RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ANTI DIABETIC DRUGS IN API DOSAGE FORM

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ABSTRACT:

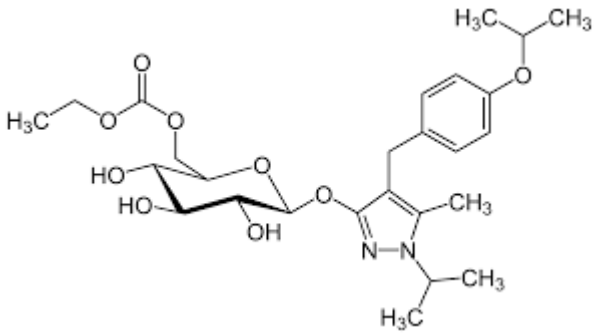
A straight forward, Exact, exact technique was created for the concurrent assessment of the Remogliflozin and Vildagliptin in drug measurement's structure. Chromatogram was gone through Phenomenon x C 18 section (150x4.6mm, 5µm). Portable stage containing Phosphate cushion, Acetonitrile and Methanol in the proportion of 30:05:65 was siphoned through section at a stream pace of 1.2 ml/min. Support utilized at pH 4.6. Temperature was kept up with at Encompassing. Upgraded frequency for Remogliflozin and Vildagliptin was 249 nm. Maintenance season of Remogliflozin and Vildagliptin were viewed as 2.102 min and 3.246 min. The % virtue of Remogliflozin and Vildagliptin was viewed as 100.348% and 100.049% separately. The framework appropriateness boundaries for Remogliflozin and Vildagliptin, for example, hypothetical plates and following component were viewed as 3569.028,4798.716, 1.27 and 1.11. The linearity study for Remogliflozin and Vildagliptin relationship coefficient (r2) was viewed as 0.999 and 0.999, % mean recuperation was viewed as 99.03 % and 100.19 %, %RSD for repeatability was 0.944 and 0.548, % RSD for middle of the road accuracy was 0.119 and 0.649 individually. The accuracy study was exact, strong and repeatable. LOD esteem was 0.35 and 0.08, and LOQ esteem was 1.08 and 0.25 separately. The consequences of study showed that the proposed RP-HPLC technique is a straightforward, exact, exact, rough, hearty, quick and reproducible, which might be helpful for the normal assessment of Remogliflozin and Vildagliptin in drug measurement structure.

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

INTRODUCTION:

Remogliflozin Etabonate is an orally accessible prodrug of remogliflozin, a benzylpyrazole glucoside-based inhibitor of renal sodium-glucose co-carrier subtype 2 (SGLT2) with antihyperglycemic movement. Upon organization and retention, the dormant prodrug is changed over completely to its dynamic structure remogliflozin and acts specifically on the sodium-glucose co-carrier subtype 2 (SGLT2). Remogliflozin etabonate has been utilized in preliminaries concentrating on the treatment and essential study 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. Atomic equation is C26H38N2O9. Atomic weight is 522.6. Remogliflozin etabonate is dissolvable in methanol and DMSO.

Vildagliptin (LAF237) is an orally dynamic antihyperglycemic specialist that specifically restrains the dipeptidyl peptidase-4 (DPP-4) protein. It is utilized to oversee type II diabetes mellitus, where GLP-1 emission and insulinotropic impacts are impaired.[3] By repressing DPP-4, vildagliptin forestalls the debasement of glucagon-like peptide 1 (GLP-1) and glucose-subordinate insulinotropic polypeptide (GIP), which are incretin chemicals that advance insulin discharge and direct blood glucose levels. Raised degrees of GLP-1 and GIP therefore results in improved glycemic control. In clinical preliminaries, vildagliptin has a moderately generally safe of hypoglycemia[4]. IUPAC name is (2S)- 1-[2-[(3-hydroxy-1-adamantyl) amino] acetyl] pyrrolidine-2-carbonitrile. Atomic Weight is 303.4. Atomic Formula is C17H25N3O2. Vildagliptin is dissolvable in natural solvents like ethanol, DMSO, and dimethyl formamide (DMF), which ought to be cleansed with an idle gas. The dissolvability of vildagliptin in ethanol and DMSO is roughly 16 mg/ml and around 20 mg/ml in DMF.



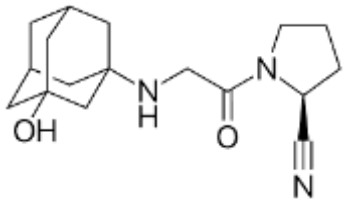


Figure 1: Structure of Remogliflozin Etabonate **Figure 2: Structure of Vildagliptin**

The writing overview uncovered that There are truly couple of approaches revealed in the abstract works for assessment of Remogliflozin and furthermore Vildagliptin alone or in blend with different medications in the unadulterated structure as well as medications plans by RP-HPLC [5-9]. Considering the interest for a suitable, practical RP-HPLC technique for routine examination of Remogliflozin and furthermore Vildagliptin synchronized assessment of in drug portion type. Endeavors were made to lay out simple, exact, precise as well as cost-proficient sensible strategy for the gauge of Remogliflozin and furthermore Vildagliptin. The prescribed methodology will be approved by ICH rules. The target of the prescribed work is to lay out a pristine, basic, sensitive, definite and conservative consistent technique as well as acknowledgment for the Synchronized assessment of Remogliflozin and furthermore Vildagliptin in drug portion kind by using RP-HPLC. To check the laid out technique in view of ICH norms for the ideal scientific application.

MATERIALS AND METHODS:

Chemicals and Reagents: Remogliflozin and Vildagliptin were Bought from market. NaH2PO4 was scientific grade provided by Finerchem restricted, Orthophosphoric corrosive (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC framework, outfitted with an auto sampler, UV locator and Engage 2 programming. Investigation was done at 249 nm with Phenomene x C 18 section (150x4.6mm, 5µm), aspects at 250C temperature. The streamlined portable stage comprises of Phosphate cushion, Acetonitril and Methanol in the proportion of 30:05:65. Stream rate was kept up with at 1 ml/min

Preparation of solutions:

Preparation of phosphate buffer solution

4.2568 gm of di-sodium hydrogen orthophosphate was gauged and adequate water (HPLC grade) was added to break down it. Then sonicate for 10 min. Then 1ml of tri ethanol amine was added, the last volume was made up to 1000ml with water and changed the pH to 3.5 with ortho phosphoric corrosive.

Preparation of mobile phase:

Methanol, Cushion and Acetonitrile were blended in the proportion of 65:30:5 and sonicated for 20minutes, Sifted with 0.45 µ layer channel.

Preparations of working standard solution:

100mg of Remogliflozin and 50 mg of Vildagliptin were precisely gauged and moved in to a different 50 ml volumetric cup and adequate versatile stage was added to break down the medication. The last volume was made up to 50 ml with portable stage (essential stock arrangement). Pipette out 2ml from the above stock arrangement into a 50ml volumetric jar and the last volume was left up to the imprint with the portable stage.

Preparation of Sample solution

20 tablets were gauged and powdered, tablets powder identical to 100mg of Remogliflozin and 50 mg of Vildagliptin was moved in to a 50 ml volumetric carafe, adequate measure of portable stage was added and broken down by 20 minutes ultrasonication. Then, at that point, made the volume sufficient with the versatile stage and separated with 0.45 µ channel paper. Pipette out 2 ml from the above arrangement and weakened to 50ml with the versatile stage.

Procedure:

20µL of the norm, test are infused into the chromatographic framework and the regions for Remogliflozin and Vildagliptin tops are estimated and the %Assay are determined by utilizing the formulae.

METHOD:

The created chromatographic technique was approved for framework appropriateness, linearity exactness, accuracy, roughness and strength according to ICH rules.

System suitability parameters: To assess framework reasonableness boundaries, for example, maintenance time, following component and USP hypothetical plate count, the portable stage was permitted to course through the segment at a stream pace of 1.2 ml/min to equilibrate the section at encompassing temperature. Chromatographic partition was accomplished by infusing a volume of 20 µL of standard into Phenomene x C 18 section (150x4.6mm, 5µm), the portable period of sythesis Support: ACN: Methanol (30:5:65), pH-3.5 was permitted to move through the segment at a stream pace of 1.2 ml each moment. Maintenance time, following component and USP hypothetical plate count of the created technique are displayed in table 1.

Assay of pharmaceutical formulation: The proposed approved strategy was effectively applied to decide Remogliflozin and Vildagliptin in their tablet dose structure. The outcome got for was similar with the relating marked sums and they were displayed in Table-2,3

Validation of Analytical method:

Linearity: Fitting volume from the stock arrangement was weakened to get the last centralization of 200, 300, 400, 500, 600 µg/mL for Remogliflozin and 5, 7.5, 10, 12.5, 15 µg/mL for Vildagliptin. The region of each level was utilized for computation of connection coefficient. Infuse each level into the chromatographic framework and measure the pinnacle region. Plot a chart of pinnacle region versus fixation (on X-hub focus and on Y-pivot Pinnacle region) and compute the relationship coefficient. The outcomes are displayed in figure 6 and 7.

Accuracy studies: The still up in the air by help of recuperation study. The recuperation technique did at three level 75%, 100 percent, 125% and 75%, 100 percent, 125% Infuse the standard arrangements into chromatographic framework. Work out the Sum found and Sum added for Remogliflozin and Vildagliptin and ascertain the singular recuperation and mean recuperation values. The outcomes are displayed in table 4.

Precision Studies: accuracy was determined from Coefficient of change for six recreate infusions of the norm. The standard arrangement was infused for multiple times and estimated the region for every one of the six Infusions in HPLC. The %RSD for the area of six duplicate infusions was found. The outcomes are displayed in table 5.

Ruggedness: To assess the transitional accuracy of the technique, Accuracy was performed on various day, different expert, different instrument. The standard arrangement was infused for multiple times and estimated the region for every one of the five infusions in HPLC. The %RSD for the area of five reproduce infusions was found. The outcomes are displayed in table 6.

Robustness: As a component of the Strength, purposeful change in the Stream rate, Versatile Stage organization, Temperature Variety was had to assess the effect on the technique. The stream rate was differed at 1.3 ml/min to 1.1 ml/min. The outcomes are displayed in table 6.

LOD and LOQ: The responsiveness of RP still up in the air from LOD and LOQ. Which were determined from the alignment bend involving the accompanying conditions according to ICH rules. The outcomes are displayed in table 7.

$LOD = 3.3\sigma/S$ and

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

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION

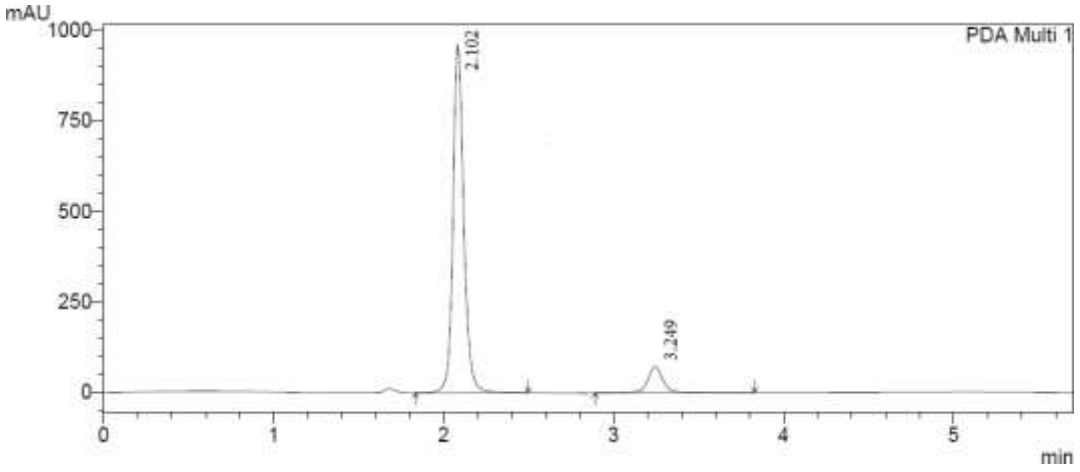


Figure 3: Standard chromatogram

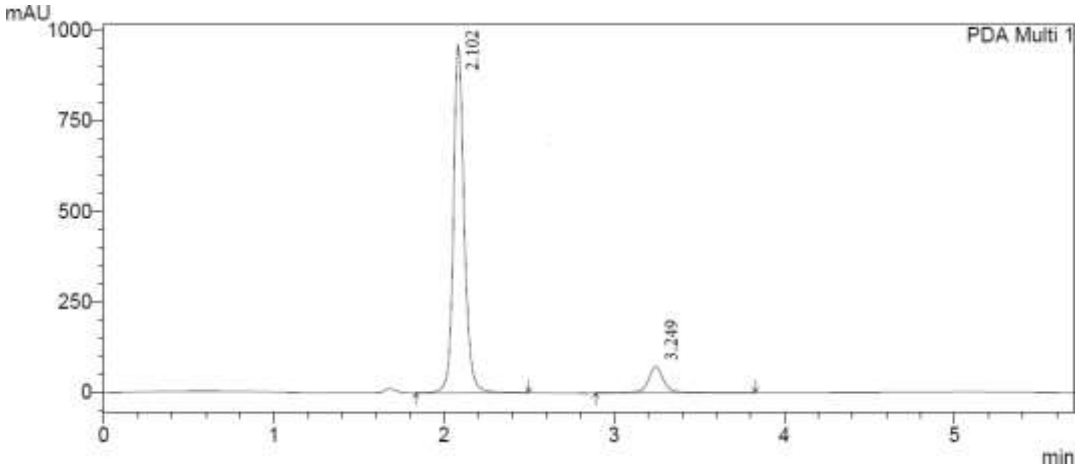


Figure 4: Sample chromatogram

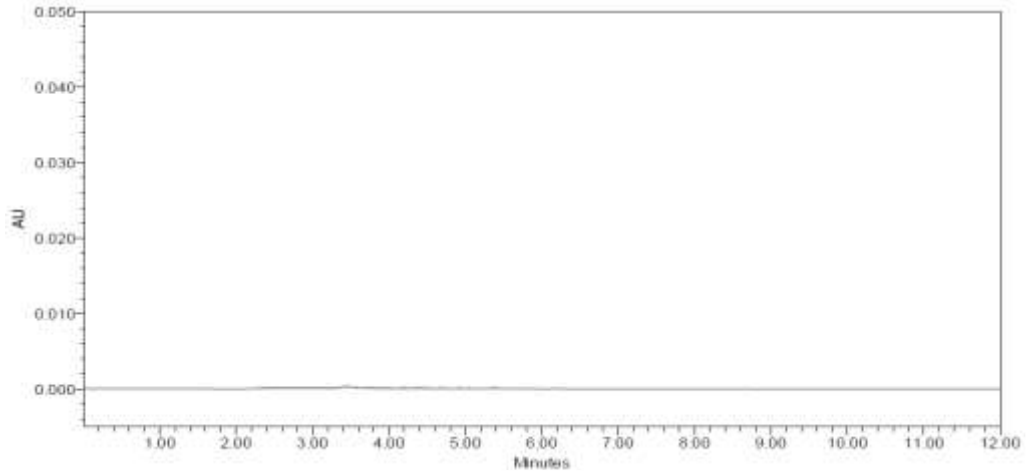


Figure 5: Blank chromatogram

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

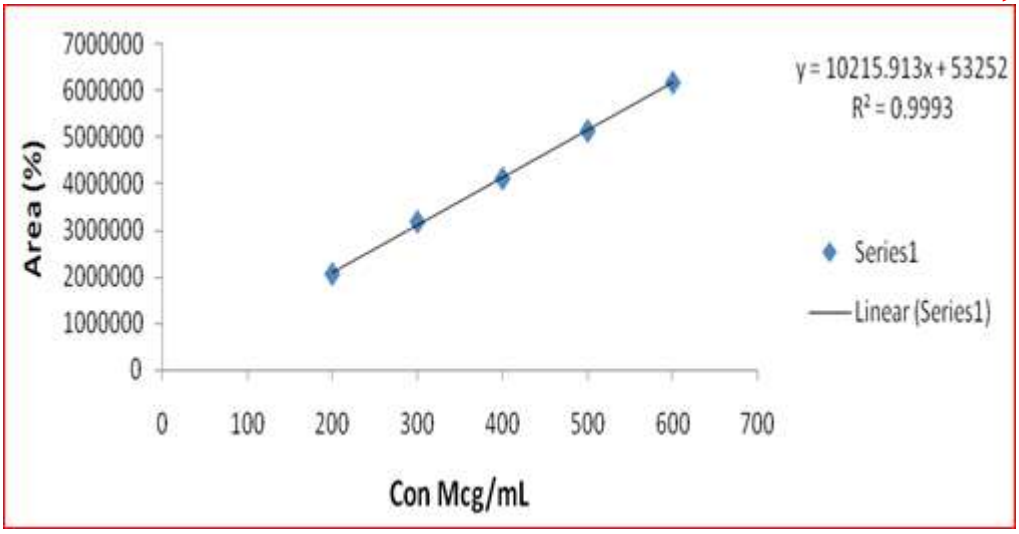


Figure 6: Linearity graph for Remogliflozin

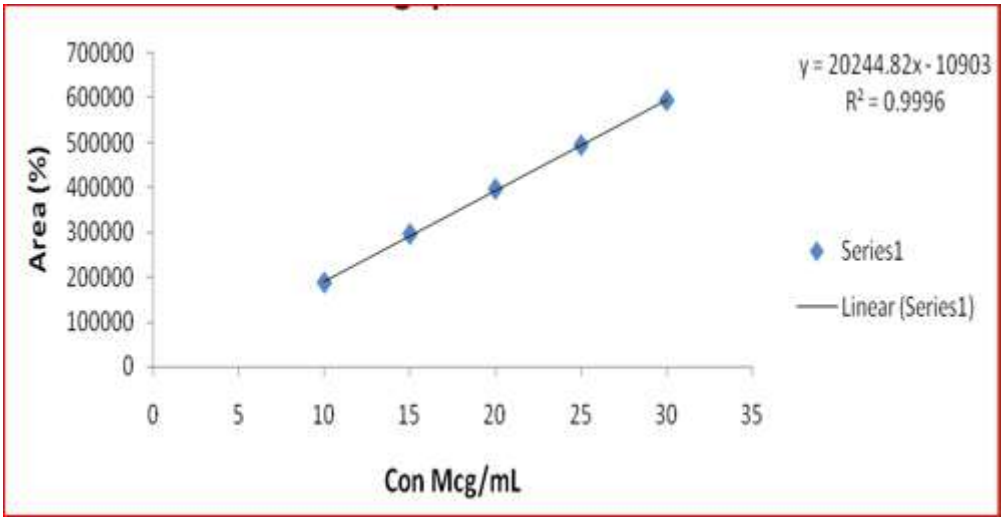


Figure 7: Linearity graph for Vildagliptin

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

		REM			VIL		
Parameters		**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 Created HPLC strategy was approved and it was viewed as basic, exact, precise and touchy for the concurrent assessment of Remogliflozin and Vildagliptin in its unadulterated structure and in its drug dose structures. Thus, this technique can undoubtedly and helpfully take on for routine quality control examination of Remogliflozin and Vildagliptin in unadulterated and its drug dose structures.

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