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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF THREE COMBINATION DRUGS REMOGLIFLOZIN ETABONATE, METFORMINE AND VILDAGLIPTIN BY RP-HPLC TECHNIQUE

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Keywords:

RP-HPLC, Metformin, Remogliflozin, Vildagliptin, Validation

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ABSTRACT: Remogliflozin Etabonate, Metformin, Vildagliptin are anti-diabetic drugs. It inhibits the sodium-glucose transport proteins (SGLT), which are responsible - for glucose reabsorption in the kidney. Blocking this transporter causes blood glucose to be eliminated through the urine. Several attempts were made on simultaneous analysis of Metformin with either Vildagliptin or Remogliflozin but no work were carried out on simultaneous analysis of all three drugs combination. In the present work, efforts are made to develop a simple new accurate, precise and linear reverse phase high performance liquid Chromatographic (RP-HPLC) method for simultaneous estimation of Remogliflozin etabonate (RGE), Metformin (MET) and Vildagliptin (VGN) in bulk drug and marketed tablet formulation Different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization ICH Q2B guidelines. Chromatographic separation was achieved on Acclaimed Mix Mode HILIC-1 column (150 mm × 4.6 mm, 5µm) applying an isocratic elution based on 20 mM ammonium acetate: acetonitrile (75:25, v/v) as a mobile phase. The ultraviolet detector was operated at 230 and 254 nm. The retention time for RGE was 3.81+0.5min, VGN was 4.86+0.5min, MET was 5.81+0.5min. The standard curve was linear over the concentration range of 3.9-62.5µg/ml with r2 close to one (1 to 0.999). The proposed method was validated for system suitability, specificity, linearity, accuracy, precision, LOD, LOQ and robustness. All parameters were found to be within the acceptance limit.

INTRODUCTION: Remogliflozin Etabonate is an anti-diabetic drug, chemically known as 5-Methyl4 -[4-(1-methyl ethoxy) benzyl]-1-(1 methylethyl)-1H-pyrazol – 3 – yl – 6 - O-(ethoxy carbonyl)-β-D glucopyranoside. It inhibits the sodium-glucose transport proteins (SGLT), which are responsible - for glucose reabsorption in the kidney. Blocking this transporter causes blood glucose to be eliminated through the urine [1-2].

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In the present work, efforts are made to develop a simple, accurate and precise RP-HPLC and solution stability method for Remogliflozin Etabonate in bulk and pharmaceutical dosage form and to validate it in accordance with ICH guidelines [4-17].

The treatment of diabetes are complicated and tedious method; hence, a multiple intervention approach such as the practice of healthy diets, physical activity, and various therapeutic strategies may help to minimize the complications of diabetes. Dipeptide peptidase-4 (DPP-4) and sodium-glucose transporter-2 (SGLT-2) inhibitors showed an enhanced HbA1c control when compared with conventional sulfonylureas and thia-zolidinediones [3].

The Food and Drug Administration (FDA) has approved a fixed-dose Remogliflozin and Vildagliptin tablet for T2DM. Remogliflozin etabonate (RGE) is an oral hypoglycemic drug [4], which acts by inhibiting the SGLT-2 enzyme and thereby decreasing the reabsorption of glucose from the glomerular filtrate back to the blood.

SGLT-2 inhibitors reduce cardiovascular events, body weight and also show a defensive effect on the renal system. These functional properties of SGLT-2 inhibitors considerably reduce the hospitalization of T2DM patients exclusively due to heart failure [5-6]. Vildagliptin (VGN), a DPP-4 inhibitor, decreases the blood sugar level by protecting the incretins from degradation, which helps in the production of insulin after food and reduces glucagon formation in the liver. The protection of incretins also helps in reducingbody weight by decreasing the appetite and prolonging the slow digestion of food [7, 8].

Few quantitative analytical procedures are illustrated in the literature for the analysis of RGE and VGN alone and with metformin from medicines and biological fluids. A quantitative determination of lone was explained using UV-Vis spectrophotometry [9-10], HPLC [11-12], and LCMS [13]. Derivative UV spectrophotometry, RP-HPLC, and UPLC procedures were stated for the concurrent estimation of RGE with metformin [14–16].

Different analytical methods have been reported in the literature for the quantification of VGN from formulations and biological samples. VGN alone was determined using UV-Vis spectrophotometry [17–19], HPTLC [20] and UPLCMS [21]. Several spectrophotometric [22–24] and HPLC methods were reported for the determination of VGN long with other drugs [24–27]. The determination of VGN and RGE alone and with other active ingredients. However, no quantitative analytical method has been described for the concurrent estimation of VGN and RGE from a formulation. Derivative UV spectrophotometric techniques are simple, accurate, fast, and may possibly be utilized for the quantification of multicomponent formulations showing overlapping spectra [28–32]. Hence, in the current work, three spectrophotometric methods were validated and applied to a concurrent determination of VGN and RGE from laboratory mixed solutions and formulations. Water was used as a dilution solvent for the samples, making the developed spectrophotometric methods ecofriendly [27-29].

Instrumentation: The high performance liquid chromatography (HPLC) of Shimadzu SCL-10A_{VP} inbuilt with binary pump (LC-10AT_{VP}), UV detector (SPD-10A_{VP}), Rheodyne 20µl loop capacity manual injector (P/N 77251) was used throughout the analysis. The LC-Solution software was used to interpret the HPLC reports. Acclaimed Mix-Mode HILIC-1 (5µm; 150 x 4.6 mm ID.) was used throughout the analysis.

EXPERIMENTAL WORK: Several attempts were made on simultaneous analysis of Metformin with either Vildagliptin or Remogliflozin but no work were carried out on simultaneous analysis of all three drugs combination. Importantly, all separation was performed using reverse phase chromatography, specifically by conventional C18 column with improved adsorbent properties. Nevertheless, there are certain drawbacks of this technique, such as first; as highly polar nature of Metformin, it elute with dead volume in RP-HPLC. Second, the Remogliflozin is moderately non-polar in nature so it retain strongly in C18 phase.

Third, the Vildagliptin has very low UV sensitivity and hence it gets detected at low UV wavelength which again causes unstable base line. In addition, while considering RP-HPLC for simultaneous estimation of Metformin with Vildagliptin and Remogliflozin, the results achieved with elongated run time and resolution, and most importantly, Metformin does not retain in ODS phase.

Alternative to this the HILIC technique was first time utilize for this simultaneous estimation of all three selected drugs. As resulted **Fig. 4**, Metformin eluted quite late and it is reverse to that of RP-HPLC whereas Remogliflozin eluted earlier with retention factor was ≤ 0.5. The total run time was shortened to 6 minutes which in results increase its separation efficiency. Prior to the HPLC analysis, the UV spectra of all selected drugs were performed at the wavelength ranging from 350 – 200 nm. In results, both Remogliflozin and Metformin shows significant maximum absorbance at 230 nm wavelength.

In contrast, the Vildagliptin has very low UV sensitivity which has optimum UV absorbance limit is 203-205 nm. However, the aqueous mobile phase with Ammonium acetate has the UV absorbance strength more than 210 nm. Beside these, the real Isobastic point for all drugs were lying between something around 250-260 nm wavelength.

Therefore, two wavelengths; 230 and 254 were selected for HILIC chromatography **Fig. 4.**

Reagents and Reference Samples: The reference standards; Metformin, Remogliflozin and Vildagliptin were obtained as a gift samples from Yarrow Pharma Chem Ltd.

Ammonium acetate from Merck Ltd. (Mumbai-India) HPLC grade acetonitrile and deionised water from Merck (Mumbai, India). 0.20µ and 0.45µ nylon membrane filters were used from UltraChrom Innovatives Pvt. Ltd. (India). All other chemicals and reagents were used of HPLC grade.

Standard Stock Solutions: Standard stock solutions of 1 mg/mL of standards, Metformin, Remogliflozin and Vildagliptin were prepared separately by dissolving 10 mg of the drug in 10 ml of Acetonitrile: Methanol: Water (3:4:3 v/v) in a 20 mL volumetric flask.

Furthermore, freshly prepared standards were mixed together to get the concentration 100 ppm each for performing validation studies like repeatability, precision and robustness studies. Standard stock solution was then ultrasonicated for 10-20 minutes and filtered through 0.20µ nylon filters prior to the HPLC analysis.

Chromatographic Conditions: Chromatographic separation was achieved on Acclaimed Mix Mode HILIC-1 column (150 mm × 4.6 mm, 5µm) applying an isocratic elution based on 20 mM ammonium acetate: acetonitrile (75:25, *v/v*) as a mobile phase. The ultraviolet detector was operated at 230 and 254 nm.

The buffer solution was filtered through 0.2 µm nylon membrane filter and degassed for 10-20 min in an ultrasonic bath prior to its use. The mobile phase was pumped through the column at a flow rate of 1 mL min^{-1}. The column temperature was adjusted to 28°C and the injection volume was 20 µL.

Sample Preparation for Linearity/Calibration Studies: 1000 ppm (1000 µg/ml) of standard stock solution of Remogliflozin, Metformin and Vildagliptin was made separately; then all three drugs solutions were mixed in order to get 100 ppm of each in a homologous mixture.

Subsequently, serial dilutions of five different concentrations such as 65 ppm, 31.50 ppm, 12.25 ppm, 6.25 ppm and 3.12 ppm were made, ultrasonicated and then analysed using the experimental section.

Furthermore, the calibration curve (linearity graph) was plotted **Fig. 1, 2, 3** & **4** by calculating the peak area against known concentration to determine LOD, LOQ and regression coefficient (R^2) value.

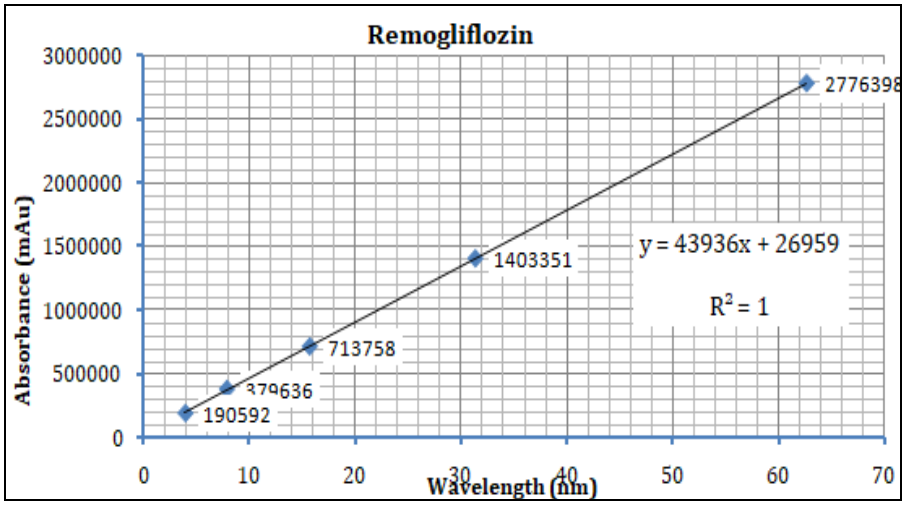

FIG. 1: CALIBRATION CURVE OF REMOGLIFLOZIN

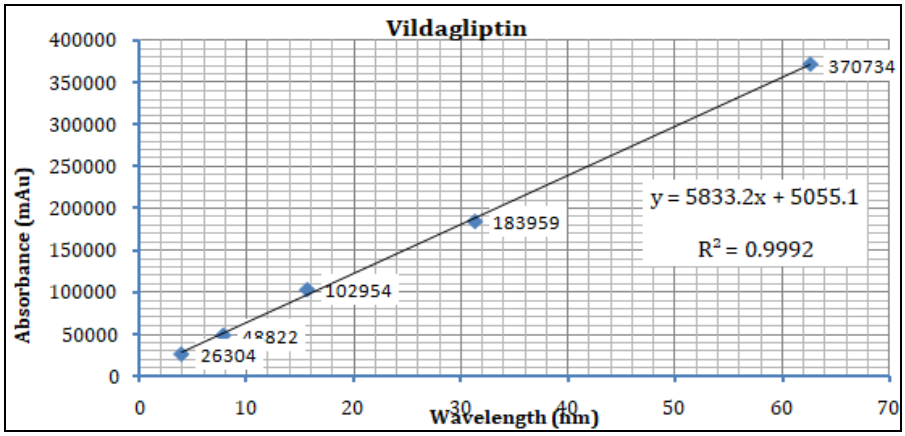

FIG. 2: CALIBRATION CURVE OF VILDAGLIPTIN

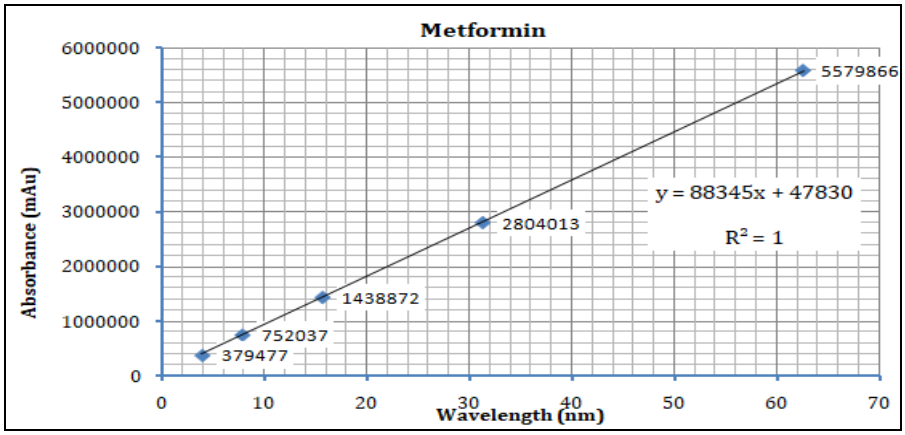

FIG. 3: CALIBRATION CURVE OF METFORMIN

Linearity (Calibration) Studies of REM, VIL and MET: The flow rate of the mobile phase was changed by 1.00 ±1 decimal from 1mL/min to 1.1 mL/min and to 0.9mL/min to evaluate the effect of the flow rate; similarly the variation of organic modifier used as acetonitrile was changed by ± 2% from 75% to 77% and 73% to monitor the peak area and retention time. Finally, the effect of wavelength was monitored by making deliberate variation from 230 to 232 and 228 nm and the differences in various system suitability parameters including retention time, peak tailing, capacity factor, resolution and theoretical plates were tested and evaluated. A new methodwas developed for the estimation of Remogliflozin, Vildagliptin and Metformin **Fig. 5** and the estimation of Remogliflozin, Vildagliptin and Metformin reported in **Table 1** & **2**. Robustness study was performed as per the procedure mentioned under the experimental section.

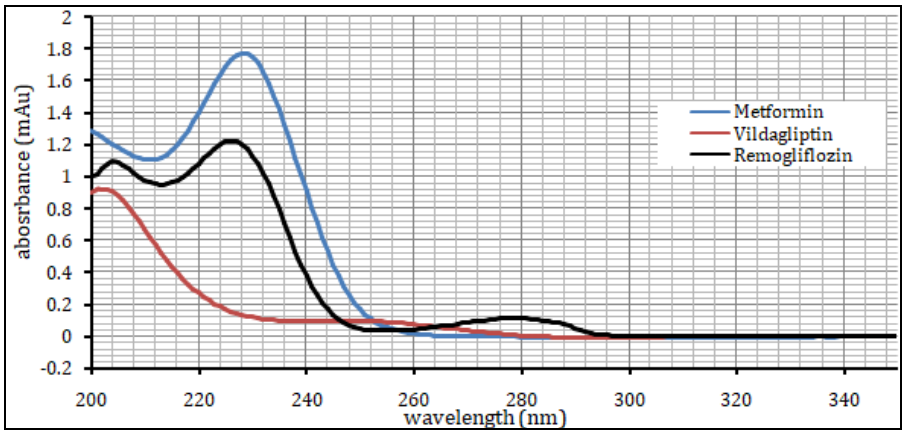

FIG. 4: OVERLAY UV SPECTRA OF METFORMIN, VILDAGLIPTIN AND REMOGLIFLOZIN

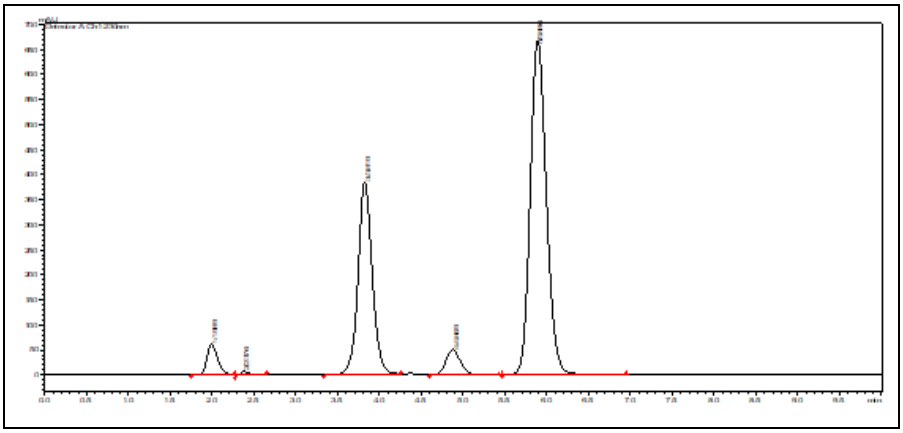

FIG. 5: DEVELOPED METHOD FOR ESTIMATION OF REMOGLIFLOZIN, VILDAGLIPTIN AND METFORMIN

TABLE 1: METHOD FOR ESTIMATION OF REMOGLIFLOZIN, VILDAGLIPTIN AND METFORMIN

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.
1	1.989	560871	60781	3.8116	1012.448	--	0	1.332
2	2.376	51178	6771	0.3478	2410.588	1.742	0.194	2.043
3	3.819	4537971	385702	30.839	2452.875	5.749	0.92	1.085
4	4.869	600834	49864	4.0831	3601.997	3.318	1.448	1.188
5	5.886	8964174	667980	60.9185	4381.955	2.991	1.959	1.176

Analytes: Remogliflozin (100 ppm); Vildagliptin (100 ppm) and Metformin (100 ppm). Column: Acclaimed Mix-Mode HILIC-1(5µ, 150 x 4.6 mm id). Solvent A; 20 Mm Ammonium acetate B; ACN (100%). Gradient elution: Isocratic elution mode with 20mM AA-ACN (25:75). Flow rate: 1 ml/min. Wavelength: 230 nm. Note: 1. All three selected analytes were well separated and follows all ICH guidelines.

System Suitability Tests for Remogliflozin, Vildagliptin and Metformin:

TABLE 2 SYSTEM SUITABILITY STUDIES OF REM, VIL AND MET

System suitability parameters	Remogliflozin (REM)	Vildagliptin (VIL)	Metformin (REM)
Theoretical plates (*N*)	2452	3601	4381
Capacity Factor (*K'*)	0.92	1.44	1.95
Resolution (*R*)	---	3.31	2.99
Asymmetry/Tailing factor (*T*)	1.08	1.18	1.17
Retention time (t_R)	3.81 min.	4.86 min.	5.88 min.
Wavelength of Detection (nm)	230 nm	230 nm	230 nm
Repeatability (%RSD)	0.33	1.30	0.41
Intra-Day Precision (%RSD)	0.29 - 0.84	0.62 - 1.95	0.43 - 0.79
Inter-Day Precision (%RSD)	0.82 - 1.10	0.30 - 1.26	0.43 - 1.47
Linearity range	3.9 – 62.5 µg.ml^{-1}	3.9 – 62.5 µg.ml^{-1}	3.9 – 62.5 µg.ml^{-1}
Regression equation	Y= 43936x + 26959	Y= 5833.2x + 5055.1	Y= 88345x + 47830
SE of intercept (S_e)	4789.20779	3113.822334	8895.354744
SD of intercept (S_a)	11731.11536	7627.275868	21789.0802
Correlation Coefficient (R^2)	1	0.999	1
LOQ[a] (µg.mL^{-1})	2.67 µg/ml	5.34µg/ml	1.01 µg/ml
LOD[a] (µg.mL^{-1})	0.80 µg/ml	1.60µg/ml	0.30 µg/ml

Sample Preparation for Drug Accuracy Studies: Exactly 5 tablets of valdiff-M consisting 500 mg of Metformin and 50 mg of Vildagliptin were weighed separately and the average weight was determined. They were mixed and crushed to fine powder into the mortar and pestle. An accurately weighed amount of the finely powdered equivalent to 10 mg was dissolved in 10 ml of Acetonitrile: Methanol: Water (3:3:4). It was then ultrasonicated and filtered through 0.45µ nylon filter. Furthermore, serial dilutions were made to get the final concentration 10 ppm of Vildagliptin and equivalent to 100 ppm of Metformin.

Accuracy Studies of Marketed Formulation: Percentage drug accuracy of three different concentrations; 80%, 100% and 120% (injected thrice) to estimate the Vildagliptin and Metformin from marketed formulation and results obtained have been reported in **Table 3-5**.

Accuracy can be studied by applying the calibration curve, the Y-intercept and the slope of the graph were used to determine the % drug recovery, attributed to the developed method for the simultaneous quantification of selected drugs or by comparing with similar concentration of reference standard. As resulted, the achieved drug recovery of both Vildagliptin and Metformin were in the range of 100.4-100.7 and 100-105, respectively. As recommended by International conferences of Harmonization (ICH) guidelines the drug recovery should be within the range of 90-110% and the RSD in percentage should be less than 2%. Hence, the calculated drug recoveries for simultaneous estimation of Vildagliptin and Metformin represents **Fig. 6** the drug recovery were in the acceptance limit given by ICH guidelines.

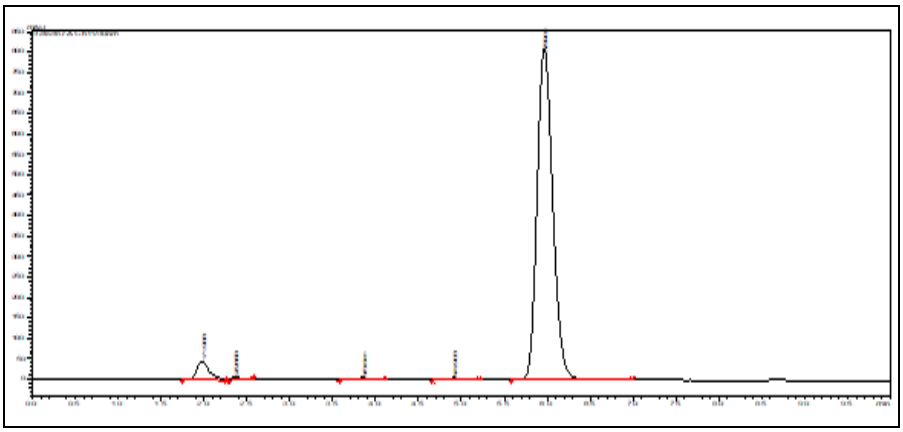

FIG. 6: ACCURACY STUDIES OF MARKETED FORMULATION

TABLE 3: ACCURACY STUDIES OF MARKETED FORMULATION

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.
1	1.968	443132	44582	4.055	946.643	--	0	1.64
2	2.358	51396	6741	0.4703	2113.191	1.69	0.198	1.779
3	3.841	42535	4108	0.3892	3144.7	6.188	0.951	1.008
4	4.903	53617	4770	0.4906	4172.946	3.679	1.491	1.157
5	5.961	10337416	812366	94.5949	4976.74	3.296	2.028	1.216

TABLE 4: ACCURACY DATA OF VILDAGLIPTIN

Drug Name: Vildagliptin			Drug content: 50 mg		Marketed formulation: Valdiff-50mg		
Std. conc. (%)	Std. (ppm)	Peak area	Drug (%)	Drug (ppm)	Peak area	Avg. peak area	Drug Rec. (%)
100%	10 ppm	53617	80	8	46114	45593	106.29
				8	45072		
			100	10	55323	55597	103.69
				10	55871		
			120	12	62036	61670	95.85
				12	61304		
			Drug recovery Range (%) as per ICH = 100±10%				95.85– 106.29 %

TABLE 5: ACCURACY DATA OF METFORMIN

Drug Name: Metformin			Drug content: 500mg		Marketed formulation: Valdiff-500mg		
Std. conc. (%)	Std. (ppm)	Peak area	Drug (%)	Drug (ppm)	Peak area	Avg. peak area	Drug Rec. (%)
100%	100 ppm	10337416	80	80	8083189	8083189	97.74
				80	8083189		
			100	100	9844520	9778076	94.59
				100	9711632		
			120	120	12484452	12484452	100.64
				120	12484452		
			Drug recovery Range (%) as per ICH = 100±10%				94.59– 100.59 %

Precision Studies of the Proposed Method: The homologous mixture of Remogliflozin, Metformin and Vildagliptin of similar concentration 100 ppm each were analyzed thrice within the same day (intraday precision) as well as, three successive days (intermediate precision) using the chromatographic condition mentioned in experimental section and then average, mean standard deviation and relative standard deviation (RSD) in percentage was calculate.

Precision Studies for REM, VIL and MET: The precision of HPLC method represents its closeness to the agreement among the series of repetitive results, derived after multiple sampling of the same homogenous mixture of selected drugs under the given conditions. As displayed in **Table 6**; for intermediate variability for precision studies, this method is significantly precise over the testing range of Remogliflozin, Vildagliptin and Metformin. Moreover, the peak area of all studied samples was also correlated with selected concentration since as observed their percentage relative standard deviation (RSD) was less than 2%. Thus it reflects, the proposed method has acceptable precision with minimum variations and can be applicable for routine analysis.

Intraday and Interday (Intermediate) Precision: Implementing the chromatographic procedure mentioned under experimental section (5.3), the homologous mixture of REM, VIL and MET of three replicates of similar concentrations; were tested within a same day. The percentage RSDs for all three drugs was calculated and they were found less than 2%. The results were shown in **Table 6 to 8.**

TABLE 6: INTRADAYPRECISION DATA OF REM

Drug Name: Remogliflozin				
S. no.	**Concentration (ppm)**	**Area**	**Mean ± SD**	**%RSD**
1	100 PPM	4237971	12288.45934	0.29
	100 PPM	4230084		
	100 PPM	4213869		
2	100 PPM	4200808	16765.31563	0.40
	100 PPM	4234048		
	100 PPM	4221243		
3	100 PPM	4490298	37533.234	0.84
	100 PPM	4451020		
	100 PPM	4526059		
	Range of %RSD			0.29 - 0.84

TABLE 7: INTRADAY PRECISION DATA OF VIL

Drug Name: Vildagliptin				
S. no.	**Concentration (ppm)**	**Area**	**Mean ± SD**	**%RSD**
1	100 PPM	560834	11047.38351	1.95
	100 PPM	580207		
	100 PPM	561320		
2	100 PPM	564947	3525.909244	0.62
	100 PPM	564161		
	100 PPM	570623		
3	100 PPM	599450	7626.440257	1.26
	100 PPM	603526		
	100 PPM	614217		
	Mean %RSD			0.62 -1.95

TABLE 8: INTRADAY PRECISION DATA OF MET

Drug Name: Metformin				
S. no.	**Concentration (ppm)**	**Area**	**Mean ± SD**	**%RSD**
1	100 PPM	8464174	39790.44931	0.47
	100 PPM	8475041		
	100 PPM	8401334		
2	100 PPM	8421736	36284.71553	0.43
	100 PPM	8407204		
	100 PPM	8476044		

3	100 PPM	8918273	70393.48489	0.79
	100 PPM	8928364		
	100 PPM	9044930		
	Mean %RSD			0.43 - 0.79

Interday (Intermediate) Precision Studies of REM, VIL and MET: Implementing the chromatographic procedure mentioned under experimental section, the homologous mixture of REM, VIL and MET of three replicates of similar concentrations (100 ppm) were tested and evaluated for three successive days (interday/intermediate precision). Furthermore, the percent RSD was calculated and found it is less than 2%; for all selected analytes in simultaneous HPLC-UV analysis **Table 9-11.**

TABLE 9: INTERDAY (INTERMEDIATE) PRECISION DATA OF REMOGLIFLOZIN

Drug Name: Remogliflozin				
S. no.	**Concentration (ppm)**	**Area**	**Mean ± SD**	**%RSD**
DAY 1	100 PPM	4490298	37533.234	0.84
	100 PPM	4451020		
	100 PPM	4526059		
DAY 2	100 PPM	4585701	37429.70483	0.82
	100 PPM	4586439		
	100 PPM	4521243		
DAY 3	100 PPM	4544530	49524.65625	1.10
	100 PPM	4451020		
	100 PPM	4526059		
		Range of % RSD		0.82-1.10

TABLE 10: INTERDAY (INTERMEDIATE) PRECISION DATA OF VILDAGLIPTIN

Drug Name: Vildagliptin				
S. no.	**Concentration (ppm)**	**Area**	**Mean ± SD**	**%RSD**
Day 1	100 PPM	599450	7626.440257	1.26
	100 PPM	603526		
	100 PPM	614217		
Day 2	100 PPM	621967	1881.010455	0.30
	100 PPM	624338		
	100 PPM	620623		
Day 3	100 PPM	617772	7414.895167	1.21
	100 PPM	603526		
	100 PPM	614217		
		Range of % RSD		0.30 -1.26

TABLE 11: INTERDAY (INTERMEDIATE) PRECISION DATA OF METFORMIN

Drug Name: Metformin				
S. No.	**Concentration (ppm)**	**Area**	**Mean ± SD**	**%RSD**
Day 1	100 PPM	8918273	70393.48489	0.79
	100 PPM	8928364		
	100 PPM	9044930		
Day 2	100 PPM	8421736	36284.71553	0.43
	100 PPM	8407204		
	100 PPM	8476044		
Day 3	100 PPM	9194615	133468.3649	1.47
	100 PPM	8928364		
	100 PPM	9044930		
		Range of % RSD		0.43 -1.47

Robustness for the Chromatographic Method: Robustness of any HPLC method represents its ability to remain unaffected by small but deliberate changes in certain separation factors to ascertain its reliability during routine HPLC analysis. The variation in separation factors such as effect of temperature, flow rate, wavelength, column length, stationary phase particle size, pH, organic modifier

composition in mobile phase and injection volume have been considered. The effects of all these variables over changes in retention pattern including effects on capacity/retention factor (k'), resolution (Rs), tailing factor (*Tf*), separation factor, theoretical plates (N) and peak area can be monitored. In this method, robustness studies was established by making deliberate changes in flow rate (1.0 ± 0.1 ml/minutes), organic modifier as acetonitrile (75±2% ml), and wavelength (230 ±2nm).

As shown in results **Fig. 6-7**, variation in flow rate and organic modifier have made slight changes in retention pattern like increase in flow rate and organic modifier have reduce the retention time, retention factor and resolution whereas decreasing the same variables have marginally extended the retention time, capacity/retention factor (k'), resolution (Rs). As noted, these variations have not made any significant changes in theoretical plates and tailing factor of all selected drugs.

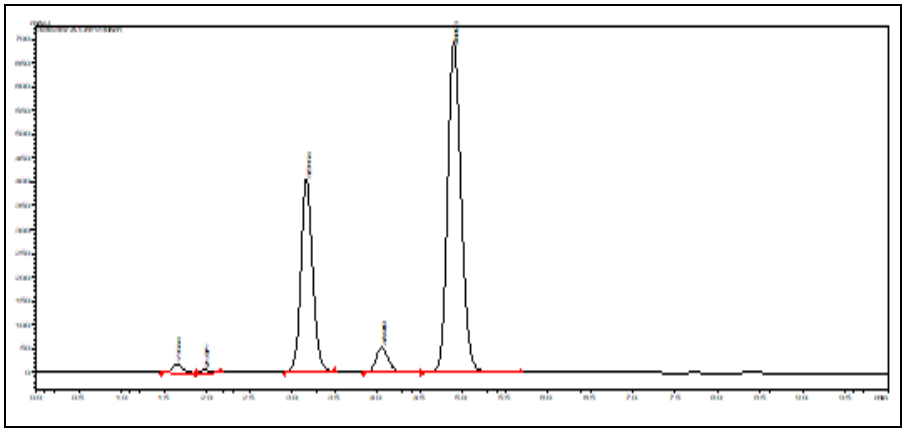

FIG. 7: EFFECT OF FLOW RATE 1.1 ML/MIN ON REM, VIL AND MET

TABLE 12: ROBUSTNESS STUDIES, EFFECT OF FLOW RATE 1.1 ML/MIN

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.
1	1.644	151277	18092	1.2321	885.499	--	0	1.455
2	1.971	43244	6429	0.3522	1922.323	1.631	0.199	1.728
3	3.164	3848261	405655	31.3415	2446.837	5.477	0.925	1.144
4	4.049	526843	53101	4.2908	3615.224	3.367	1.462	1.202
5	4.896	7708842	692296	62.7834	4296.881	2.984	1.978	1.187

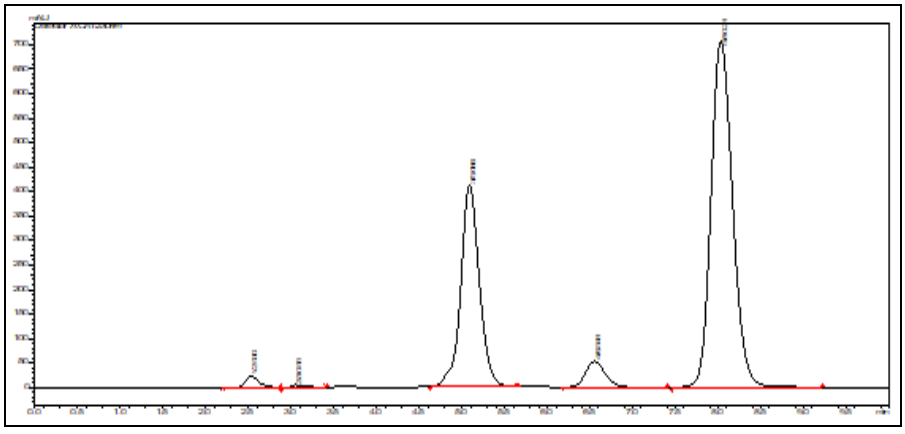

FIG. 8: EFFECT OF FLOW RATE 0.9 ML/MIN ON REM, VIL AND MET

TABLE 13: ROBUSTNESS STUDIES, EFFECT OF FLOW RATE 0.9 ML/MIN

Peak#	Ret. Time	Area	Height	Area%	T. Plate#	Resolution	k'	Tailing F.
1	2.53	284120	23870	1.4357	1143.558	--	0	1.579
2	3.055	68088	6208	0.3441	2124.637	1.862	0.208	1.647
3	5.086	6206012	411322	31.3605	2755.32	6.221	1.01	1.009
4	6.548	848840	52961	4.2894	3755.931	3.59	1.588	1.187
5	8.028	12382193	707638	62.5703	4814.898	3.325	2.173	1.179

RESULTS AND DISCUSSION: UV-Vis spectroscopic methods are extensively used analytical techniques due to their simplicity, accuracy, and reproducibility. Many reports have demonstrated the use of derivative spectroscopic methods for the analysis of multicomponent formulations without a prior separation. RGE showed UV absorption in the range of 200 to 300 nm due to the presence of individual five and six membered rings whereas VGN showed below 230 nm due to an absence of an aromatic ring and the presence of an aliphatic nitrile and carbonyl group. However, both analytes did not show any absorption above 300nm hence the analytes were scanned in the wavelength rage of 200 to 300 nm.

In the present work, three processed UV spectroscopic procedures were validated for the concurrent quantification of VGN and RGE. The first procedure was established on the measurement of absorption at zero-crossings of one of the analytes where another analyte had a degree of absorption. For the determination of VGN and RGE, normal absorption spectra were processed into first derivative spectra utilizing 4 nm as $\Delta\lambda$ and a scaling factor of 10. Different wavelengths of 2, 4, 8, and 10 nm were envisaged during the first derivative spectrum; however, 4 nm resulted in smooth spectra so 4 nm was selected. A scaling factor of 10 demonstrated sufficient peak amplitude at a low concentration of VGN; hence, a scaling factor of 10 was selected.

CONCLUSION: The proposed RP-HPLC method was validated as per the International Conference on Harmonization (ICH) Q2B Guiding principle and was found to be appropriate for routine quantitative analysis of Metformin, Vildagliptin and Remogliflozin Etabonate by HILIC chromatography. The fallouts of linearity, precision, accuracy and specificity, were demonstrated to be within the limits. The method makes available selective quantification of Metformin, VGN and RGE with no interference. The method was highly reproducible, reliable, rapid, robust and specific. Consequently, a high proportion of recovery.

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CONFLICTS OF INTEREST: Nil

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