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Analytical Methods For Detecting And Quantifying Vildagliptin And Remogliflozin In Bulk And Combined Dosage Form And Their Potentiality

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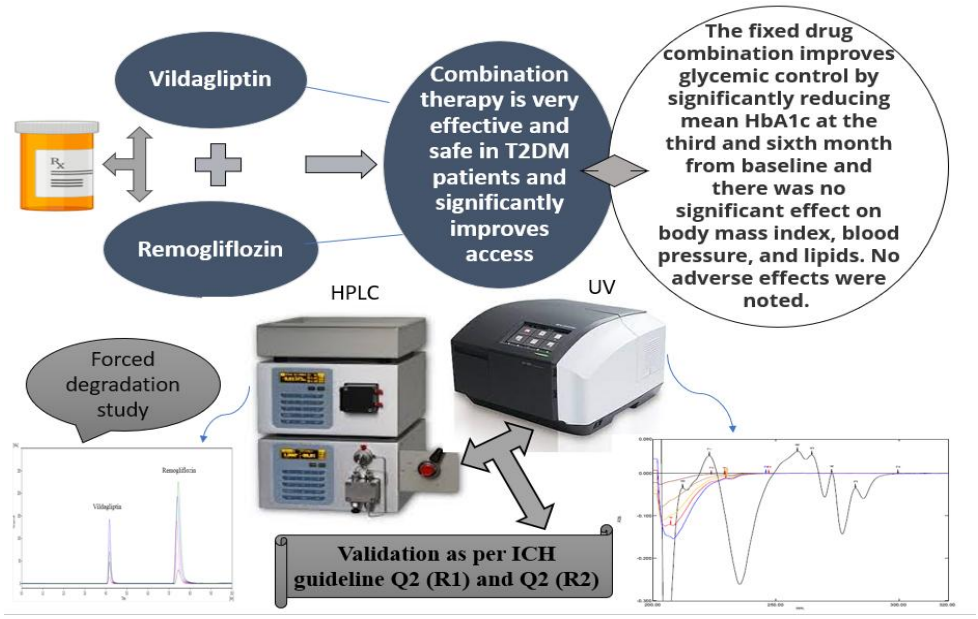
ABSTRACT
In reference to Q2 (R1 and R2) set of guidelines issued by the International Conference on Harmonization (ICH), a novel, straightforward, accurate, and specific Stability indicating Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) and First-order derivative Ultraviolet Visible (UV) spectrophotometric approach was established and validated. The separation of isocratic chromatography was possible. Considering it was discovered to separate the peaks more effectively, acetonitrile: phosphate buffer ratio of 60:40 percent volume by volume having pH 3.6 modified using 10% orthophosphoric acid was employed as mobility phase. Utilizing a Kromstar C18 (250*4.6 mm, 5 µm) column with a speed of flow 1.0 mL/min at 222 nm, the chromatographic separation was performed. The recommended dosage limits for vildagliptin and remogliflozin are 5-25 micro grams per milliliter and 10-50 ppm, subsequently. have been demonstrated to be linear for the methods. The two medications have been evaluated against pH-dependent, basic, oxidizing, photo reactive, and thermal decomposition. There were no appreciable differences between the outcomes of both of these methods, according to a statistical examination using the student's t-test. Vildagliptin and Remogliflozin were shown to be more vulnerable to heat and acidic degradation, correspondingly. These techniques were incredibly sensitive, exact, accurate, and efficient for reliable assessment when used together.

GRAPHIICAL ABSTRACT



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INTRODUCTION:

In 2014, 8.5 percent of people over the age of 18 had diabetes. 1.5 million individuals died from diabetes in 2019. In contrast to 0.98 percent of all fatalities in 1990, deaths associated with diabetes made up 3.1% of the total fatalities in India in 2016. Diabetes attributable 10 million of the 27.5 million disability-adjusted life years triggered by both diabetes in 2020 [1]. In the current situation, the death rate of hyperglycemic patients with cardiovascular and microvascular complications is higher than other disease. A number of clinical trials have found that in the treatment of type 2 diabetes mellitus (T2DM) individuals, Sodiumglucose co-transporter-2 (SGLT2) inhibitors, also known as gliflozins, (Remogliflozin) demonstrated a beneficial combination with dipeptidyl peptidase-4 enzyme blockers such as gliptins (Vildagliptin), improving quality of existence and lowering the chance of problems. Vildagliptin is a DPP-4 inhibitory agents, also known as (2S)-1-[(3-hydroxyadamantan-1-yl) amino] acetyl] pyrrolidine-2-carbonitrile. Glyco Lipoprotein-1 (GLP-1), a class of incretins that are digestive hormones secreted in reaction to food, is broken down by the DPP-4 enzyme. It increases the responsiveness of islet cells in the pancreas to blood sugar [2–4]. Remogliflozin, 5-methyl-4-[4-(1-methylethoxy) benzyl]-1-(1-methyl ethyl) Ethoxycarbonyl-1H-pyrazol-3-yl 6-O-Non-alcoholic steatohepatitis (NASH) and type 2 diabetes (T2DM) are two conditions for which -D-glucopyranoside is a recommended medication [5,6]. The sodium-glucose transportation proteins, whose function is in charge of reabsorbing sugar in the renal system, are inhibited by it. Blood sugar is excreted via urine when this pathway is blocked [7]. Individually, Vildagliptin is available in tablet dosage form in the market as well as in combination with metformin and with other drugs but Vildagliptin and Remogliflozin combination therapy is very effective and safe in T2DM patients and significantly improves access. It's well-researched combined dosage form provided a reasonable cost to Indian patients [8]. The Drugs Controller General of India (DCGI), India's pharmaceutical regulatory body, recently granted approval for this combo of medications when metformin and the fixed- dosage combinations individual components are insufficient for controlling blood glucose levels or whenever Vildagliptin and Remogliflozin were previously administered in different doses [9]. Vildagliptin and Remogliflozin were analyzed using a variety of techniques, including spectroscopic techniques like UV [10,11] and mass spectroscopy (MS), as well as chromatographic approaches like high-performance liquid chromatography (HPLC) [12–17] according to the extensive scientific review. HPLC stability [18,19]; HPTLC (High-Performance Thin Layer Chromatography) [20,21] have been documented both separately and as a distinct class of mixtures like Vildagliptin alone Vildagliptin and Glimepiride [22,23], Metformin and many more [24]. Remogliflozin and Vildagliptin cannot be estimated simultaneously because no technique has been developed and validated for this. With the goal to simultaneously estimate Vildagliptin and Remogliflozin in conjunction, the current study sought to develop and assess first-order derivative UV spectrophotometric methods and stability indicating RP-HPLC technique**.**

EXPERIMENITAL:

Experimental:

Chemical compounds and Substances

Vildagliptin and Remogliflozin became available from Torrent Research Pharmaceuticals Ltd. in Ahmedabad as gift samples. Finar Chemicals, Ahmedabad provided the HPLC-grade methanol, acetone, and water that had been utilized. We bought 75 percent AR grade orthophosphoric acid and potassium dihydrogen phosphate from India's Astron Pharmaceuticals Company Limited. Every day, fresh remedies originated for every problem.

Scientific instrumentation and its tools

Reversed phase liquid chromatography procedure was successfully carried out using the Clarify® software, a Rheodyne injector tool for injection with twenty µl cartridge, a Systronic liquid chromatography 138 for RP-HPLC included a UV detector. Reverse phase techniques were employed during the method's execution. Using a 60:40 percent v/v ratio of acetonitrile to a phosphate buffer mixture (pH 3.6 amended using ten percent ortho phosphoric acid) along with an inflation speed of 1 milliliter per minute, both medicines were isocratically eluted. The UV-vis Detector's detecting wavelength has been configured at 222 nm. Prior being used, preparations comprising mobile phase were made each day and filtered via a Sonicator (Equitron, India) and 0.45 µm Millipore filtration using membranes. Both a Systronics® pH meter and a Column Kromstar® C_{18} having 250 mm length, 4.6 mm diameter, 5 µm particle size were utilized. At 25 1°C ambient humidity, the high-performance liquid chromatography setup had operated.

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Shimadzu Ultraviolet visible model 1900 dual beams spectrometer and quartz cells (1.0 cm) were used for the UV Spectrophotometric technique. All tests of absorbance were performed using UV Probe 2.7 version software. Balancing was done throughout on a Wensar Dab 13-220 digital analytical balance.

Development of Reference solution

Vildagliptin and Remogliflozin standard were assigned and accurately measured 10 mg each before being fully liquified in 100 millilitre methanol. Vildagliptin and Remogliflozin had been dissolved in a solution that contained 100 µg/ml.

Sample Solution creation

5 milligram of Vildagliptin and Remogliflozin 10 mg have been precisely balanced at equal amounts with introduced to 100 millilitre vol. flask before being partially reconstituted with methanol. Methanol was added to this solution and it had been sonicated until the medication disintegrates. Using Whatman filter paper, the resulting mixture was screened. The concentrations of vildagliptin and remogliflozin were 50 ppm and 100 ppm, consequently. To make highest possible concentration 10 parts per million of Vildagliptin and 20 ppm of Remogliflozin, 2.0 ml of the aforesaid mixed solutions were placed into 10 milliliter Vol flask and quantity raised up to marked with mobile phase.

Detection wavelength chosen:

Both the drugs 10 ppm Vildagliptin and 20 µg/ml for Remogliflozin have been tested to recognizing spectrum. These solutions were scanned, and the spectrum of their 200–400 nm wavelength were captured versus a blank reagent composed of methanol.

RP-HPLC Method:

The right choice of the detection wavelength determines the degree of sensitivity of the UV-detection RP-HPLC process. When measured at 222 nm, Vildagliptin and Remogliflozin showed excellent shape and impressive resolution. Therefore, 222 nanometer was chosen as the wavelength of interest for additional research. Vildagliptin (10 ppm) and Remogliflozin (20 ppm) coupled UV spectra [zero order (D_0)] in methanol are displayed in Figure 8.

UV (First order derivative) technique:

Aspects full zero-degree spectrum (D_0) was converted to a first differential spectrum (D_1) employing δ lambdas 2.0 and the scaling rate 4. Vildagliptin and Remogliflozin first derivative spectra at various concentrations had been observed. Vildagliptin's and Remogliflozin's zero-crossing points (ZCPs) were discovered at 224 and 246 nanometer. The 10 ppm Vildagliptin and 20 ppm Remogliflozin beneath UV spectrum in methanol (first sequence D1) are displayed in the two figure 6 and 7.

Technique Assessment:

The procedures were approved in accordance with ICH guideline Q2 (R1) [28]. The approach has undergone rigorous validation in terms of tests for system appropriateness, robustness, accuracy, precision, detection limit, linearity and range, accuracy, and precision.

Specificity

To confirm deterioration as well as disruptions, sample solutions containing 10 ppm of Vildagliptin and Remogliflozin 20 ppm were produced and introduced into a Chromatographic injector for testing. Vildagliptin and Remogliflozin impedance was examined when analyzing pharmaceuticals using blank chromatogram.

Linearity and Range:

The Calibration curve The combination of Vildagliptin levels ranging from 5-25 µg/ml and Remogliflozin strengths of 10-50 µg/ml (n=6) were used to generate the test curves with respect to RP-HPLC and UV methods.

The portions of the original solutions 0.5, 1.0, 1.5, 2.0, and 2.5 ml of Vildagliptin and Remogliflozin 1.0, 2.0, 3.0, 4.0, and 5.0 ml were aliquots was filled in 10 ml from 100 µg/ml of both drugs reference. Vildagliptin and Remogliflozin were further dissolved using solvent to reach various concentrations, including 5, 10, 15, 20, and 25 ppm for Vildagliptin and 10, 20, 30, 40 and 50 ppm for Remogliflozin. Through use of sloped lines, correlation factor, and interception, linearity was assessed.

Accuracy:

According to ICH guidelines, a recovery study of both analytical techniques were carried out to assess accurateness across various concentration levels such as 50%, 100%, and 150%. Vildagliptin 10 ppm and Remogliflozin 20 ppm solutions have been taken as 100% analyzable. This performance was duplicated three times. The accuracy was calculated using the conventional addition approach as a percentage of recovery.

Precision:

The level of precision investigations of both techniques were conducted through the following phases: repeatable, reproducible, and intermediate. For shorter-term preciseness, mixtures

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comprising 5, 10, and 15 micro grams per milliliter regarding Vildagliptin and 10, 20, and 30 micro grams per milliliter Remogliflozin have been assessed on three separate occasions during precise same day. Strategies holding 10 ppm of Vildagliptin and Remogliflozin (20 µg/ml) have been analyzed on six separate occasions to gauge repeatability, whereas 3 successive days were used to analyze formulations featuring 5, 10, and 15 micro grams per milliliter Vildagliptin and 10, 20, and 30 ppm about Remogliflozin. Every result has been reported as a percentage R.S.D.

Detection Limit (DL) and Quantitative Limit (QL):
According toward ICH recommendations, detection and quantification limits for the RP-HPLC and UV methods had been determined using this equation.
Limit of detection = 3.30 × (σ/S); Limit of quantification: 10 × (σ/S); Where, σ = standard variation, and S stands for mean slope of calibration curve.

Robustness :
Through sample analysis were done at multiple conditions, such as identification wavelength change within ± 2 nm, 0.2 ml/min change in flow rate, and 2% v/v change in proportion of the mobile phase. Durability of Liquid chromatography technique had been assessed thrice times.

System suitability tests :
Resolution and repeatability of the chromatography system were confirmed employing a framework appropriateness evaluation like no. of theoretical plates, resolution, column efficiency, and asymmetry factor).

Investigations on forced degradation:
By conducting tests on forced deterioration, selectivity was evaluated. 10 ppm of Vildagliptin and 20 parts per million of Remogliflozin were merged to be sample and constrained in a variety of circumstances, including acid, alkaline, oxidative, photo, thermal, and others [29–32]. Both drugs were essentially soluble in mobile phase, and which is why it was used for the entire investigation.

Acid degradation:
Acid degradation: Exactly 1 ml of the test remedy had been pipetted from the sample into volumetric flask with a capacity of 10 milliliters. Each of the flasks was filled with 1 cc of 0.1 normal HCl and stored on forty degrees Celsius for two hours. Each of the vessels had been neutralized by adding one milliliter within 0.1 normal NaOH, which had been subsequently concentrated alongside a solution of two content. Filter the solution using membrane filters with a 0.45-micron pore size, inject it into the chromatography, and then capture the chromatogram.

Base Destruction:
Precisely Pour One milliliter of test mixture into 10-milliliter volume flask using a pipette. Each flask was felled through 1 cc of 0.1 normal sodium hydroxide and maintained at fourty ounce calcium degree for two hours. Each flask received one milliliter of HCl (0.1 N), which was then dissolved using methanol to the desired volume to neutralise. Prior to introducing the mixture to chromatography, purify it utilizing 0.45-micron filter media to keep track of a chromatogram.

Degradation by oxidation:
Exact examining the blended mixture, precisely 1 milliliter of the sample mixture was assigned to ten milliliter capacity bottle. Individual flask contained with 1 cc of 3% hydrogen peroxide and heated to 40 ˚C for 2 hours. Filter the solution using membrane filters with a 0.45-micron pore size before injecting it into hplc and gather its chromatogram.

Photolytic degradation Corrosion by photolysis:
For two hours, medication were made vulnerable to intense UV rays in a photo stability chamber. To ascertain the degree of drug destruction, the medications were extracted, suitably diluted, and then introduced onto liquid chromatography at various time frames.

Degradation by heat*:*
One milliliter of the experiment test was carefully drawn out the mixture to ten milliliter vol. flask, and it was then heated to 80 degrees Celsius for two hours. To find out how much medications have degraded, fill the chromatography column with methanol at various frequencies to reach desired volume.

Student t-test Statistical assessment of both Ultraviolet and RP-HPLC techniques
Aforementioned equation was used to calculate the student's t-test:
$t=(\bar{x}_1-\bar{x}_2)/\sqrt{((S^2\ (1/n_1+1/n_2)))}$
wherever, s2 represents total average deviation for two tech groups, n1 and n2 are shown total no. of measurements in every set, test value as t , and x1 and x2 are average of HPLC and UV, correspondingly. [33, 34].

RESULTS AND DISCUSSION:
Development and validation for HPLC Technique:
Vildagliptin and Remogliflozin were both subjected to creation and verification of high-performance liquid chromatographic technique. C_{18} column (Kromstar) was taken as fixed segment. For choice of movable stage, a variety of fluids in varying ratios, such as methanol: water, acetonitrile: water, and acetonitrile: phosphate buffer, have been

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investigated, precisely an outcome, acetonitrile and potassium dihydrogen phosphate buffer mixture ratio 60:40 percent volume by volume with pH 3.6 had been chosen, and the moving phase was optimized according to peak parameters that adhere to desirable system compatibility parameters such as appropriate migration, separateness, and resolution at one milliliter per minute movement rate.

Spectrum analysis in checking mode across a UV area of 200-400 nm was performed for estimation of wavelength findings, and spectras were taken using a UV Spectrophotometer Shimadzu-1900 and UV probe 2.7 version software. The medication was noticeably absorbed in 222 nm, according to the overlain spectra. Consequently, it was chosen as the point of detection, as seen in Figure 1.

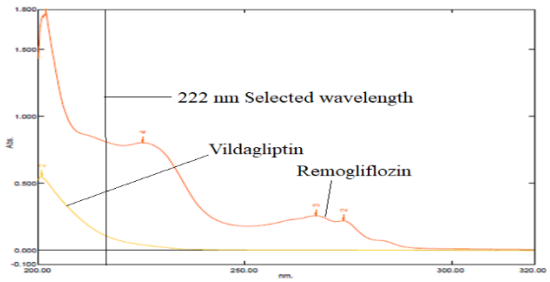

Fig. 1. UV Spectra of Vildagliptin (10 µg/ml) and Remogliflozin (20 µg/ml).

Vildagliptin & Remogliflozin's respective time to retention of 4.1 and 7.2 minutes allow for a quick assessment of the medicines, which is crucial for regular analysis. Table 1 contains the findings of the framework's compatibility metrics.

Table 1: Criterion for system appropriateness

Parameters	Retention Time (min)	Tailing Factor	Number of Theoretical plates	Resolution
Vildagliptin	4.1	1.0	7432	6.8
Remogliflozin	7.2	1.2	13425	
Abbreviation: Rt: Retention time (min).				

For specificity, the chromatograms of Blank, Remogliflozin, and Vildagliptin revealed no signs of interference, indicating that the RP-HPLC procedure proved specific. Eluted and creating symmetrical peaks, Vildagliptin and Remogliflozin found also well away from the liquid front. As seen in Figure 2, the proportionality was achieved at concentrations of 5-25 ppm for Vildagliptin and 10-50 ppm for Remogliflozin.

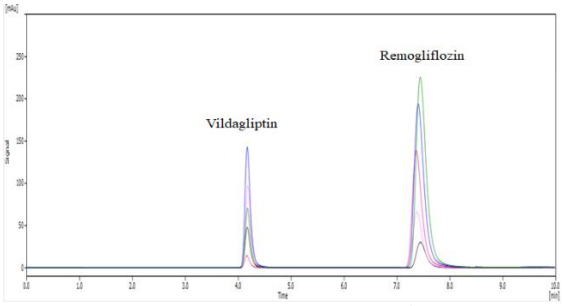

Fig. 2. Overlain RP-HPLC chromatogram of Vildagliptin (5-25 µg/ml) and Remogliflozin (10-50 µg/ml) at 222 nm

Plot of mean peak area versus dosages conc constructed measurement contour. From calibration arc, straight line mathematical equations had been derived. Vildagliptin's linear regression formula stated r = 0.9983 over y = 134.84x - 289.97 and for Remogliflozin, the correlation coefficient is 0.999 within regression equation y = 26.814x - 146.52 gave extremely vital results which disclosed in Table 2.

Table 2: Regression analysis of data of Vildagliptin and Remogliflozin

Statistical parameters	HPLC Method		UV Method	
Parameters	**Vildagliptin**	**Remogliflozin**	**Vildagliptin**	**Remogliflozin**
Concentration range (µg/ml)	5-25	10-50	5-25	10-50
Wavelength (nm)	222 nm		224 nm	246 nm
Regression equation (y = mx +c)	y = 134.84x -289.97	y = 26.814x - 146.52	y = 0.0006x - 0.0004	y=0.0003x + 0.0003
Correlation coefficient (r)	0.9983	0.999	0.9972	0.9956

HPLC: High performance liquid chromatography; UV:Ultra-violet visible spectroscopy

Vildagliptin with Remogliflozin were shown to have recoveries level in percentage ranging from 99.57 to 99.88% and 99.74 to 99.84%, for each. According to outcomes, satisfactory sensitivity was attained, demonstrating delineation strategies' superior effectiveness.

Vildagliptin along with Remogliflozin's intraday, interday, and repeatable precision has been recorded in safe ranges and given as a percentage of the mean variance. This result signifies the process is precise and accurate.

Vildagliptin's detection and quantification values turned out 0.1809 ppm and 0.603 ppm, correspondingly, for Remogliflozin 0.1407 ppm and 0.469 ppm as well, both of which were both within the safe limits at 222 nm. Vildagliptin and Remogliflozin's % assays were discovered to be 99.95 and 100.17 percent, respectively.

Minor but intentional modifications have been

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implemented to the approach's variables, including mobile phase ratio, The longueur of the detection device and speed of flow, to carry out robustness investigation. Robustness had been assessed across an extensive number of circumstances, including ± 0.2 milliliter per minute change in rate of flow, ± 2 nanometer wavelength variation, ± 2 percent vol by volume ratio difference to mobile phase, with outcomes represented as a percentage of RSD. The Robustness data showed that method was robust.

UV Method:

Vildagliptin and Remogliflozin combination were simultaneously estimated using a UV spectrophotometric approach that was designed and verified to be trustworthy, precise, and accurate. Between 200 and 400 nm, 10 and 20 ppm of Vildagliptin and Remogliflozin, respectively had been investigated. The measured wavelengths of Vilda and Remo constituted at 246 and 224 nanometer as their detection wavelength (λ). Each of the measurements took place at these specific wavelength. UV spectra of Vildagliptin's (10 ppm) & Remogliflozin's (20 ppm) were generated. Figures 3 and 4 showed the consistency of the linear range.

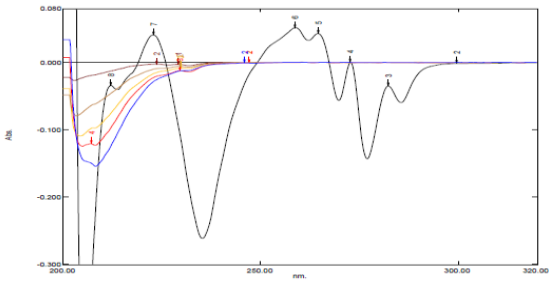

Fig. 3. Overlain UV Spectra of Vildagliptin (5-25 µg/ml) at 224 nm.

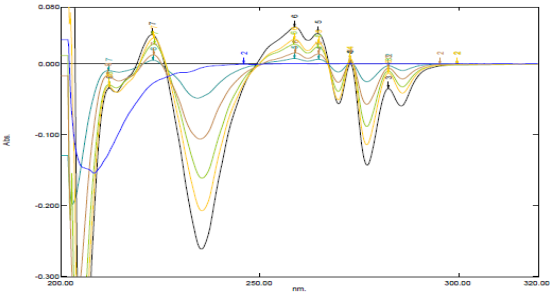

Fig. 4. Overlain UV Spectra of Remogliflozin (10-50 µg/ml) at 246 nm.

Beer's theory was followed across a range of concentrations of 5-25 micro grams per milliliter for Vildagliptin and from 10 to 50 ppm for Remogliflozin, according to calibration contours that were created. Vildagliptin's linear predictive formula (coefficient of linkage) is y = 0.0006x - 0.0004 with 0.9972 at 224 nanometer, while Remogliflozin's is y = 0.0003x + 0.0003 having 0.9956 at 246 nm (Table 2). The two drugs Vildagliptin and Remogliflozin were reported to have recovery rates of 99.57 to 99.88% and 99.72 to 99.84%, accordingly. The findings obtained fall within guidelines that are appropriate. Vildagliptin in addition Remogliflozin's intraday, interday, and repeatability precision were reported in normal ranges and given as a percentage RSD. This outcome as implemented technique is accurate and precise. The precision facts of Vildagliptin & Remogliflozin showed that method were precise.

Detection and Quantitation level of Vildagliptin & Remogliflozin had been originated 0.602 ppm and 1.986 ppm at 224 nm & 0.638 ppm and 1.958 ppm at 246 nm, respectively it fell inside the permissible bounds. The effectiveness within assay of Vildagliptin and Remogliflozin test percentages were 99.60 and 99.77 percent, to be exact.

Forced Degradation Studies:

Acid-base hydrolysis, stress from oxidative degradation, photo degradation, and dry heat degradation were all used to execute enforced deterioration. The devised approach was used to analyse the deteriorated specimens. The peak areas for standard Remogliflozin and Vildagliptin were determined to be 319.484 and 954.262, subsequently. Employing equation 1.4, the percentage destruction of Vildagliptin and Remogliflozin was determined.

% degradation=100-((Degradation area)/(Standard area))×100

Investigation of acidic hydrolysis:

When exposed to 0.1 normal concentrated HCl at forty degrees Celsius for two hours, mixture demonstrated enough deterioration. Vildagliptin showed 9.83 percent and 12.67% degradation at 1 and 2 hours, distinctly; whereas 9.78 percent and 19.50 percent degradation for Remogliflozin at 1 and 2 h.

Basic hydrolysis context:

Using 0.1 normal sodium hydroxide treated through forty °C, enough decomposition took place in 2 hours, much like with acidic solutions. At 1 and 2 hours, Vildagliptin shown 5.28% and 9.57% deterioration correspondingly, while Remogliflozin demonstrated 6.73 percent and 12.86 percent loss.

Oxidative deprivation framework:

Upon completion conditioning via three percent hydrogen peroxide at ambient temperature, depreciation became apparent after two hours. Remogliflozin demonstrated 3.84 & 9.32 percent deterioration at the first and second hours, correspondingly, while Vildagliptin shown 1.56 and

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2.96 percent loss at same times.

Analysis of photolytic decline:
For two hours, substances were subjected to intense ultraviolet radiation. At both one and two hours, Vildagliptin shown 7.35 and 11.98% decomposition subsequently, while Remogliflozin demonstrated 8.94 and 18.97% degraded.

Investigation of temperature deterioration:
For two hours, pharmaceuticals underwent exposure to heat at 80 ºC. Remogliflozin demonstrated 5.49 and 10.11 percent deterioration at one and two hours, specifically, while Vildagliptin demonstrated 9.46 and 13.87 percent depreciation at equivalent times.

Statistical Evaluation of Analytical Methods:
A statistical approach was used to differentiate between the proposed analytical approaches. There were no discernible differences among the quantities measured acquired in the subject matter evaluation through the two separate procedures based on student t-test findings. At the 5% significance level, the estimated t-value (from formula) proved less than the critical t-value (from the statistics database).

CONCLUSION:
On account of regular testing of Vildagliptin and Remogliflozin, both RP-HPLC and UV spectroscopy techniques were devised and proven to be straightforward, fast, stability indicating, specific, accurate, and precise. These medications were forced to degrade under a variety of stressful situations. When used separately for concurrent estimate without any interference, the offered approaches were appropriate and effective. The ICH guideline Q2 (R1) was followed for validating the suggested approaches. All outcomes were discovered to meet the standards for acceptance. The suggested approaches can therefore be modified for routine surveillance of quality analysis. A statistical analysis demonstrated the repeatability and selectivity of the suggested methodologies for the combined assessment of Vildagliptin and Remogliflozin.

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CONFLICT OF INTEREST:
There is not any conflict between the interests of the authors.

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