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RESEARCH ARTICLE

RP-HPLC Method Development and Validation for the Estimation of Antidiabetic Drugs Remogliflozin Etabonate and Vildagliptin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Introduction: The present study was done to develop a rapid and precise method by using reverse-phase high-performance liquid chromatography.

Materials and Methods: This method was developed for the validation of Remogliflozin etabonate and vildagliptin in pharmaceutical dosage forms. Chromatography was carried out on a hypersil C18 (4.6×150 mm, 5 µm) column using a mixture of acetonitrile: Water (50:50% v/v) as mobile phase at a flow rate of 1.0 mL/min. The detection was carried out at 259 nm.

Results: The retention times of remogliflozin etabonate and vildagliptin were found to be 2.344 and 3.282 minutes, respectively. This method produced linear responses in the concentration range of 10 to 50 ppm of remogliflozin etabonate 5 to 25 ppm for vildagliptin. The precision for the determination of assay was below 2.0% RSD.

Discussion: This method can be used in the quality control tests of both bulk and pharmaceutical formulations.

Keywords: Remogliflozin etabonate, Vildagliptin-HPLC, Validation.

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INTRODUCTION

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound›s partition coefficient result in differential retention on the stationary phase and thus affect the separation.[1-14]

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High-Performance Liquid Chromatography

Using this chromatography technique, it is possible to perform structural and functional analysis and purification of many molecules within a short time. This technique yields perfect results in the separation and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules. In HPLC, the mobile phase passes through columns under 10 to 400 psi, and with a high (0.1–5 mL/min) flow rate. In this technique, use of small particles and the application of high pressure on the rate of solvent flow increases the separation power of high-performance liquid chromatography (HPLC)and the analysis is completed within a short time.[15-24]

Remogliflozin chemically known as 5-methyl-4-[4-(1-methylethoxy) benzyl]-1-(1-methlethyl)-1H-pyrazol-3-yl 6-O-(ethoxy carbonyl)-B-D-glucopyranoside with an empirical formula $C_{26}H_{38}N_2O_9$ (Figure 1), remogliflozin etabonate is a prodrug of remogliflozin. Remogliflozin etabonate is an antidiabetic drug. Remogliflozin inhibits sodium-glucose co-transport proteins (SGLT) responsible for glucose reabsorption in the kidney. It works by removing excess glucose from the body through urine excretion to reduce hyperglycemia for the treatment of type 2 diabetes. Remogliflozin is selective for SGLT2.

Vildagliptin chemically known as (2S)-1-{2-[(3-hydroxyadamantan-1-yl) amino acetyl} pyrrolidine-2-carbonitrile with empirical formula $C_{17}H_{25}N_3O_2$ (Figure 2). Vildagliptin is an orally active anti-hyperglycemic agent of di peptidyl peptidase-4(DPP-4) enzyme. It is used in type 2 diabetes, which inhibits the inactivation of GLP-1 by DPP-4, allowing GLP-1 to potentiate the secretion of insulin in the beta cells. Vildagliptin has oral bioavailability of 85%, which melts at 138-140°C with a molecular weight 303.399 g/mole.[25-26]

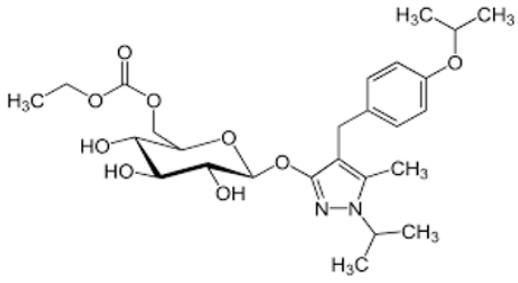

Figure 1: Structure of remogliflozin

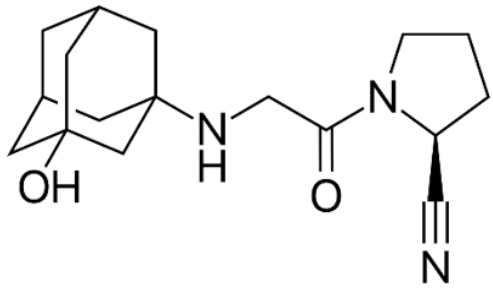

Figure 2: Structures of vildagliptin

Based on our literature review, various methods were developed by HPLC, UPLC, HPTLC for remogliflozin and vildagliptin. The already developed methods were carried out both individually and in combination with other drugs. The retention time of the already developed methods was more along with being less sensitive. Hence, a stability-indicating sensitive, rapid, accurate and valid HPLC method for the simultaneous determination of remogliflozin and vildagliptin for combined dosage form in generic drug development was developed and validated.[27-47]

MATERIALS AND METHOD

Chemicals and Reagents

The reference drugs remogliflozin etabonate and vildagliptin were acquired as generous gift samples from Fourrts India Pvt Laboratories. All of the chemicals utilized were water and methanol for HPLC (LICHROSOLV) and Acetonitrile for HPLC (MERCK) as a mobile phase. The water used in buffer preparation was freshly prepared from Milli Q filtered using a membrane filter.

Equipment

Water HPLC Alliance 2695 separation module, software: Empower 2, 996 PDA Detector. The column used for separation was hypersil C18 (4.6×150 mm, 5.0 μm), UV spectrophotometer (Lab India), Analytical balance (Sartorius), Digital ultra sonicator (Lab man), P^H meter (Lab India), Volumetric flask (Borosil), pipettes and burettes (Borosil), Beakers (Borosil), was used during the study.

Chromatographic Conditions

The optimized conditions for the simultaneous estimation of remogliflozin etabonate and vildagliptin were performed by using the hypersil c18 column (4.6×150 mm, 5.0 μm) as the stationary phase at ambient temperature. The elution was isocratic and the mobile phase comprising of a blend of acetonitrile: water (50:50 v/v) at a flow rate of 1-mL/min was used. The eluent was monitored by 259 nm with run time of 5 minutes and analysis was performed over a wavelength range of 200 to 400 nm. The injection volume was 10 μL. Prior to injecting the solutions, the column was equilibrated using the mobile phase for a minimum of 30 minutes through the system. The retention times of remogliflozin etabonate and vildagliptin under the chromatographic conditions was found to be 2.34 and 3.28 minutes, respectively as shown in Figure 3.

Preparation of Analytical Solutions

Preparation of mobile phase

Accurately measured 500 mL (50%) of acetonitrile and 500 mL of HPLC grade water, degassed in a digital ultrasonic water bath for 10 min, filtered under vacuum filtration through 0.45 μ filter.

Preparation of standard solution (marketed formulation):

Accurately weighed 10 mg of remogliflozin etabonate and vildagliptin, transferred into a10 mL of clean dry volumetric flask, add 5 mL of ACN and sonicate to dissolve and remove air completely and make volume up to the mark with water. Pipette 0.3 mL of the above remogliflozin etabonate and 0.15 mL of vildagliptin stock solutions into a 10 mL volumetric flask and dilute up to the mark with mobile phase.

Inject the samples and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Selection of analytical wavelength

Remogliflozin etabonate and vildagliptin were prepared separately by sufficient dilution of each standard solution with mobile phase, while the blank solution taken is the mobile phase was scanned at 200 to 400 nm and the maximum absorption was found at 259 nm.

Development and validation of HPLC method

The current research was investigated in order to obtain a new, reliable, cost-effective and convenient method for remogliflozin etabonate and vildagliptin, simultaneous determination using HPLC in bulk dosage form that is employed in routine analysis. The method developed has

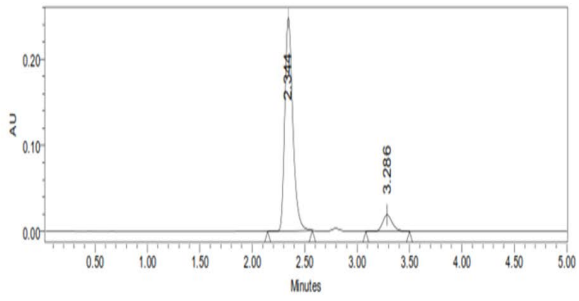

Figure 3: Chromatogram of standard

been validated for parameters such as system suitability, specificity, linearity, detection limit (LoD), quantitation of limit (LoQ), precision, and robustness and for all the parameters, %RSD was determined.

System suitability

System suitability tests on freshly prepared standard solutions of remogliflozin etabonate and vildagliptin were carried out by injecting in five replicates. The standard chromatogram evaluated parameters like plate number (N), retention time, resolution, and tailing factor.

Specificity

Specificity was ascertained whether the method is affected by the presence of other components, marketed formulation, blank and placebo solutions. To determine specificity, remogliflozin etabonate and vildagliptin were studied with parameters like retention time and resolution factor.

Linearity

The linearity of the method developed was elucidated by linear regression analysis and is measured by using the least square method. A series of standard solutions of remogliflozin etabonate and vildagliptin were prepared and injected into the HPLC system at six different concentrations, i.e., 10 to 50 µg/mL (Remogliflozin) 5 to 25 µg/mL (Vildagliptin). Calibration curves for the standard solutions were plotted against respective concentrations with their peak areas. Slope-a, intercept-b and correlation coefficient-R^2 were determined.

Precision

System precision was achieved using six standard concentration replicates. Intermediate precision, and ruggedness were obtained by analyzing the sample under modifying normal test conditions such as analyst and equipment. Retention time and peak area were determined and expressed as mean and %RSD from the data collected.

Accuracy

Accuracy in terms of assay and percent recovery was evaluated for the developed method. The study was performed by standard addition method at 50, 100, and 150% levels. Known amounts of standard solutions of drugs remogliflozin etabonate and vildagliptin were spiked to pre-analyzed sample solutions injected to HPLC system in triplicate and percentage recoveries were calculated using area observed for each level.

Robustness

Robustness of the optimized method was examined by evaluating the effect of small deliberate variations in procedural variables such as flowrate (± 2 mL/min), shift in wavelength (± 2 nm) and proportion of the organic content in the mobile phase (± 2 mL) and % RSD.

Sensitivity

The detection limit (LoD) and quantification limit (LoQ) were determined as the quantities for which the signal-to-noise ratio were 3:1, and 10:1, respectively.

Procedure for assay

The optimized chromatographic conditions were reported with a steady baseline. About 10 µL standard (pure drug) and sample (extracted from tablets) solutions were injected into the HPLC system separately and the chromatogram was recorded. The amount of the drug in the sample may be determined from the peak area of remogliflozin etabonate and vildagliptin.

RESULTS AND DISCUSSION

An effort has been made for a simple, rapid, accurate and precise method for the simultaneous estimation of remogliflozin etabonate and vildagliptin in pure form and in formulation by an isocratic RP-HPLC method. The absorption maximum (λ) remogliflozin etabonate and vildagliptin was found to be 259 nm and same was selected as the detection wavelength in the method development and validation process of the RP-HPLC system.

By changing the chromatographic parameters such as mobile phase composition and pH, an optimized method was developed.

Parameters such as theoretical plates(N), tailing factor (T) and retention time were calculated from the standard chromatogram to evaluate system suitability as shown in Table 1. The process was deemed suitable as the parameters were studied in the range of acceptance criteria.

The specificity of the method suggests that no interferences of mobile phase and placebo or any other excipients co-eluted with the drug and the drug peak as presented in Figure 4, indicating it to be pure in nature and hence the developed method is specific without any interferences of impurities or excipients. The method developed showed linear correlation at different

concentration levels 10 to 50 µg/mL for remogliflozin etabonate and 5 to 25 µg/mL for vildagliptin as shown in Table 2. The calibration curve obtained by plotting peak area *versus* concentration is presented in Figures 5 and 6. The precision of the method was assessed from peak area determinations of six sample solution replicates and %RSD for system precision tabulated in Table 3 was found to be 0.26 and 0.78 and that for intermediate precision Table 4 was found to be 0.25 and 0.42 for remogliflozin etabonate and vildagliptin respectively.

The results reveal that the proposed method is repeatable, reproducible and precise as %RSD was found to be less than 2%. The accuracy of the optimized method was calculated by assay and recovery studies at three concentration levels 50, 100, 150%. The mean percentage recovery values for remogliflozin etabonate and

Table 1: System suitability parameter

Parameter	*Remogliflozin etabonate*	*Vildagliptin*
Theoretical plate (n)	4506	6387
Tailing factor (T)	1.2	1.2
Retention time (R_t)	2.343	3.281

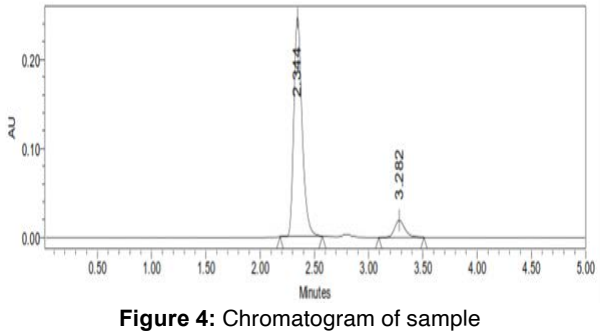

Figure 4: Chromatogram of sample

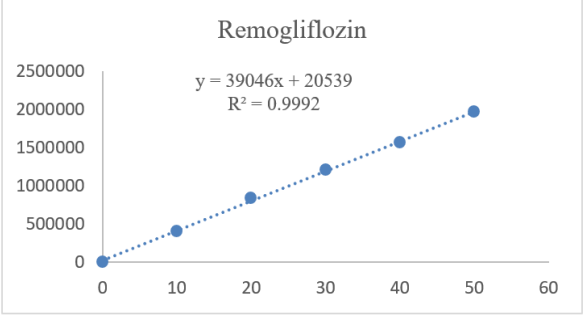

Figure 5: Linearity plot of remogliflozin etabonate

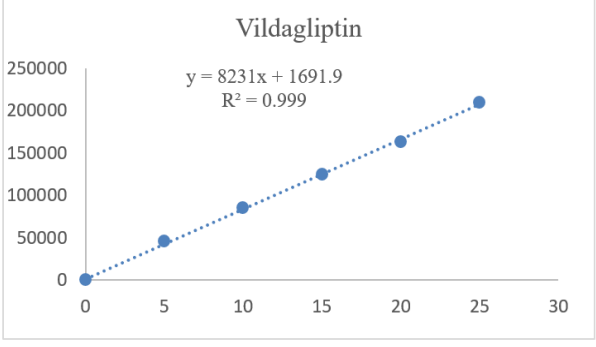

Figure 6: Linearity plot of vildagliptin

Table 2: Linearity data of remogliflozin etabonate and vildagliptin by the proposed method

Remogliflozin etabonate		*Vildagliptin*	
Concentration (g/ml)	*Mean peak area (n = 5)*	*Concentration (g/mL)*	*Mean peak area (n = 5)*
10	408934	5	45510
20	836781	10	84701
30	1203873	15	124802
40	1563458	20	162731
50	1967084	25	209732
Standard deviation(s)	20990.9	Standard deviation(s)	2739.313
Slope (a)	39046	Slope(a)	8231
Intercept(b)	20539	Intercept(b)	1691
Correlation coefficient	0.99	Correlation coefficient	0.999
Regression equation	Y = 39046x + 20539	Regression coefficient	Y=8231×+1691

Table 3: Method precision data of remogliflozin etabonate and vildagliptin by the proposed method

	Area	
S. NO.	*Remogliflozin etabonate*	*vildagliptin*
1	1102729	14149
2	1102947	14066
3	1103236	14271
4	1103977	14291
5	11.9759	14056
Mean	1104530	14166.6
Standard deviation	29610.88	110.721
%RSD	0.26	0.78

Table 4: Intermediate precision data of remogliflozin etabonate and vildagliptin by the proposed method

S.no	*Remogliflozin etabonate*		*Vildagliptin*	
	Day-1	*Day-2*	*Day-1*	*Day-2*
1	1100148	113151	14487	14041
2	1104520	113996	14626	14093
3	1105937	114390	14632	14198
4	1106476	115191	14702	14032
5	1108271	114951	14962	14098
6	1106582	113161	14972	14100
Average	1105322	114140	14730.17	14093.67
Standard deviation	2807.405	869.724	196.2859	59.19685
%RSD	0.25	0.76	1.33	0.42

Table 5: Accuracy data of remogliflozin etabonate and vildagliptin by the proposed method

%concentration (at specification level)	Area	Amount added (ppm)	Amount found (ppm)	%Recovery	Mean recovery
50%	7833	7.5	7.47	99.6	99.4
100%	14023.3	15	14.8	98.6	
150%	20306	22.5	22.5	100	
50%	606659.3	75	74.8	99.7	
100%	1192925	150	149.7	99.8	99.8
150%	1774609	225	224.8	99.9	

Table 6: Robustness data of remogliflozin etabonate

Parameterused for sample analysis	Peak area	Retention time	Theoretical plates	Tailing factor
Actual flow rate of 1.0 mL/min	1128848	2.344	4558	1.3
Less flow rate of 0.8 mL/min	1569971	2.911	7036.3	1.3
More flow rate of 1.2 mL/min	1114875	2.014	4389	1.4
Less organic phase	1120197	2.361	4508.4	1.4
More organic phase	1107845	2.038	4417	1.4

Table 7: Robustness data of vildagliptin

Parameter used for sample analysis	Peak area	Retention time	Theoretical plate	Tailing factor
Actual flow rate of 1.0 mL/min	14391	3.282	6031	1.2
Less flow rate of 0.8 mL/min	15550	4.075	70.6.3	1.2
More flow rate of 1.2 mL/min	13951	3.089	6215	1.2
Less organic phase	14406	4.422	6387.7	1.2
More organic phase	14589	3.015	6285	1.2

vildagliptin was found to be 99.4 and 99.8%, respectively, which showed better recovery values compared to the reported recovery results and are outlined in Table 5. The method was accurate and %recovery limits were in the range of 98 to 102% for Remogliflozin Etabonate and 98 to 102% vildagliptin.

Robustness has been studied with minor but deliberate modifications in parameters such as detection wavelength, pH of buffer in mobile phase and flow rate shown in Tables 6 and 7. Remogliflozin etabonate and vildagliptin LoD were found to be 1.177 and 1.0 µg/mL and LoQ were found to be 5.37 and 3.3 µg/mL, respectively. The data depicts that the method is sensitive (Table 8).

Table 8: Sensitivity data of remogliflozin etabonate and vildagliptin by the proposed method

Parameter	Remogliflozin etabonate	Vildagliptin
LoD (µg/mL)	1.177	1.0
LoQ (µg/mL)	5.37	3.3

CONCLUSION

For the determination of remogliflozin etabonate and vildagliptin from bulk pharmaceutical dosage form a simple, precise, reliable, accurate, economical and rapid method was developed. The developed method that was validated to parameters such as specificity, linearity, accuracy, precision, robustness, LoD, LoQ and system suitability according to ICH guidelines shown values within limits.

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