SIMULTANEOUS ESTIMATION OF REMOGLIFLOZIN ETABONATE AND VILDAGLIPTIN IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A Simple rapid and accurate RP-HPLC method was developed for simultaneous estimation of Remogliflozin etabonate and Vildagliptin in tablet dosage form. A Sheisedo C18 (4.6 mm x 250 mm, 5 µm) column was used with a flow rate of 1 ml/min and detection wavelength of 210 nm. Mobile phase of Buffer (adjusting to pH 9.5 using orthophosphoric acid) – Methanol (20:80 %v/v) was used. The retention time of Remogliflozin etabonate & Vildagliptin was found to be 5.9 min and 3.22 min respectively. The Linearity was observed in the concentration range of 25-150 µg/ml and 12.5-75 µg/ml for Remogliflozin etabonate and Vildagliptin. The proposed method was simple, rapid, accurate, precise and useful for the routine analysis.

Keyword : - *Remogliflozin etabonate and Vildagliptin, RP-HPLC, Validation*.

1. INTRODUCTION:

Remogliflozin etabonate is chemically 5 methyl-1-(propan 2-yl)-4-[-4-(propan 2-yl oxy) benzyl] 1H pyrazol-3-yl-6-O(ethoxy carbonyl)-b-D glucopyranoside hemihydrate. It is a SGLT-2 inhibitors. Remogliflozin etabonate is prodrug of remogliflozin.By inhibit SGLT2, gliflozin prevent the kidneys reuptake of glucose from the glomerular filtrate and subsequently lower the glucose level in the blood and promote the excretion of glucose in the urine (glucosuria).

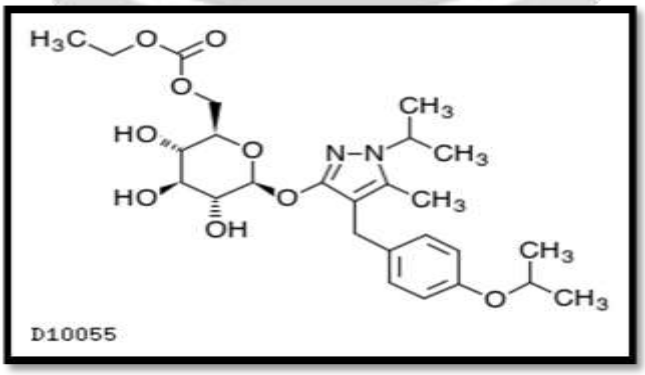

Fig -1: Chemical structure of Remogliflozin etabonate

Vildagliptin is chemically 1-2-[(3-Hydroxy-1-adamantyl) amino] acetyl] pyrrolidone-2-carbonitrile.It is antidiabetic –Dipeptidyl Peptidase-4 Inhibitors (DPP-4).Vildagliptin binds covalently to the catalytically site of DPP-4, eliciting prolonged enzyme inhibition. This raises intact GLP-1 level, both after meal ingestion and in the fasting state. Vildagliptin has been shown to stimulate insulin secretion and inhibit glucagon secretion in a glucose- dependent manner.

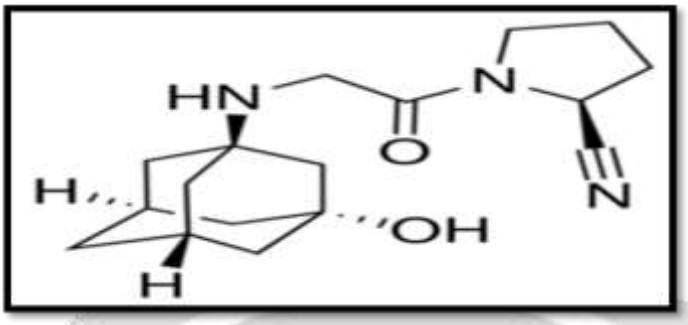

Fig -2: Chemical structure of Vildagliptin

2. MAERIALS AND METHODS:

Remogliflozin etabonate was supplied as gift sample from Torrent Pharmaceutical, Vildagliptin was provided by Exemed Pharma. All chemicals and reagents used were of analytical grade and purchased from Merck Chemicals, India. REMO-V tablet formulation containing Remogliflozin etabonate 100mg and Vildagliptin 50 mg was produced from local market.

2.1 Instrumentation:

- HPLC (Model: Shimadzu, Software: LC Solution, Pump: Isocratic) column used was Sheisedo C18 (4.6 mm x 250 mm, 5 µm)
- UV spectrophotometer (Make: Labindia, Model: UV3000 with UV win 5 software).
- Digital analytical balance

2.2 CHROMATOGRAPHIC CONDITION:

- Stationary Phase: Sheisedo C18 (4.6 mm x 250 mm, 5 µm)
- Mobile Phase:Buffer (adjusting to pH 9.5 using orthophosphoric acid) : Methanol (20:80)
- Flow rate:1.0 ml/min
- Injection volume:20 µl
- Detection: 210 nm

2.3 VALIDATION METHOD: The validation method was developed as per the ICH guidelines and accordingly the parameters evaluated were specificity, precision, accuracy, linearity, ruggedness, robustness and system suitability studies. For all the parameters %RSD were calculated.

2.4 System Suitability: It is an essential portion of chromatographic method. System suitability tests are established on the idea that the equipment, electronics, analytical operations and samples constitute an integral system that can be assessed as a whole. System suitability testing offers assurance that the process will offer accurate and precise data for its planned use.

2.4 Specificity: Specificity of the pharmaceutical analysis is the ability to measure accurately and specifically the concentration of API, without interference from other active ingredients, diluents, mobile phase. Solutions of mobile phase, sample solution, standard solution were injected into liquid chromatography. Retention times of samples and standard were compared.

2.5 Linearity: The linearity is expressed in term of correlation co- efficient of linear regression analysis. The linearity of response for Remogliflozin etabonate and Vildagliptin was assessed by analysis of six independent level of calibration curve in range of 25-150 µg/ml and 12.5-75 µg/ml respectively. Graph of peak area (Y-axis) versus concentration (X-axis) was plotted. Correlation co-efficient of Remogliflozin etabonate and Vildagliptin was found to be 0.993 and 0.994 respectively.

2.6 Accuracy: Accuracy is the closeness of the obtained results to its true value. The % recovery experimentation was performed by the Standard Addition Method. Fixed quantities of sample mixture of Remogliflozin etabonate and Vildagliptin and increasing quantity of its working standard solutions were spiked at 50, 75,100 and 125 % level of the accuracy.

2.7 Precision: Precision of developed method was evaluated by performing repeatability, interday, intraday precision studies. Interday precision was carried out by analyzing three replicates of three different concentration of active ingredient. The peak area was measured and % RSD was calculated at each concentration level. Intraday precision studies were carried out on same day of different time intervals whereas interday studies were carried out on three different consecutive days using mentioned concentration for all two drugs.

2.8 LOD AND LOQ: Limit of detection (LOD) is the lowest amount of analyte that can be detected but not necessarily quantified. The LOD can be calculated as,

$$LOD = 3.3 \times (SD / Slope)$$

Limit of Quantification (LOQ) Quantitation limit is the lowest amount of sample that can be detected as well as quantified. The LOQ can be calculated as,

$$LOQ = 10 \times (SD/Slope)$$

2.9 Robustness: The solution containing concentration of 50 % & 25% was analyzed at altered mobile phase, pH and flow rate. The peak area found for each solution was measured and % RSD was calculated.

2.10 Assay: Applicability of proposed method was tested by analyzing the commercially available Tablet formulation Remo V.

3.RESULTS AND DISCUSSION: Remogliflozin etabonate and Vildagliptin can be effectively analyzed by the RP- HPLC method with Buffer and Methanol composition of Buffer: Methanol (20: 80 v/v) at a flow rate of 1.0 ml/minute and detection wavelength of 210 nm.The retention time of the drugs was 3.227 and 5.973 minute for vildagliptin and Remogliflozin etabonate respectively. The assay limits for Remogliflozin etabonate and vildagliptin was found to be 98.89 and 99.64% hence the results were within the limits.

3.1 System Suitability:

Table No. 1: System Suitability Data

System Suitability	Remogliflozin etabonate	Vildagliptin
Retention time(min)	5.920	3.207
Resolution(R)	-	10.24
Theoretical plate number(N)	3345	3702

3.2 Specificity: was determined by examining standard drugs and sample of Remogliflozin etabonate and Vildagliptin. The results suggested that proposed method is specific the excipient present in the formulation does not affect the result. The chromatogram taken by running only with mobile phase and after injection of the sample and standard are given below.

3.3 Linearity: The linearity range was found to be 25-150 µg/ml and 12.5-75 µg/ml for Remogliflozin etabonate and Vildagliptin. Calibration curves were plotted between the peak area and the concentrations and the linear regression coefficients for both drugs Remogliflozin etabonate and Vildagliptin were found to be 0.994 and 0.993 respectively . Hence the results obtained within the limits.

Table No. 2: Linearity of Remogliflozin etabonate and Vildagliptin

Sr no.	Remogliflozin etabonate			Vildagliptin		
	Conc.	Mean area ±SD (n=3)	% RSD	Conc.	Mean area ±SD (n=3)	%RSD
1.	25	142617.7963 ±319.10	0.22	12.5	308766.76 ± 252.84	0.081
2.	50	291972.92 ± 782.76	0.26	25	667314.40 ± 799.17	0.119
3.	75	418310.64 ± 424.16	0.10	37.5	916368.587±169.92	0.030
4.	100	572659.78 ± 790.09	0.13	50	1202765.576 ±440.54	0.03
5.	125	788871.40 ± 974.03	0.12	62.5	1620007.059 ± 823.19	0.050
6.	150	920642.42 ± 214.01	0.02	75	1838332.059 ± 235.62	0.012

3.4 Accuracy: The accuracy studies were shown as % recovery for Remogliflozin etabonate and Vildagliptin at four levels; 50 %,75%, 100 % and 150 % (Table 3). The mean % recovery of the Remogliflozin etabonate was 99.6% and Vildagliptin 99.04 %. The limits of % recovery of drugs were 98-102 % and the above results which indicates that the method was accurate the limits of % recovered should be in range of 98-102 %.

Table No.3: Accuracy of Remogliflozin etabonate and Vildagliptin

Targeted Conc. %	Spiked Conc. %	Final Conc.%	Remogliflozin etabonate			Vildagliptin		
			Mean area ±SD	% Recovery	% RSD	Mean area ±SD	% Recovery	% RSD
50	0	50	286078.14±1267.41	98.12	0.44	664144.02±3513.08	99.04	0.52
50	0	50						
50	0	50						

50	25	75	428184.63±4281.63	99.64	0.99	969792.48±1490.47	98.97	0.15
50	25	75						
50	25	75						
50	50	100	574447.87±2228.47	98.72	0.38	1320269.64±5448.86	98.21	0.41
50	50	100						
50	50	100						
50	75	125	713375.20±1930.56	102.14	0.27	1592146.014±6573.23	101.31	0.41
50	75	125						
50	75	125						

3.5 Precision: A binary mixture was obtained of 75%, 100%, 125% and thus each concentration mixture was repeated 3 times during the analysis. Mean area was calculated and % RSD.

Table No. 4: Precision of Remogliflozin etabonate and Vildagliptin

Drug	Conc. (µg/ml)	Repeatability		Intraday		Interday	
		Mean area± SD (n=3)	% RSD	Mean area± SD (n=3)	% RSD	Mean area± SD (n=3)	% RSD
Remogliflozin etabonate	75	418573.98 ±606.57	0.14	418544.31±1434.62	0.34	418185.40 ± 2272.26	0.54
	100	571622.742 ± 545.32	0.095	572093.084 ± 1359.59	0.23	572970.452 ± 2636.97	0.46
	125	789565.791 ± 543.18	0.068	788621.794 ± 1157.14	0.14	786714.844 ± 2554.81	0.32
Vildagliptin	37.5	916121.87 ± 522.44	0.057	916334.776 ± 1201.84	0.13	918853.7733 ± 2522.57	0.27
	50	1202567.6 ± 612.19	0.050	1202669.275 ± 1227.78	0.10	1203969.68 ± 2864.61	0.23

	62.5	1619149.521 ± 425.30	0.026	1620245.955 ± 1386.55	0.085	1616688.956 ± 2760.57	0.17

3.6 LOD and LOQ:

LOD was found to be 0.304 for the Remogliflozin etabonate and 0.923 for Vildagliptin.

LOQ was found to be 0.063 for the Remogliflozin etabonate and 0.191 for Vildagliptin

3.7 Robustness:

Robustness was performed and calculated for each drug. Acceptance criteria – Less than 2%.

Table No. 5: Robustness of Remogliflozin etabonate and Vildagliptin

Sr no.	Remogliflozin etabonate(50 µg/ml)			Vildagliptin(25 µg/ml)		
	pH	Flow rate	Mobile Phase	pH	Flow rate	Mobile Phase
	+ 0.2 units	+0.2 units	+2%	+ 0.2 units	+0.2 units	+2%
	-0.2 units	-0.2 units	-2%	-0.2 units	-0.2 units	-2%
1.	281977.92	282359.84	282475.56	669219.40	669366.41	669622.42
	281473.79	282399.74	282458.78	669286.41	669642.42	669837.44
2.	281689.85	282679.89	282285.73	669589.407	669495.42	669684.42
	281648.63	282559.56	282589.69	669388.40	669684.42	669829.47
3.	282075.8	282882.73	282172.83	669782.409	669595.42	669857.42
	281856.74	282734.54	282597.65	669585.40	669797.42	669985.48
Mean	281914.52	282640.82	282311.37	669530.40	669485.75	669721.42
	281659.72	282564.6	282548.70	669420.07	669708.09	669884.13
SD	163.81	263.62	152.98	286.09	114.81	121.79
	191.71	167.45	77.98	151.99	80.16	87.86

% RSD	0.058	0.093	0.054	0.042	0.0171	0.0181
	0.068	0.059	0.027	0.022	0.011	0.0131

3.8 Analysis of Marketed Formulation:

Table no. 6: Assay result of marketed formulation

Formulation (tablet)	Total amount (mg)		Amount Found (mg)		%Assay	
	RGE	VLD	RGE	VLD	RGE± SD (n=3)	VLD ±SD (n=3)
1.	100	50	98.93	49.82	98.93 ± 1.29	99.64 ± 0.40
2.	100	50	99.05	49.23		
3.	100	50	102.45	50.00		

4. CONCLUSION: The developed HPLC method is accurate, precise and specific. It can be routinely applied for the analysis of Remogliflozin etabonate and Vildagliptin in their combined pharmaceutical formulation.

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