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STABILITY INDICATING ISOCRATIC RP-HPLC AND SECOND DERIVATIVE UV SPECTROSCOPIC METHODS FOR SIMULTANEOUS DETERMINATION OF REMOGLIFLOZIN ETABONATE AND VILDAGLIPTIN HYDROCHLORIDE

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ABSTRACT

Remogliflozin and Vildagliptin are the latest drug combination in order to treat type 2 diabetes. The present work describes a comparative study of 2nd-ordered derivative UV and isocratic stability indicating RP-HPLC methods for simultaneous determination of Remogliflozin and Vildagliptin in the drug and its formulation. The HPLC method was established with a C18 column and the mobile phase with Buffer (pH-6.50): Acetonitrile: Methanol ratio of 55:44:1, at 1.2 ml/min flow and wavelength of 210 nm. The UV spectrophotometric method works on spectrophotometry with 2nd order derivative spectra(2D) using a zero-line cross method. The absorbance determination was performed at 246.0 nm for REM and at 219 .6 nm for VIL. The methods were validated for Linearity, Precision, Recovery, Robustness, and Forced degradation as per ICH-Q2(R1). The linear range was established in the range of 4-80 ppm for REM and 2-40 ppm for VIL for the UV method and 2-200 ppm for REM and 1-100 ppm for VIL for the HPLC method with regression coefficient ≥0.990. The accuracy was 99.37% & 99.62% for UV and 100.98 & 100.65 for the HPLC method for REM and VIL respectively. The proposed methods have been effectively used for the quantification of REM and VIL in API mixture and formulation dosage forms. Both methods are economical, fast, simple, and accurate which can be easily adopted for laboratory use.

Keywords: Remogliflozin, Vildagliptin, HPLC, UV, Validation, Forced Degradation.

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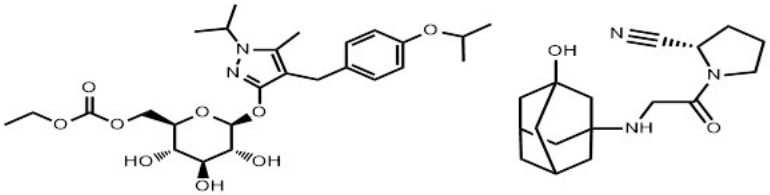
INTRODUCTION

Type-2 diabetes is now a very common disease. 10% of the total population and 25% of adults up to the age of 65 years of the population are suffering from this type of hyperglycemia. The death rate was higher in COVID-19-infected patients having diabetes.[1-4] Maintaining a stable blood glucose level is crucial in order to avoid complications and organ failure. Several medications are being studied to maintain normal blood glucose levels and achieve low Hb1AC levels.[5-8] Remogliflozin Etabonate (Fig.-1) is the newest accumulation of the recently approved SGLT2 inhibitor for type-2 diabetes. Remogliflozin Etabonate, the prodrug of Remogliflozin is a selective sodium-dependent glucose transporter 2 (SGLT2) inhibitor that enhances glycemic management through increased urine glucose excretion. The sodium-glucose co-transporter subtype 2 is the only target of the inactive prodrug's selective action after administration and absorption, which transforms it into the active form Remogliflozin (SGLT2).[9-11]



Remogliflozin Vildagliptin

Fig.-1: Structures of Remogliflozin Etabonate and Vildagliptin

Vildagliptin, (Fig.-1) formerly known as LAF237, is a dipeptidyl peptidase 4 (DPP-4) inhibitor with hypoglycemic action based on cyano-pyrrolidine. The cyano moiety of Vildagliptin is hydrolyzed, and the

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resulting inactive metabolite is primarily eliminated through the urine.[12] The combination of VIL and REM improved glycemic control and provided additional benefits.[13] The analytical method is an essential part of any organization to evaluate the exact content of the formulation. Few methods are reported for Remogliflozin and its combinations with other drugs, but all have a narrow linear range.[14-25] Based on this, the study's goal was to develop fast and economical methods to quantify the newest combination of Remogliflozin with Vildagliptin by different techniques and with accurate results.

EXPERIMENTAL

Chemicals and Reagents

Marketed formulation (REMO-V) procured from the local medical store. Remogliflozin and Vildagliptin API standard samples were received as gifts from Glenmark Life Science Pvt. Ltd. Acetonitrile (AR grade) and Methanol (AR grade) from SD fine Chemical purchased from a local supplier. The combination drug from the market REMO-V tablet (Remogliflozin Etabonate - 100 mg and Vildagliptin Hydrochloride – 50 mg) was used.

Instrumentation

Spectrophotometric experiments were made using a UV-Vis double beam spectrophotometer, model number UV-1800, with a slit width of 1 nm and UV probe 2.4 data processor software installed. Ultra-bath sonicator (4.5 Litre, PCI analytics) was utilized to properly combine and sonicate the stock solution. Analytical Balance (Mettler Toledo, XS205) was used for weighing. The Shimadzu model LC2010CHT HPLC system was used to conduct the analysis. Phenomenex Luna C18 (2) column (4.6mmx 250mm, 5µ) was used for separation. Chromleon 6.8 software was used to capture and analyze chromatographic results. All the equipment and apparatus were calibrated and verified.

HPLC Conditions

Wavelength was selected as 210 nm based on zero-order spectra of REM and VIL, which were obtained by scanning the 10ppm solution of each REM and VIL. LUNA C18(250 x 4.6 mm and 5µ, Agilent Eclipsed XDB-C18 (150 x 4.6mm,5µ) and Unisphere' Aqua C18 (4.6 x 150 mm, 3µ) columns ware explore during experiments and LUNA C18(250 x 4.6mm, 5µ) of Phenomenex brand was selected. 0.01M Na_2HPO_4 was used for buffer preparation and the pH of the buffer was adjusted from 2.5 to 7.0 with ortho-phosphoric acid and mobile phase composition was set for a different ratio of buffer with acetonitrile and methanol. The oven temperature for the column was tried at 40°C to get a sharp peak shape. The final optimized method was validated for the combination of REM, and VIL. For buffer preparation 0.01M Na_2HPO_4 was prepared, and the pH of the buffer solution was set to 6.5 by ortho-phosphoric acid. Phosphate Buffer, acetonitrile, and methanol were combined having a ratio of 55: 44: 1 to create the mobile phase. This phase was filtered from Millipore filter paper (0.45 µ). Luna C18 (2) Column (4.6 mm x 250 mm, 5µ) was selected to get separate peaks for analysis. The column oven temperature was kept at 40°C. 1.2 ml/min flow rate was kept and 210 nm wavelength was set for the detector channel. The volume of injection was adjusted to 20 µL. Autosampler temperature was set at 10°C for better solution stability.

UV Conditions

The standard solutions containing 10 ppm of REM and VIL were prepared and scanned between 400-200 nm. Lambda-max, zero-order spectra, First-order spectra, and 2nd order spectra were obtained from the scanned data. Zero order spectra of REM and VIL overlay on each other, and responses interfered hence 1st order and 2nd order spectra were considered. First-order spectra also interfered, and no zero-line crossing was observed. In 2nd order spectra at 219.6 nm and 246.0 nm, the REM and VIL had zero crossing points, respectively. To determine the individual absorbance wavelengths of 246.0 nm for REM and 219.6 nm for VIL were chosen (Fig.-2). From initial trials, experimental conditions were optimized to get the final conditions. AR-grade Methanol and HPLC-grade water were used as diluents. Scanning was done from 200-300 nm with fast scanning speed. Slit Width was set at 1.0 nm. Absorbance was measured from 200-300 nm and data transformed to 2nd derivative spectra at a 219.6 nm and 246.0 nm for VIL and REM respectively using 8.0000 Delta Lambda and 20.0000 scaling factor. The REM and VIL had no points of crossing at 219.6 nm and 246.0 nm respectively. Thus, wavelength frequencies were chosen as 219.6 nm for VIL and 246.0 nm for REM.

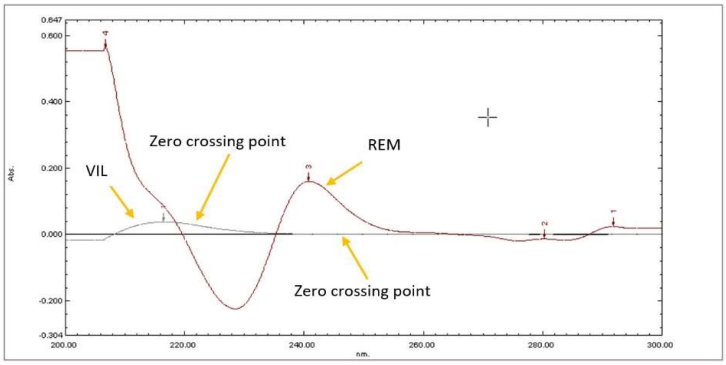

Fig.-2: Overlay Spectra of 2nd Order Derivatives of REM and VIL

Standard and Sample Preparation for UV Method

Twenty tablets containing 50 mg VIL, and 100 mg. were crushed to a fine powder. AR-grade Methanol and HPLC-grade water were used to make a standard laboratory solution for UV analysis. The stock solutions were prepared by dissolving 10mg of each standard drug in methanol and diluting to 10 ml separately. 2 ml of VIL stock solution and 4 ml from REM stock solution were diluted to 100 ml in water and the final standard solution obtained containing 20 ppm of VIL and 40 ppm of REM. The sample solution was made by dissolving a synthetic mixture and drug powder in AR-grade Methanol. Weighed samples conforming to 50 mg of VIL and 100 mg of REM in a 50ml flask with 30ml methanol, kept for 15 minutes for sonication and diluted to 50ml using methanol. Filtered the above solution from whatman filter paper .2ml of the above solution was diluted to 100 ml with water which contained 20 ppm VIL and 40 ppm REM.

Standard and Sample Preparation for HPLC Method

HPLC-grade water and AR-grade acetonitrile at 70: 30 ratio were used as diluents for analysis in the HPLC instrument. The stock solutions were prepared by dissolving 50mg VIL and 100 mg REM in a diluent and diluted to 100 ml separately. 5 ml from each was diluted to 100 ml in diluent and the final standard solution was obtained containing 50 ppm of VIL and 100 ppm of REM. Weighed samples equal to 50 mg VIL and 100 mg REM in a 100ml flask having 50ml methanol, kept 15 minutes for sonication and diluted up to 100ml with methanol. 5 ml of the above solution, diluted to 100 ml with diluent which contained 50 ppm of VIL and 100 ppm of REM. Method evaluation parameters were performed according to ICH guideline Q2(R1) with above mentioned finalized instrument conditions.[26-31]

RESULTS AND DISCUSSION

Linearity

The proposed methods were tested for linearity for a variety of analyte concentrations in the range of 1-100 ppm for VIL and 2-200 ppm for REM by HPLC and 2-40 ppm for VIL and 4-80 ppm for REM by 2nd order UV method. (Fig.-3 to 5) The square of Correlation Coefficient(R^2) was achieved more than 0.990 for UV and HPLC method proving the excellent linear relationship between concentration and response in the above range (Table-1).

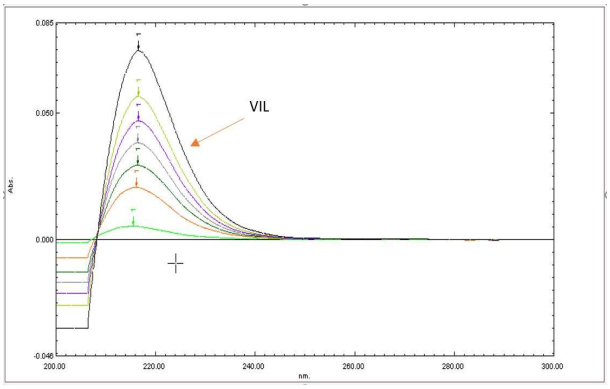

Fig.-3: Overlay Spectra of Linearity of VIL

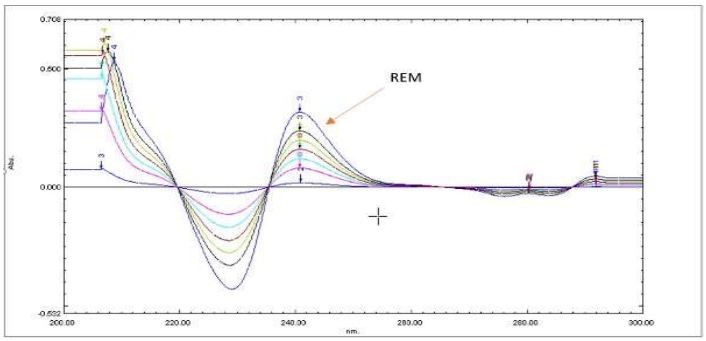

Fig.-4: Overlay Spectra of Linearity of REM

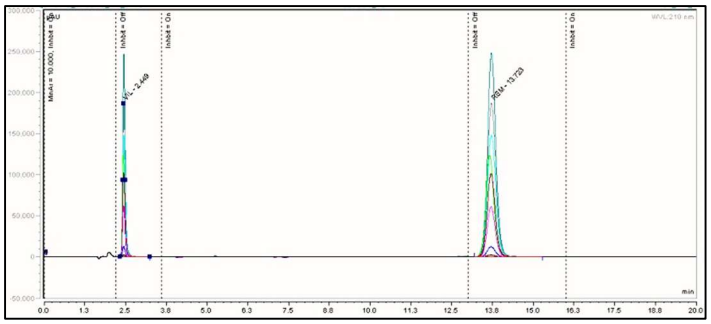

Fig.-5: Overlay Chromatogram of Linearity of REM and VIL

Limit of Detection and Limit of Quantitation

The LOD and LOQ were established from the Slope and Standard deviation of Linearity. LOD and LOQ were 3.3 times and 10 times the intercept's standard deviations and slope of the linearity, respectively. LOD and LOQ were determined using both approaches and observed a very low LOQ indicated the sensitiveness of the proposed analytical techniques (Table-1).

Precision

The repeatability of the two approaches was examined by performing a determination of precision of the concentration of the synthetic mixture. The synthetic mixture was analyzed on the same day in six replicates, and precision was checked in terms of the relative standard deviation of the content of the drugs. The intraday(intermediate) precision of the methods was determined by analyzing the above synthetic mixture on another day. The (% RSD) relative standard deviation values of intraday precision and inter-day precision were lower than 2.0 in both methods which represents the good precision of both UV and HPLC methods. (Table-1).

Accuracy

Proposed methods were tested for accuracy by the spiking method (standard addition method) at three different concentrations (50,100, and 150%). Both methods showed excellent recovery i.e. 99.1-99.82 for UV and 99.56-101.72 for the HPLC method. The accuracy results indicate that both the proposed UV and HPLC procedures are very much capable of producing accurate results (Table-2 and Table-3).

Table-1: Resulting Parameter of UV and HPLC Method

Validation Parameter	UV method		HPLC Method	
	VIL	REM	VIL	REM
Wavelength-λ(nm)	219.6	246.0	210	210
Linearity range(ppm)	2-40	4-80	1-100	2-200
Coefficient of Determination(r^2)	0.99971	0.99992	0.99989	0.99991
Slop(b)	0.00162	0.00232	13287.31428	22005.9045
Intercept(a)	0.00137	0.00225	2085.31386	301.82685

LOD (ppm)	0.715	0.733	1.173	2.179
LOQ (ppm)	2.166	2.222	3.555	6.602
Precision (% RSD) n=6				
Intraday	0.47	0.29	0.24	0.30
Inter-day	0.14	0.15	0.21	0.28

Table-2: Recovery Studies Results by UV Method

Concentration	VIL			REM		
	Amt. added (ppm)	Amt. recovered (ppm)	% Recovery	Amt. added (ppm)	Amt. recovered (ppm)	% Recovery
50%	5.30	5.28	99.62	10.01	9.93	99.20
100%	9.95	9.90	99.50	20.04	19.86	99.10
150%	15.22	15.18	99.74	30.00	29.79	99.82
Mean			99.62			99.37
RSD(n=3)			0.12			0.39

Table-3: Recovery Studies Results by HPLC Method

Concentration	VIL			REM		
	Amt. added (ppm)	Amt. recovered (ppm)	% Recovery	Amt. added (ppm)	Amt. recovered (ppm)	% Recovery
50%	25.1	25.5	101.72	50.0	50.8	101.60
100%	50.2	50.5	100.67	100.0	100.9	100.90
150%	75.4	75.1	99.56	150.0	150.7	100.44
Mean			100.65			100.98
RSD(n=3)			1.07			0.58

Forced Degradation

A forced degradation study was carried out for the HPLC method by Physical and Chemical degradation of the dry samples and their dilutions. Chemical degradation was done on the sample solution by adding 0.1 N HCl, 0.01N NaOH, and 3% H_2O_2 solutions. Thermal degradation was done by heating the samples at 60°C for 24 hours. Photolytic degradation was done by exposure of 1.2 million lux hours for dry samples. Humidity degradation was done by exploring ammonium hydroxide solution in the dry sample in a closed container and kept for eight hours. The degradation was calculated for all conditions and describe in Table-4.

Table-4: Forced Degradation Study

Sr. No	Stress parameter	VIL		REM	
		Assay (%)	Degradation (%)	Assay (%)	Degradation (%)
1	As such	100.2	-	100.2	-
2	Acid degradation	100.5	0.0	97.1	2.9
3	Alkali degradation	99.8	0.2	72.5	27.5
4	Peroxide degradation	83.6	16.4	96.2	3.8
5	Thermal degradation	98.5	1.5	99.0	1.0
6	Photolytic	97.9	2.1	97.6	2.4
7	Humidity degradation	99.2	0.8	99.1	0.9

Specificity

The specificity of the methods was measured by comparing the interference of the blank and placebo in both methods. In the UV method absorbance was checked at working wavelengths 219.6 nm and 246 nm which were used for VIL and REM respectively. In the HPLC method, blank and placebo were injected in

the same HPLC system and the peak area was checked at the retention time of VIL and REM. No interference in blank and Placebo was observed in both methods.

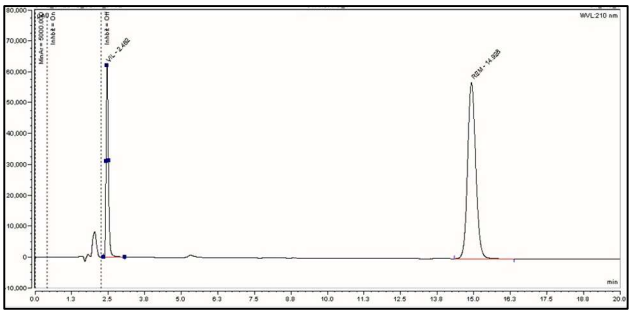

Fig.-6: Standard Chromatogram of REM and VIL

Robustness

The proposed HPLC method was tested for robustness study of instrumental parameter including wavelength for 2 nm, Flow rate by 0.1 ml, and oven temperature by 5°C. The pH of the buffer was also altered to 0.20 units. The mobile phase ratio was altered to 2% absolute. The results were complied with as such chromatographic conditions (Table-5).

Table-5: The Results of the Robustness Study of the HPLC Method

S. No.	Change in Method parameter	VIL			REM		
		Mean Area Standard	Mean Area Sample	Assay (%)	Mean Area Standard	Mean Area Sampled	Assay (%)
1	As such	674438	663160	100.4	2199425	2172503	100.3
2	-WL(-2nm)	793526	781718	100.6	2454331	2417950	100.0
3	+WL(+2nm)	521342	513241	100.5	2049027	2016219	99.9
4	-Flow(-0.1ml)	731875	721272	100.6	2386497	2349865	100.0
5	+Flow(+0.1ml)	622092	611970	100.4	2030769	1996707	99.8
6	-Oven Temp.(-5°C)	671933	660969	100.4	2187058	2149437	99.8
7	+Oven Temp.(+5°C)	675251	663852	100.4	2196472	2156352	99.7
8	-pH (-0.2 unit)	659099	648369	100.4	2189722	2147706	99.9
9	+pH (+0.2 unit)	665093	654228	100.4	2197599	2156949	99.8
10	-buffer ratio (-2%)	673626	661537	100.3	2189722	2149347	99.6
11	+buffer ratio (+2%)	675079	662728	100.2	2197599	2156949	99.6

The proposed methods have been successfully validated according to ICH-Q2(R1). Validated methods are accurate, precise, robust, and selective but easy, economical, and advantageous to previously reported work. Mahesh Attimarad *et.al.* has used methanol as a final diluent in their reported UV method while in proposed UV method's final diluent is water has the advantage of cheaper solvent. The proposed method has lower LOD and LOQ for REM which is advantageous for detection at a low level.[14] R. V. Patel *et.al.* used has buffer: Acetonitrile: Methanol (35:10:55) as mobile phase.[17] There is 20% less solvent used which is an advantage to the greenness of the method in terms of solvent disposal for the environment. Mahesh Attimarad et.al. have utilized Acetonitrile: Buffer (pH=5) (55:45) and Acetonitrile: Buffer (pH=4.9)(58:42) in their previous reported work.[18,25] The proposed HPLC method has Buffer: Acetonitrile: Methanol (55:44:1) having the advantage of using the lowest solvent concentration making it the most economical.

Application of the Proposed Method

The proposed methods were checked for the correctness of the results by verifying the marketed formulations. The results obtained were having excellent accord with the API quantity mentioned on the label of the medicine and verified that the presence of excipients had no influence on the assay of either drug. The excipient effects are eliminated by the proposed procedures. The proposed methods are very

simple for laboratories to use and any quality control laboratory can easily adapt them for routine sample analysis. (Table-6).

Table- 6 : Results of dosage tablet by UV and HPLC

Drug name	Content(mg)	UV method		HPLC method	
		Amount (mg)	Assay(%)	Amount(mg)	Assay(%)
VIL	50	50.20	100.40	50.12	100.25
REM	100	100.12	100.13	100.15	100.15

CONCLUSION

UV-Spectrophotometer and stability indicating RP-HPLC methods for the simultaneous determination of REM and VIL pharmaceutical dosage and synthetic mixture were developed and validated in line with ICH-Q2(R1).[30] The UV technology proved to be quick, accurate, and easy. The assay values achieved using all this methodology are in satisfactory correlation. In the case of pharmaceutical dosage forms or other matrices, the HPLC technique might give benefits above the UV method for the selective analysis of REM and VIL. The derivative method is a supplementary method for determining REM and VIL in commercial samples. A comparison of the resolution of a binary mixture of REM and VIL utilizing Chromatographic and spectrophotometric techniques has been conducted. Both the HPLC approach and the second-order derivative method provide values with significant accuracy. Despite being more specific than the UV 2nd order derivative approach, the HPLC technique requires expensive materials and equipment. UV spectrophotometer methodologies are much less costly and do not really require complex instruments, or any special conditions. The synthetic mixture and pharmaceutical formulation were determined by UV and HPLC methods for the content determination of REM and VIL and the results were found successful. Both methods were having simple and fast operation, good specificity, and higher accuracy. The advantage of the methods is that they can be easily adapted for laboratory use.

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List of Abbreviations:

REM= Remogliflozin Etabonate
VIL= Vildagliptin
UV= Ultraviolet
HPLC=High-performance Liquid chromatography
LOD= Limit of Detection
LOQ= Limit of Quantification
Nm= nanometer
µg= microgram
mL= milliliter
SGLT2= Sodium-glucose cotransporter-2
ppm= parts per million

CONFLICT OF INTERESTS

The authors state that they do not have any conflicts of interest in this article's content.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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