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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF REMOGLIFLOZIN AND VILDAGLIPTIN IN API DOSAGE FORM

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ABSTRACT

Objective: A simple, Accurate, precise method was developed for the simultaneous estimation of the Remogliflozin and Vildagliptin in pharmaceutical dosage form. **Methods:** Chromatogram was run through Phenomene x C 18 column (150x4.6mm, 5µm). Mobile phase containing Phosphate buffer, Acetonitril and Methanol in the ratio of 30:05:65 was pumped through column at a flow rate of 1.2 ml/min. Buffer used at pH 4.6. Temperature was maintained at Ambient. Optimized wavelength for Remogliflozin and Vildagliptin was 249 nm. **Results:** Retention time of Remogliflozin and Vildagliptin were found to be 2.102 min and 3.246 min. The % purity of Remogliflozin and Vildagliptin was found to be 100.348% and 100.049% respectively. The system suitability parameters for Remogliflozin and Vildagliptin such as theoretical plates and tailing factor were found to be 3569.028,4798.716, 1.27 and 1.11. The linearity study for Remogliflozin and Vildagliptin correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 99.03 % and 100.19 %, %RSD for repeatability was 0.944 and 0.548, % RSD for intermediate precision was 0.119 and 0.649 respectively. The precision study was precise, robust and repeatable. LOD value was 0.35 and 0.08, and LOQ value was 1.08 and 0.25 respectively. **Conclusion:** The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Remogliflozin and Vildagliptin in pharmaceutical dosage form.

KEYWORDS: Remogliflozin, Vildagliptin, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Remogliflozin Etabonate is an orally available prodrug of remogliflozin, a benzylpyrazole glucoside-based inhibitor of renal sodium-glucose co-transporter subtype 2 (SGLT2) with antihyperglycemic activity. Upon administration and absorption, the inactive prodrug is converted to its active form remogliflozin and acts selectively on the sodium-glucose co-transporter subtype 2 (SGLT2). Remogliflozin etabonate has been used in trials studying the treatment and basic science of Type 2 Diabetes Mellitus and Diabetes Mellitus, Type 2. IUPAC name ethyl [(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[5-methyl-1-propan-2-yl-4-[(4-propan-2-yloxyphenyl) methyl] pyrazol-3-yl] oxyoxan-2-yl] methyl carbonate. Molecular formula is $C_{26}H_{38}N_2O_9$. Molecular weight is 522.6. Remogliflozin etabonate is soluble in methanol and DMSO.

Vildagliptin (LAF237) is an orally active antihyperglycemic agent that selectively inhibits the dipeptidyl peptidase-4 (DPP-4) enzyme. It is used to manage type II diabetes mellitus, where GLP-1 secretion and insulinotropic effects are impaired.[3] By inhibiting DPP-4, vildagliptin prevents the degradation of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are incretin hormones that promote insulin secretion and regulate blood glucose levels. Elevated levels of GLP-1 and GIP consequently results in improved glycemic control. In clinical trials, vildagliptin has a relatively low risk of hypoglycemia.[4] IUPAC name is (2S)-1-[2-[(3-hydroxy-1-adamantyl) amino] acetyl] pyrrolidine-2-carbonitrile. Molecular Weight is 303.4. Molecular Formula is $C_{17}H_{25}N_3O_2$. Vildagliptin is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), which should be purged with an inert gas. The solubility of vildagliptin in ethanol and DMSO is approximately 16 mg/ml and approximately 20 mg/ml in DMF.

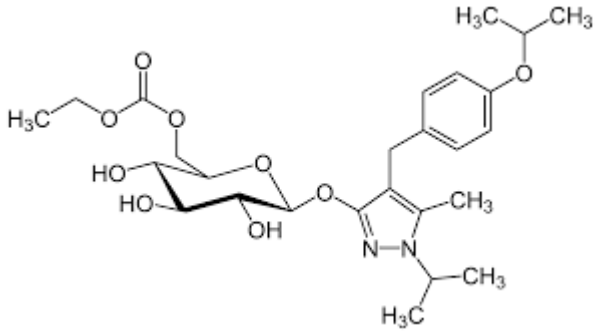

Figure 1: Structure of Remogliflozin Etabonate.

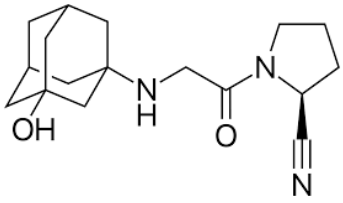

Figure 2: Structure of Vildagliptin.

The literature survey revealed that There are really few approaches reported in the literary works for evaluation of Remogliflozin and also Vildagliptin alone or in combination with various other drugs in the pure form as well as drugs formulations by RP-HPLC.[5-9] In view of the demand for an appropriate, cost-effective RP-HPLC method for routine analysis of Remogliflozin and also Vildagliptin synchronized evaluation of in pharmaceutical dose type. Attempts were made to establish easy, precise, accurate as well as cost-efficient logical method for the estimate of Remogliflozin and also Vildagliptin. The recommended approach will be validated according to ICH guidelines. The objective of the recommended work is to establish a brand-new, simple, delicate, exact and economical logical method as well as recognition for the Synchronized evaluation of Remogliflozin and also Vildagliptin in pharmaceutical dose kind by utilizing RP-HPLC. To verify the established method based on ICH standards for the desired analytical application.

MATERIALS AND METHODS

Chemicals and Reagents: Remogliflozin and Vildagliptin were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 249 nm with Phenomene x C 18 column (150x4.6mm, 5µm), dimensions at 25^0C temperature. The optimized mobile phase consists of Phosphate buffer, Acetonitril and Methanol in the ratio of 30:05:65. Flow rate was maintained at 1 ml/min.

Preparation of solutions

Preparation of phosphate buffer solution

4.2568 gm of di-sodium hydrogen orthophosphate was weighed and sufficient water (HPLC grade) was added to dissolve it. Then sonicate for 10 min. Then 1ml of tri ethanol amine was added, the final volume was made up to 1000ml with water and adjusted the pH to 3.5 with ortho phosphoric acid.

Preparation of mobile phase

Methanol, Buffer and Acetonitrile were mixed in the ratio of 65:30:5 and sonicated for 20minutes, Filtered with 0.45 µ membrane filter.

Preparations of working standard solution

100mg of Remogliflozin and 50 mg of Vildagliptin were accurately weighed and transferred in to a separate 50 ml volumetric flask and sufficient mobile phase was added to dissolve the drug. The final volume was made up to 50 ml with mobile phase (primary stock solution). Pipette out 2ml from the above stock solution into a 50ml volumetric flask and the final volume was made up to the mark with the mobile phase.

Preparation of Sample solution

20 tablets were weighed and powdered, tablets powder equivalent to 100mg of Remogliflozin and 50 mg of Vildagliptin was transferred in to a 50 ml volumetric flask, sufficient amount of mobile phase was added and dissolved by 20 minutes ultrasonication. Then made the volume up to the mark with the mobile phase and filtered with 0.45 µ filter paper. Pipette out 2 ml from the above solution and diluted to 50ml with the mobile phase.

Procedure

20µL of the standard, sample are injected into the chromatographic system and the areas for Remogliflozin and Vildagliptin peaks are measured and the %Assay are calculated by using the formulae.

METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.2 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Phenomene x C 18 column (150x4.6mm, 5µm), the mobile phase of composition Buffer: ACN: Methanol (30:5:65), pH- 3.5 was allowed to flow through the column at a flow rate of 1.2 ml per minute. Retention

time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Remogliflozin and Vildagliptin in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2,3.

Validation of Analytical method

Linearity: Appropriate volume from the stock solution was diluted to get the final concentration of 200, 300, 400, 500, 600 µg/mL for Remogliflozin and 5, 7.5, 10, 12.5, 15 µg/mL for Vildagliptin. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in figure 6 and 7.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 75%, 100%, 125% and 75%, 100%, 125% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Remogliflozin and Vildagliptin and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day, different analyst, different instrument. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 1.3 ml/min to 1.1 ml/min. The results are shown in table 6.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 7.

$$LOD = 3.3\sigma/S \text{ and}$$

$LOQ = 10\ \sigma/S$, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION

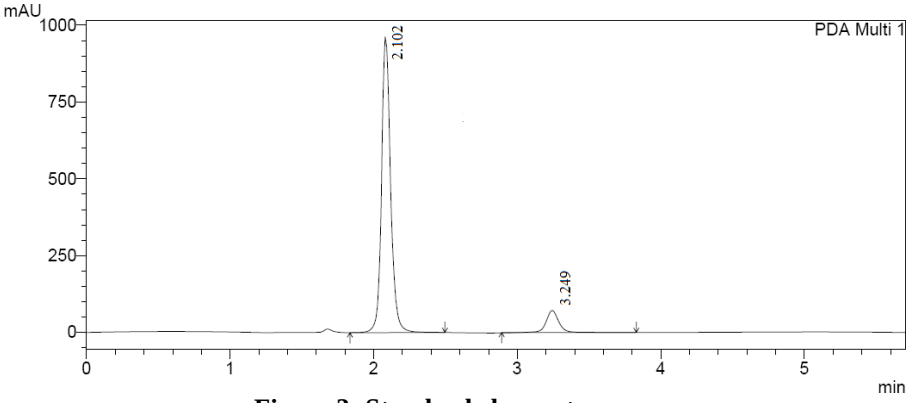

Figure 3: Standard chromatogram.

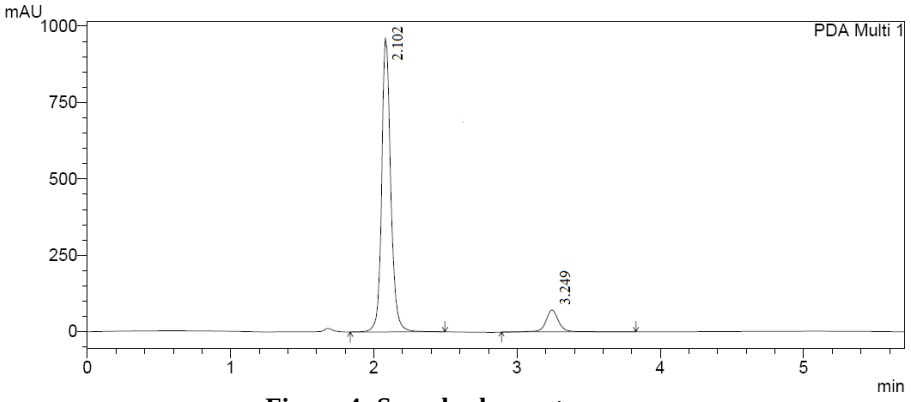

Figure 4: Sample chromatogram.

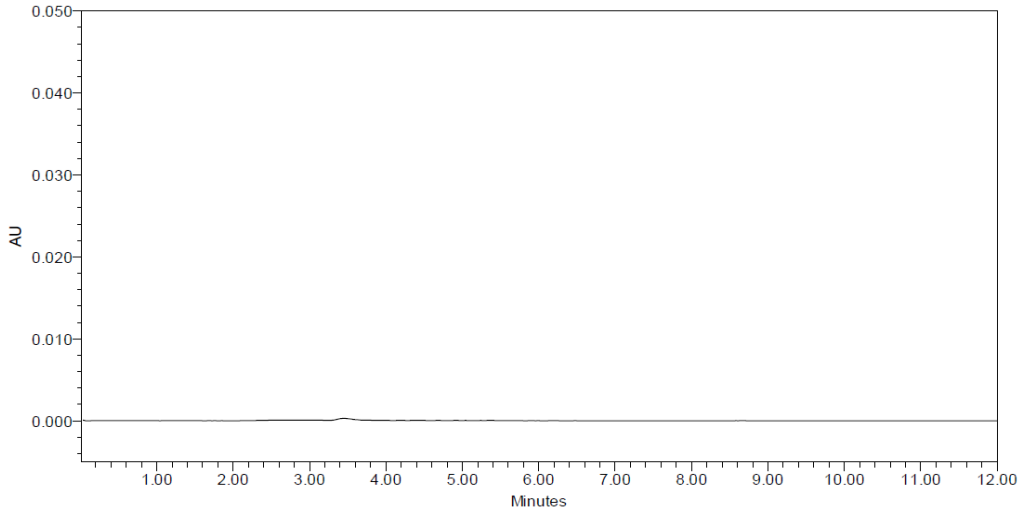

Figure 5: Blank chromatogram.

Table 1: System suitability parameters.

	REM Area	VIL Area	REM Theoretical plates	VIL Theoretical plates	REM Tailing factor	VIL Tailing factor
	4208745	400587	3568.305	4836.127	1.276	1.105
	4208746	396574	3586.231	4836.241	1.285	1.118
	4198754	398567	3528.97	4863.727	1.256	1.126
	4184764	397854	3594.212	4758.963	1.274	1.113
	4207841	399852	3567.422	4698.521	1.293	1.133
AVG	4201770	398686.8	3569.028	4798.716	1.2768	1.119
SD	10397.99	1592.19	25.1889	68.2888	0.01388	0.0109
% RSD	0.247467	0.3993	0.7057	1.4230	1.0872	0.9769

Table 2: Assay results for Remogliflozin.

SAM Area	STD Area	Amt present	% Amt present
4257964	4201770	501.772	100.354
4287561	4201770	505.26	101.052
4287956	4201770	505.306	101.061
4281863	4201770	504.588	100.918
4178293	4201770	492.383	98.4766
4187956	4201770	493.522	98.7044
	AVG	500.472	100.094
	SD	5.97758	1.19552
	% RSD	1.19439	1.19439

Table 3: Assay results for Vildagliptin.

SAM Area	STD Area	Amt present	% Amt present
403156	398687	12.5403	100.322
406321	398687	12.6387	101.11
403652	398687	12.5557	100.445
406328	398687	12.6389	101.111
401357	398687	12.4843	99.8744
398741	398687	12.4029	99.2234
	AVG	12.5435	100.348
	SD	0.09121	0.72966
	% RSD	0.72713	0.72713

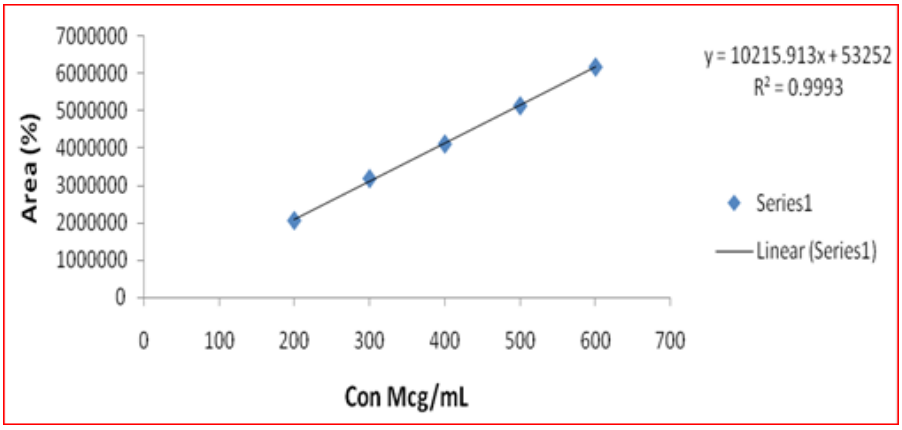

Figure 6: Linearity graph for Remogliflozin.

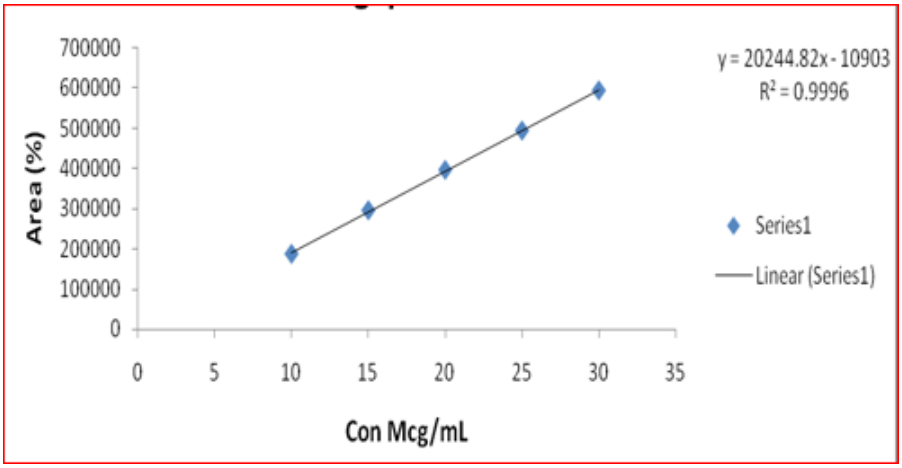

Figure 7: Linearity graph for Vildagliptin.

Table 4: Showing accuracy results for Remogliflozin and Vildagliptin.

Concentration (%)	Added AMT amount (mg)		Amt recovered (mg)		Amt recovered (%)	
REM/ VIL	REM	VIL	REM	VIL	REM	VIL
75	375	9.375	374.65	9.394	99.90	100.20
100	500	12.5	495.17	12.649	99.03	101.19
125	625	15.625	621.83	15.487	99.49	99.11

Table 5: Precision and intermediate precision results for Remogliflozin and Vildagliptin.

Parameters	Sampling time	REM			VIL		
		Amount present (mg)	Amount present (%)	RSD (%)	Amount present (mg)	Amount present (%)	RSD %
Repeatability	0 hrs	495.11	99.02	0.0920	12.62	100.97	1.4542
	8^{th} hrs	499.69	99.93	0.9449	12.37	100.62	0.5498
	16^{th} hrs	503.98	100.79	0.3633	12.60	100.83	0.7566
Intermediate precision	I^{st} Day	504.63	100.92	0.4993	12.55	100.42	0.7712
	2^{nd} day	503.59	100.71	0.3197	12.63	101.06	0.6141
	3^{rd} day	497.53	99.50	0.1257	12.70	101.64	0.1250
	Analyst -1	502.26	100.45	0.1907	12.63	101.07	0.8081
	Analyst -2	504.35	100.87	0.1197	12.61	100.94	0.6498
	Instrument -1	501.00	100.20	0.7276	12.66	101.30	0.1559
	Instrument -2	504.86	100.97	0.1219	12.61	100.94	0.4287

Table 6: Robustness results of Remogliflozin and Vildagliptin.

Parameters		REM			VIL		
		Amount present (mg)	Amount present (%)	RSD %	Amount present (mg)	Amount Present (%)	RSD %
Wavelength (nm)	248	493.04	98.60	0.1139	12.64	101.16	0.0549
	250	505.57	101.11	0.1237	12.63	101.11	0.0504
Flow Rate (mL/min)	1.3	502.87	100.57	0.3725	12.61	100.94	0.4278
	1.1	502.90	100.58	0.7906	12.65	101.23	0.0153
Mobile phase (% of (Methanol)	67	502.99	100.59	0.3907	12.65	101.27	0.1750
	63	504.86	100.97	0.09942	12.58	100.66	0.3853
pH	3.55	498.76	99.75	1.1828	12.64	101.18	0.0634
	3.45	500.30	100.06	1.3808	12.63	101.08	0.0801

Table 7: LOD, LOQ of Remogliflozin and Sitagliptin.

	REM	VIL
	2056745	188634
	2057246	187858
	2058874	187658
SD	1113.106	515.5502
Slope	10215.91	20244.82
LOD (µg/mL)	0.359561	0.084037
LOQ (µg/mL)	1.08958	0.254658

CONCLUSION

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Remogliflozin and Vildagliptin in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Remogliflozin and Vildagliptin in pure and its pharmaceutical dosage forms.

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