelines

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# INTRODUCTION

A metabolic condition known as diabetes mellitus (DM) is typified by high blood glucose levels. 90% of individuals have type 2 diabetes, and one in eleven people worldwide are thought to have DM (T2DM). Experts estimate that by the end of 2040, there would be around 642 million instances of DM worldwide, up from the current 422 million cases [1,2].

Remogliflozin etabonate (RGF), with the chemical term [5-methyl-4-(4-(1-methylethoxy) benzyl)-1-(1-methylethyl)-1H-pyrazol-3-yl 6-O-(ethoxy carbonyl)-b-D glucopyranoside] is classed as SGLT2 inhibitor. The transporter known as SGLT2 plays a major role in the kidneys' capacity to reabsorb glucose. Vildagliptin (VGT) also recognized as (S)-1-[N-(3-hydroxy1-adamantyl) glycyl] pyrrolidine-2-carbonitrile. Metformin hydrochloride (MTH) is also known by its chemical name, 1,1-dimethyl biguanide hydrochloride. [3,6]

The newer combination of RGF, VGT, and MTH was launched in October 2021 by Glenmark Pharmaceuticals Ltd., India. It works through three synergistic mechanisms to help control glycemic levels in people with diabetes (type 2) peoples may benefit from consuming RGF and VGT together with MTH.

RGF, VGT, and MTH alone and in combination with other drugs was determined using ultraviolet (UV) spectrophotometry [7,8,17,18], HPLC [4,5,6], LCMS [9], HPTLC [12,15,19,20] UPLC [13,14] and RP-HPLC [10,11,16]. However, only two research articles reported simultaneous estimation of all three drugs under study.

Based on the available literature it can be confirm that no UPLC method was available for the separation and simultaneous quantification of vildagliptin and remogliflozin etabonate. Hence the present work aimed to develop simple and precise analytical UPLC method for the separation and simultaneous estimation of vildagliptin remogliflozin etabonate and metformin in bulk drug as well as in pharmaceutical formulations.

# MATERIALS AND METHOD

## Chemicals and Reagents

Remogliflozin etabonate (REM), Vildagliptin (VLD), Metformin hydrochloride (MEH) Water, $NaHPO_4$, sodium hydroxide (NaOH), Acetonitrile and Hydrochloric acid (HCl) chemicals are made use in the research.

## Instruments

UPLC system from “Waters Alliance” corporation

Detector - Photodiode array from “Waters Alliance” corporation

Empower 2nd software from “Waters Alliance” corporation

Inertsil ODS 3V (250x4.6 mm, 5µm)

**Preparation Mobile phase:**

In a 1000 ml beaker HPLC water, then adjust the pH with $NaHSO_4$ to 3.8.

500 mL of acetonitrile and 500 mL of $NaHSO_4$ was used as mobile phase. They are mixed and sonicate for 20min.

**Preparation Diluents:**

With 50:50 volume/volume proportionality, $NaHSO_4$ and Acetonitrile are combined.

**Preparation of stock solution:**

To make a stock REM, VLD, and MEH solution, 100 mg REM, 50 mg VLD, and 500 mg MEH were carefully balanced in a flask (100 ml), then diluted appropriately in diluting fluid (NaHPO4 and Acetonitrile are mixed in a 50:50 volume/volume proportionality). The concentrations are as follows: 100 microg/ml for REM, 50 microg/ml for VLD, and 500 microg/ml for MEH.

**Preparation of working solution**

5 ml stock REM (100 µg/ml), VLD (50 µg/ml), and MEH (500 µg/ml) combined with 45 ml dissolving solvent $NaHPO_4$ and Acetonitrile (pure) merged in a 50:50 v/v fraction. REM - 100 µg/ml, VLD - 50 µg/ml, and MEH – 500 µg/ml.

**OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS:**

After a series of trials, the final chromatographic conditions were determined as follows. The mobile phase was a buffer with pH 4.5 and acetonitrile: $NaHSO_4$ (50:50% v/v), and the stationary phase was a inertsil ODS 3V column with dimensions 5 µm, 4.6 mm x 250 mm to obtain the best peak shape. The separation of remogliflozin, vildagliptin and metformin hydrochloride was good at 260 nm, a flow rate of 0.4 mL/min, and Run time is 5 min as shown in graph 1.

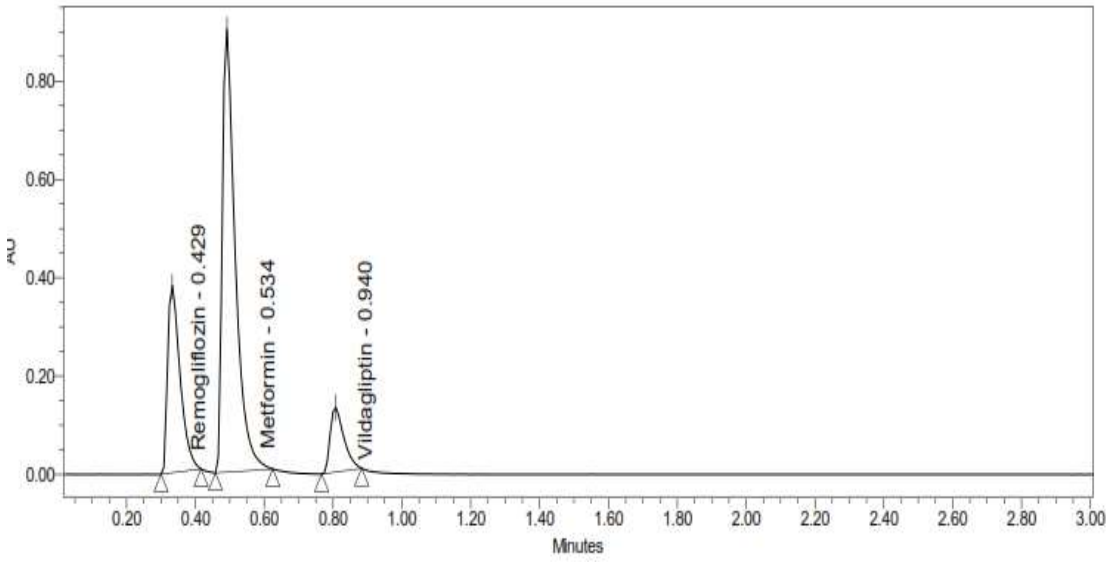


**Figure :1 Chromatograms - REM, VLD, & MEH**

## VALIDATION OF THE ANALYTICAL METHOD

### LINEARITY

The method linearity was verified with 5 dilutions of the 100% concentration: 50µg/ml, 75µg/ml, 100µg/ml, 125µg/ml and 150 µg/ml for remogliflozin and 250µg/ml, 375µg/ml, 500µg/ml, 625 µg/ml and 750µg/ml for metformin hydrochloride. And 25 µg/ml,37 µg/ml,50 µg/ml,62 µg/ml and 75 µg/ml for vildagliptin. The acceptance criterion of the regression coefficient ($R^2$) was NLT 0.99 as shown in table 1.

**Table 1: Linearity of REM, VLD AND MEH**

| REM | | VLD | | MEH | |
|---|---|---|---|---|---|
| **µg/ml** | **Area** | **µg/ml** | **Area** | **µg/ml** | **Area** |
| 50 | 1428645 | 25 | 939075 | 250 | 1870170 |
| 75.00 | 2151622 | 37.5 | 1393759 | 375.00 | 2820502 |
| 100.00 | 2871012 | 50 | 1867469 | 500.00 | 3768618 |
| 125 | 3590232 | 62.5 | 2334467 | 625 | 4710338 |
| 150 | 4317678 | 75.00 | 2808166 | 750 | 5669957 |

### SELECTIVITY

Metformin hydrochloride 500 ng/mL and remogliflozin 100 ng/mL were injected in triplicate preparations of 100% concentration to confirm the method validation's exactness and selectivity. To check for carryover, another blank was injected as shown in graph 2.

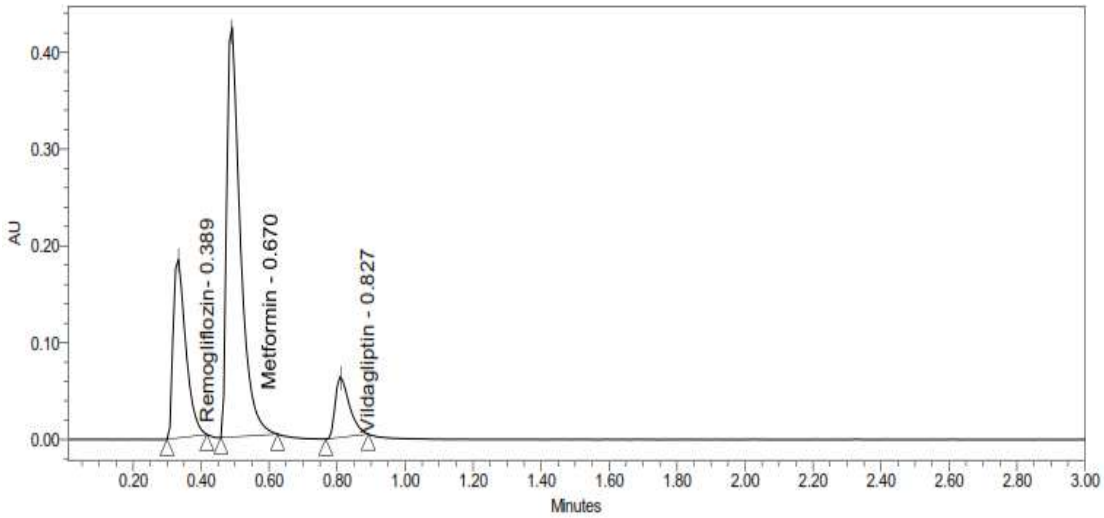


**Figure 2: Chromatograms - REM, VLD, & MEH selectivity**

### PRECISION:

After passing the specificity and system suitability criteria, the method was verified for system precision and method precision with the limit of % RSD for the RT and area NMT 2%. The intermediate precision was verified on the next day with another column by setting the limit as % RSD for the RT and NMT 2% for the area as shown in the table 2 and graph 3.

**Table: 2 Precision of REM, VLD AND MEH**

| REM | | VLD | | MEH | |
|---|---|---|---|---|---|
| **Area** | **Data** | **Area** | **Data** | **Area** | **Data** |
| 2877948 | Mean | 1861503 | Mean | 3760348 | Mean |
| 2870091 | 2879061 | 1879535 | 1868493 | 3771365 | 3766333 |
| 2888678 | SD | 1868574 | SD | 3765778 | SD |
| 2873609 | 7671.2 | 1863788 | 6219.3 | 3765331 | 4213.3 |
| 2875986 | RSD | 1868384 | RSD | 3764186 | RSD |
| 2888054 | 0.3 | 1869171 | 0.3 | 3770991 | 0.1 |

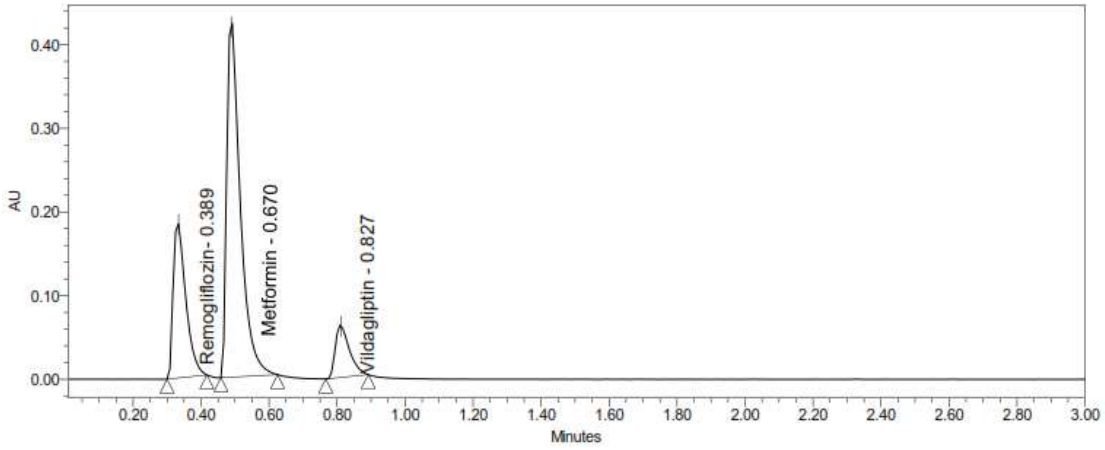


**Figure 3: Chromatograms - REM, VLD, & MEH selectivity**

**ACCURACY AND RECOVERY:**

To verify the method accuracy, triplicate preparations were prepared at 50%, 100%, and 150% of the 100% concentrations. (100 µg/ml for remogliflozin, 50 µg/ml for vildagliptin and 500µg/ml for metformin hydrochloride) The percent recovery was calculated with acceptance criteria of 95%-105% as shown in the table 3.

**Table 3: Accuracy and recovery of REM, VLD AND MET**

| S.NO | Drug Name | % of Concentrations | Add in (µg/ml) | Area |
|---|---|---|---|---|
| 1 | Remogliflozin | 50 | 49.5 | 1422423 |
| | | | 49.5 | 1431257 |
| | | | 49.5 | 1436080 |
| | | 100 | 99 | 2882003 |
| 2 | | | 99 | 2879440 |
| | | | 99 | 2877690 |
| | | 150 | 148.5 | 4315520 |
| 3 | | | 148.5 | 4313699 |
| | | | 148.5 | 4322146 |
| | | | | |
| 1 | Vildagliptin | 50 | 24.75 | 939618 |
| | | | 24.75 | 939319 |
| | | | 24.75 | 940555 |
| 2 | | 100 | 49.5 | 1872092 |

| | | | | |
|---|---|---|---|---|
| | | | 49.5 | 1869064 |
| | | | 49.5 | 1862787 |
| 3 | | 150 | 74.25 | 2814481 |
| | | | 74.25 | 2807805 |
| | | | 74.25 | 2803777 |
| 1 | Metformin | 50 | 247.5 | 1870330 |
| | | | 247.5 | 1875722 |
| | | | 247.5 | 1882941 |
| 2 | | 100 | 495 | 3769731 |
| | | | 495 | 3767081 |
| | | | 495 | 3775878 |
| 3 | | 150 | 742 | 5665164 |
| | | | 742 | 5676346 |

**LOD AND LOQ:**

By considering the 10% concentration of the target concentration, the sample was injected into the system with the acceptance criteria S/N ratio NLT 10. From the LOQ and LOD, preparations of different concentrations were injected to identify the detectability with the acceptance criteria 3:1 as shown in graph 4.

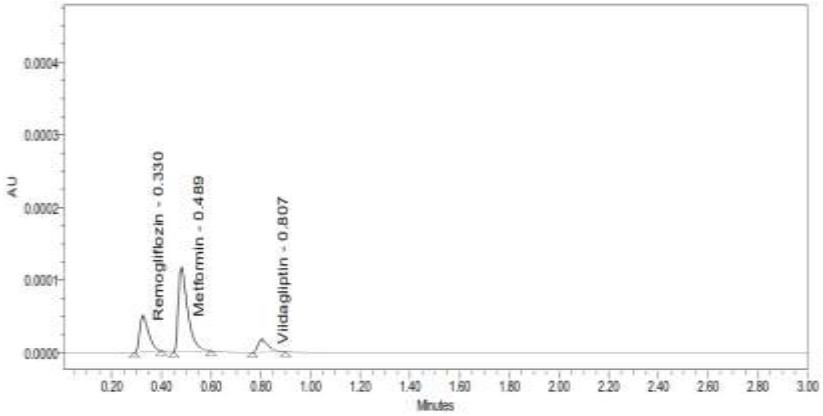


**Figure 4: Chromatograms - REM, VLD, & MEH sensitivities**

**ROBUSTNESS:**

To verify the method efficiency when minor changes occurred in optimized method parameters such as mobile-phase composition, column temperature and flow, and buffer PH. These parameters were tested with the criteria that they should pass the system suitability criteria as shown in graph 5.

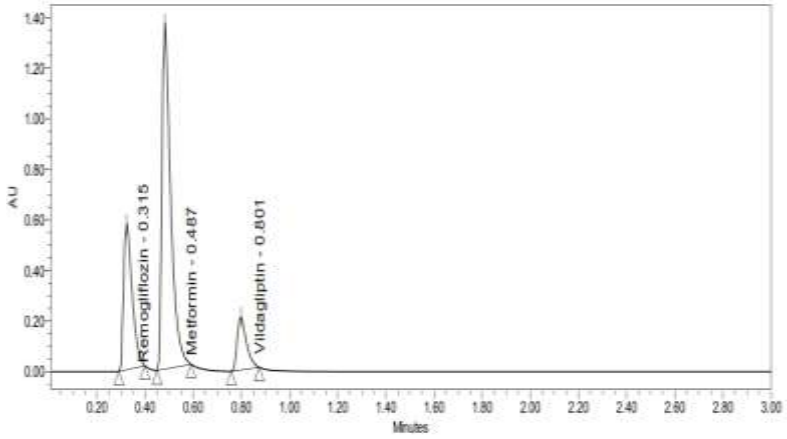


**Figure 5: Chromatograms - REM, VLD, & MEH robustness**

**DEGRADATION STUDIES:**

To test the developed method for stability indicating method the formulation sample was subjected to acid and base, and thermal, photo, and peroxide degradation were carried with the aim of detection of degradants in the chromatogram. Acid degradation was carried out by adding 10 mL of 0.1N HCL to the stock solution, and from that 1 mL was removed and added to a 1000 mL volumetric flask and the volume adjusted to the mark. In the same way, 6 mL 1N NaOH was added to test for base degradation. To test for thermal degradation, the sample was subjected to heat at 105°C for 3 hours and the sample prepared as per the assay procedure. For photo degradation, the sample was exposed to ultraviolet light with an intensity NLT 2000 lux power for 6 hours and the sample prepared as per the assay procedure. For peroxide degradation, 8mL $H_2O_2$ were added to the stock 1000 mL volumetric flask, 1 mL was removed and added to a 1000 mL flask, the volume adjusted to the mark with the diluent, and the sample was injected as shown in the table 4 and graph 6,7,8,9 and 10.

**Table 4: Degradation outcomes of REM, VLD, & MEH**

| S.no | Drug Name | Condition | Area | % Recovery | %Degradation |
|---|---|---|---|---|---|
| 1 | Remogliflozin | Acid | 2590632 | 89.37 | 10.63 |
| | | Base | 2712950 | 93.59 | 6.41 |
| | | Peroxide | 2655568 | 91.61 | 8.39 |
| | | Heat | 2615862 | 90.24 | 9.76 |
| | | Sun light | 2751275 | 94.91 | 5.09 |
| 2 | Vildagliptin | Acid | 1710901 | 90.66 | 9.34 |
| | | Base | 1791933 | 94.95 | 5.05 |
| | | Peroxide | 1764278 | 93.49 | 6.51 |
| | | Heat | 1658657 | 87.89 | 12.11 |
| | | Sun light | 1730808 | 91.71 | 8.29 |
| 3 | Metformin | Acid | 3411175 | 89.48 | 10.14 |
| | | Base | 3499230 | 92.18 | 7.82 |

| | | | | |
|---|---|---|---|---|
| | Peroxide | 3615080 | 95.24 | 4.76 |
| | Heat | 3320727 | 87.48 | 12.52 |
| | Sun light | 3539251 | 93.24 | 6.76 |

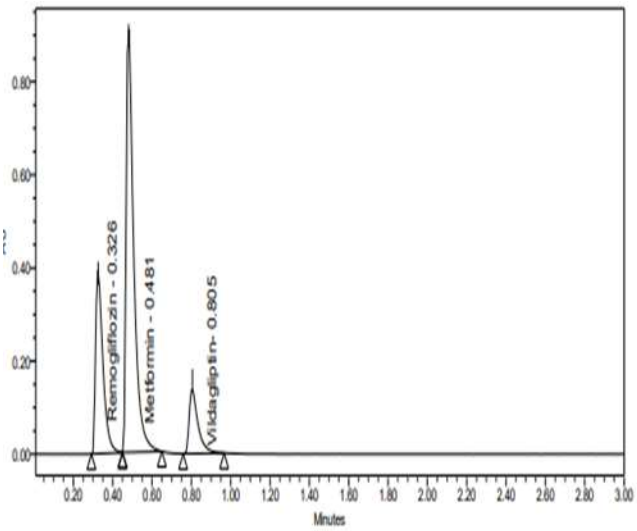


**Figure 6**

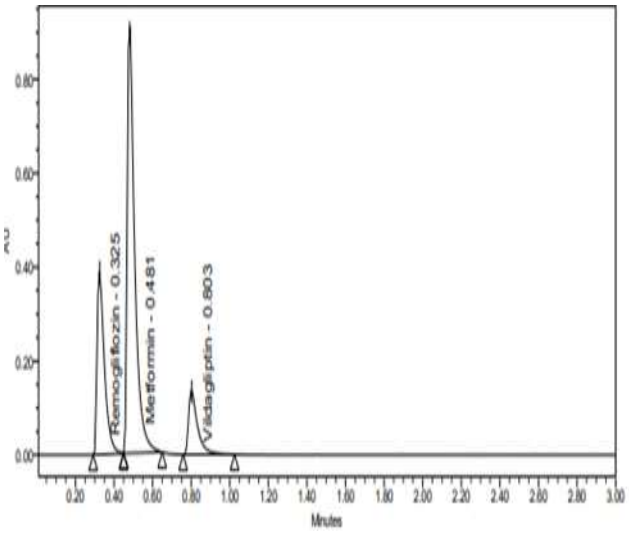


**Figure 7**

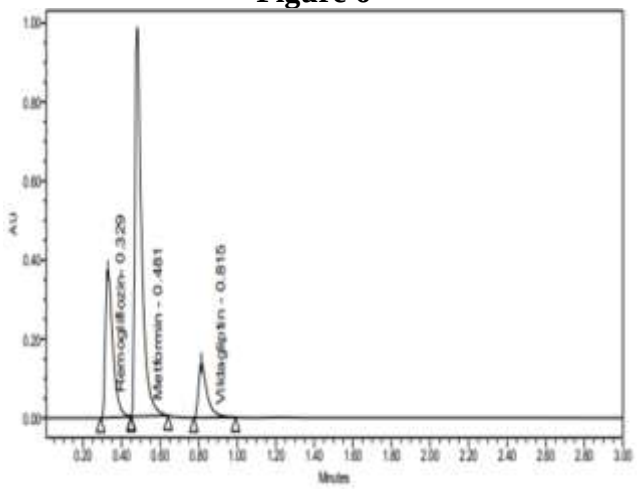


**Figure 8**

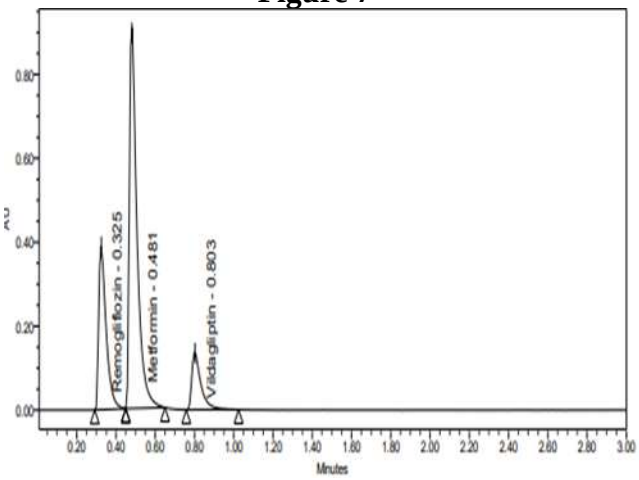


**Figure 9**

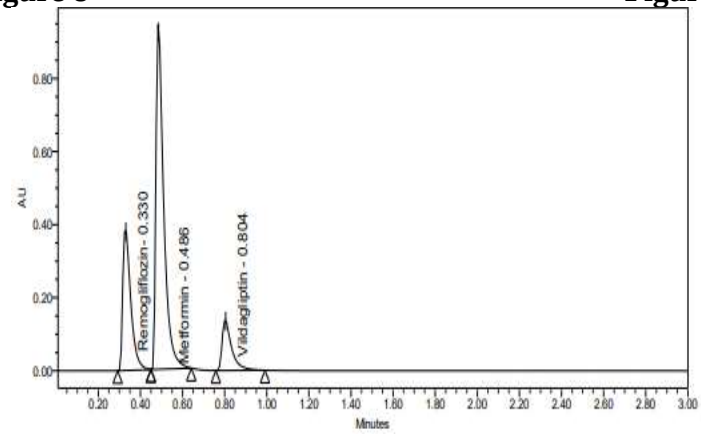


**Figure 10: Chromatograms (Acid, Base, Peroxide, Thermal and photo stability) REM, VLD, & MEH degradation**

## CONCLUTION

Based on the results obtained in the current study, the developed method was very sensitive, accurate, linear, and economical. Due to the short duration of the chromatographic program, more samples can be analysed within a short period, which will be helpful in the industry at a time when multiple products are manufactured continuously. The method met all the predefined acceptance criteria. With this method, the sample of bulk and formulation samples and surface cleaning samples can be analysed. As the method is capable of detecting degradants formulations, bulk shelf-life samples can also be analysed by using this method.

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