Research article   *Analytical research*

Development and Validation of a RP-HPLC Method for Simultaneous Determination of Remogliflozin Etabonate and Vildagliptin in Pure Form and Its Pharmaceutical Dosage Form

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ABSTRACT

A simple, rapid, economical, precise and accurate RP-HPLC method for simultaneous estimation of Remogliflozin and Vildagliptinin API form and its Marketed Tablet Dosage Form has been developed and validated as per ICH Guidelines. The separation was achieved by Develosil (250mm x 4.6mm) 5µm Particle size Column and Methanol: Acetate Buffer pH-5.6 (35:65 v/v) used as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 262 nm. Retention time of Remogliflozin and Vildagliptin were found to be 2.345 min and 3.287 min, respectively. The developed method was validated in terms of system suitability, selectivity, linearity, precision, accuracy, limits of detection and quantification for the impurities following the ICH guidelines. Linearity observed for Remogliflozin (10-30µg/ml) and for Vildagliptin (30-70µg/ml). The percentage recoveries of Vildagliptin and Remogliflozin were 100.06% and 100.373% respectively. The developed method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in quality control laboratories and pharmaceutical industries.

Keywords: Remogliflozin and Vildagliptin, RP-HPLC, Validation, Accuracy, Precision.

INTRODUCTION

Analytical chemistry is the branch of chemistry involved in separating identifying and determining the relative amount of the components making up a sample of material. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation. Measurements of physical properties of analytes such as conductivity electrode potential, light absorption or emission mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and preparation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. Most of the instruments methods fit into one of the three following categories viz spectroscopy, electrochemistry and chromatography.

Advantages of instrumental methods

- Small sample can be used.
- High sensitivity is obtained
- Measurements obtained are reliable
- Determination is very fast
- Even complex samples can be handled easily.

Limitation of instrumental methods

- An initial or continuous calibration is required
- Sensitivity and accuracy depends on the onstrument
- Cost of equipment is large
- Concentration range is limited
- Specialized training is needed
- Sizable space is required

High Performance Liquid Chromatography[4,5]

HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch.

The rate of distribution of drugs between Stationary and mobile phase is controlled by diffusion process. If diffusion is minimized faster and effective separation can be achieved. The techniques of high performance liquid chromatography are so called because of its improved performance when compared to classical column chromatography advances in column chromatography into high speed efficient, accurate and high resolved method of separation.

For the recent study metform and Sitagliptin was selected for estimation of amount of analyte present in formulation and bulk drug. The HPLC method is selected in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages

- Speed many analysis can be accomplished in 20min (or) less.
- Greater sensitivity (various detectors can be employed).
- Improved resolution (wide variety of stationary phases).
- Reusable columns (expensive columns but can be used for many analysi)
- Ideal for the substance of low viscosity.
- Easy sample recovery, handling and maintenance.
- Instrumentation leads itself to automation and quantification (less time and less labour)
- Precise and reproducible.
- Integrator itself does calculations
- Suitable for preparative liquid chromatography on a much large scale.

1-Reservior
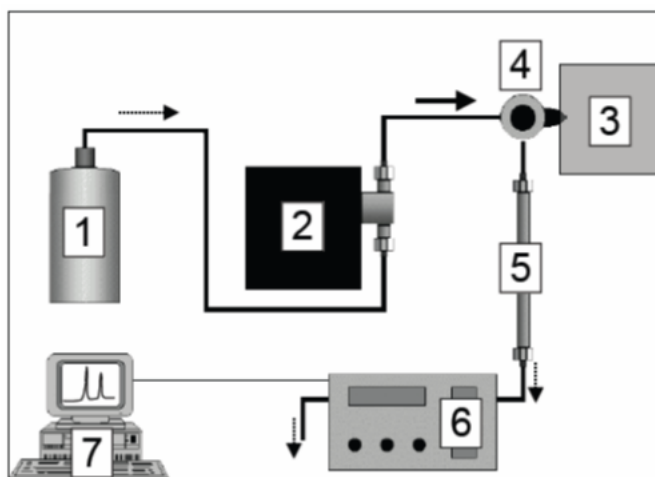2-Pump
3-Autosampler
4-Injectionvalve
5-Column
6-Decetor

Fig 1: HPLC Basic Instrumentation[6,7]:

MATERIALS AND METHODS

Table1: Instruments used

S.No.	Instruments And Glass wares	Model
1	HPLC	WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table 2: Chemicals used

S.No.	Chemical	Brand Names
1	Remogliflozin	Sura labs
2	Vildagliptin	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC
METHOD DEVELOPMENT
TRAILS
Preparation of standard solution: Accurately weigh and transfer 10 mg of Remogliflozin and Vildagliptin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.2ml of Remogliflozin and 0.5ml of Vildagliptin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

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

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water, Acetonitrile and water with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetate Buffer pH-5.6 in proportion 35:65 v/v respectively.

Optimization of Column: The method was performed with various columns like C18 column, Symmetry and X-Bridge. Develosil (250mm x 4.6mm) 5µm Particle size Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS
Instrument used: Waters HPLC with auto sampler and PDA Detector 996 model.

Temperature : 40ºC
Column : Develosil (250mm x 4.6mm) 5µm Particle size Column
Mobile phase : Methanol: Acetate Buffer pH-5.6 (35:65% v/v)
Flow rate : 1ml/min
Wavelength : 262nm
Injection volume : 20 µl
Run time : 5 min

METHOD VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE
Preparation of Acetate Buffer (pH-5.6): Prepare 800mL of distilled water in a suitable container. Add 3.859 g of Sodium Acetate (anhydrous) to the solution. Add 0.176 g of Acetic Acid to the solution. Adjust solution to final desired pH 5.6 using diluted orthophosphoric solution. Add distilled water until volume is 1 L. Filter and sonicate the solution by vaccum filtration and ultra sonication.

Preparation of mobile phase: Accurately measured 350 ml (35%) of Methanol and 650 ml of Acetate Buffer (65%) a were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)
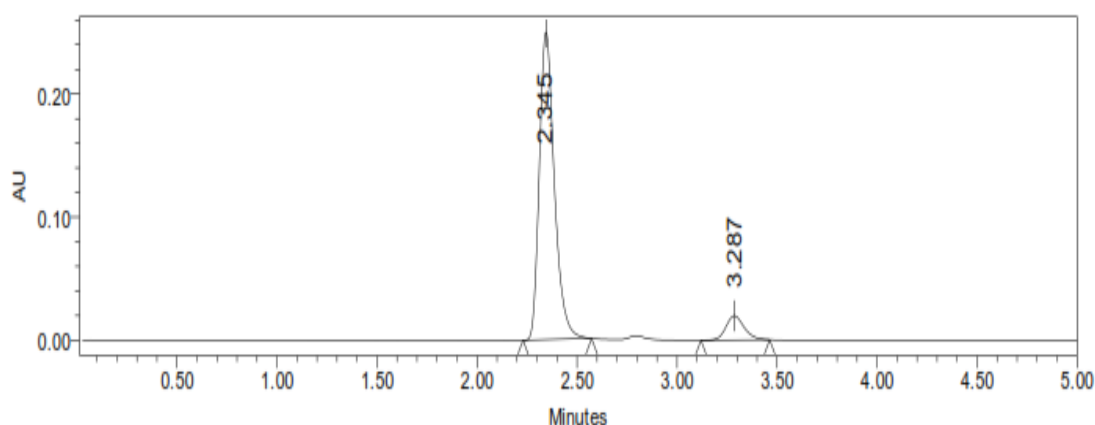
Fig 1: Optimized Chromatogram

Table 3: Peak Results For Optimized Chromatogram

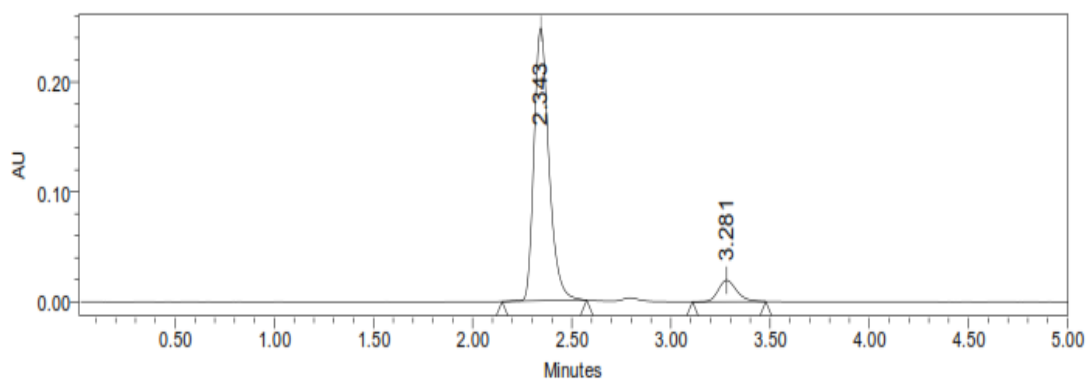| S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Remogliflozin	2.345	1268547	325854		1.48	6587
2	Vildagliptin	3.287	65842	7583	6.48	1.76	8524

Fig 2: Optimized Chromatogram (Sample)

Table 4: Optimized Chromatogram (Sample)

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Remogliflozin	2.343	1357485	336854		1.49	6685
2	Vildagliptin	3.281	66584	7685	6.49	1.77	8657

METHOD VALIDATION

System Suitability

Table 5: Results of system suitability for Remogliflozin

S No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Remogliflozin	2.343	1268598	326586	6598	1.48
2	Remogliflozin	2.343	1269854	325874	6523	1.49
3	Remogliflozin	2.342	1268547	326598	6584	1.48
4	Remogliflozin	2.344	1269856	324857	6597	1.49
5	Remogliflozin	2.342	1263584	325864	6542	1.48
Mean			1268088			
Std. Dev			2598.142			
% RSD			0.204887			

Table 6: Results of system suitability for Remogliflozin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Vildagliptin	3.281	65898	7658	8569	1.76	6.48
2	Vildagliptin	3.285	65974	7596	8574	1.77	6.49
3	Vildagliptin	3.282	65835	7652	8596	1.76	6.48
4	Vildagliptin	3.282	65982	7856	8641	1.76	6.49
5	Vildagliptin	3.282	65368	7729	8499	1.77	6.48
Mean			65811.4				
Std. Dev			255.0506				
% RSD			0.387548				

Specificity

Table 7: Peak Results for assay standard

S.No.	Peak Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Remogliflozin	2.344	1268546	325643		1.48	6582	1
2	Vildagliptin	3.286	65824	7569	6.49	1.77	8547	1
3	Remogliflozin	2.344	1265847	326854		1.49	6596	2
4	Vildagliptin	3.283	65985	7584	6.48	1.76	8569	2
5	Remogliflozin	2.344	1265345	326587		1.49	6574	3
6	Vildagliptin	3.283	65384	7634	6.49	1.78	8573	3

Table 8: Peak results for Assay sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Remogliflozin	2.344	1365845	336582		1.50	6685	1
2	Vildagliptin	3.282	66854	7584	6.50	1.78	8569	1
3	Remogliflozin	2.342	1354282	336895		1.51	6675	2
4	Vildagliptin	3.282	66239	7485	6.49	1.80	8652	2
5	Remogliflozin	2.342	1325485	335242		1.50	6624	3
6	Vildagliptin	3.282	66875	7534	6.50	1.79	8628	3

$$\%\text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$
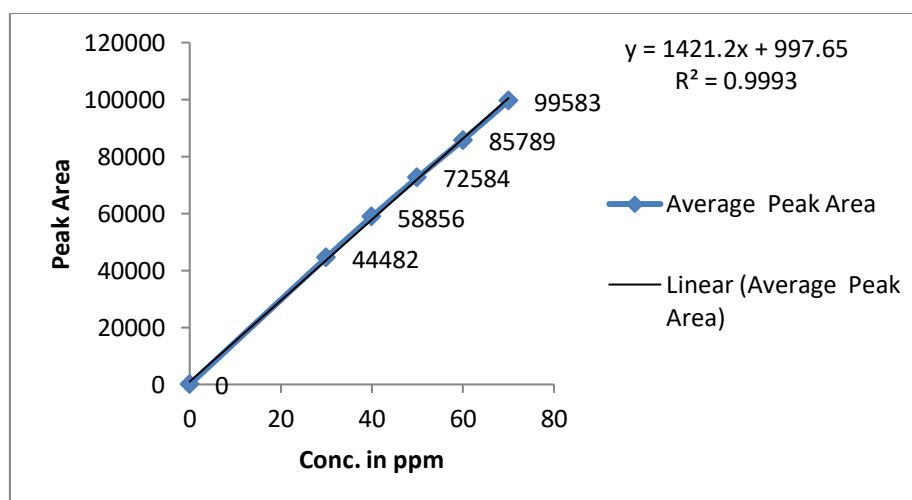
The % purity of Remogliflozin and Vildagliptin in pharmaceutical dosage form was found to be 100.14 %.

Linearity
Chromatographic Data For Linearity Study

y = 63010x + 11037
R² = 0.9998
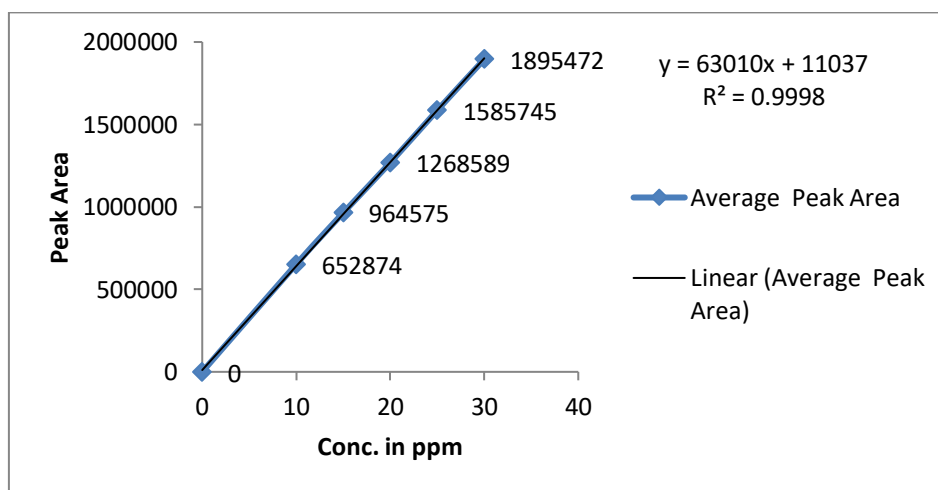
Fig 3: Calibration graph for Remogliflozin

Correlation Coefficient (r) is 0.99, and the intercept is 11037. These values meet the validation criteria.

y = 1421.2x + 997.65
R² = 0.9993

Fig 4: Calibration graph for Vildagliptin
Correlation Coefficient (r) is 0.99, and the intercept is 997.6. These values meet the validation criteria.

Precision
Repeatability

Table 9: Results of Repeatability for Remogliflozin:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Remogliflozin	2.345	1265784	326854	6589	1.48
2	Remogliflozin	2.344	1269853	326523	6587	1.48
3	Remogliflozin	2.343	1265875	326547	6593	1.49
4	Remogliflozin	2.344	1265846	326584	6582	1.49
5	Remogliflozin	2.345	1269852	326985	6598	1.48
Mean			1267442			
Std. Dev			2200.721			
% RSD			0.173635			

Table 10: Results of Repeatability for Vildagliptin:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Vildagliptin	3.287	65874	7569	8569	1.76	6.49
2	Vildagliptin	3.287	65879	7625	8547	1.77	6.50
3	Vildagliptin	3.288	65982	7598	8596	1.76	6.49
4	Vildagliptin	3.285	65289	7581	8621	1.77	6.50
5	Vildagliptin	3.288	65878	7563	8595	1.76	6.49
Mean			65780.4				
Std. Dev			278.4444				
% RSD			0.423294				

Intermediate Precision

Table 11: Results of Intermediate precision Day 1 for Remogliflozin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Remogliflozin	2.344	1358754	336582	6485	1.47
2	Remogliflozin	2.343	1365825	335879	6496	1.47
3	Remogliflozin	2.345	1374582	335682	6478	1.46
4	Remogliflozin	2.344	1385754	334254	6489	1.47
5	Remogliflozin	2.342	1369856	336241	6493	1.47
6	Remogliflozin	2.343	1358542	335898	6524	1.46
Mean			1368886			
Std. Dev			10362.82			
% RSD			0.757026			

Table 12: Results of Intermediate precision Day 1 for Vildagliptin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Vildagliptin	3.281	66985	7689	8659	1.77	6.50
2	Vildagliptin	3.281	66258	7692	8647	1.78	6.51
3	Vildagliptin	3.283	66479	7625	8625	1.78	6.50
4	Vildagliptin	3.281	66358	7648	8692	1.77	6.51
5	Vildagliptin	3.278	66259	7693	8675	1.78	6.51
6	Vildagliptin	3.281	66986	7645	8692	1.78	6.50
Mean			66554.17				
Std. Dev			343.8217				
% RSD			0.516604				

Table 13: Results of Intermediate precision Day 2 for Remogliflozin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Remogliflozin	2.343	1245125	315268	6425	1.46
2	Remogliflozin	2.343	1236589	316895	6398	1.45
3	Remogliflozin	2.342	1241542	315872	6487	1.46

4	Remogliflozin	2.344	1226898	316524	6485	1.45
5	Remogliflozin	2.343	1236524	316598	6426	1.45
6	Remogliflozin	2.344	1245853	314857	6489	1.46
Mean			1238755			
Std. Dev			7056.674			
% RSD			0.569658			

Table 14: Results of Intermediate precision for Vildagliptin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Vildagliptin	3.281	64859	7458	8453	1.75	6.45
2	Vildagliptin	3.285	64985	7496	8365	1.74	6.46
3	Vildagliptin	3.282	64258	7465	8496	1.75	6.45
4	Vildagliptin	3.286	64598	7425	8357	1.73	6.47
5	Vildagliptin	3.283	64535	7463	8345	1.74	6.45
6	Vildagliptin	3.287	63258	7524	8485	1.75	6.47
Mean			64415.5				
Std. Dev			621.8073				
% RSD			0.965307				

Accuracy

Table 15: The accuracy results for Remogliflozin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	641242	10	10.001	100.01%	
100%	1269095	20	19.966	99.83%	100.06%
150%	1907722	30	30.101	100.336%	

Table 16: The accuracy results for Vildagliptin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	36760.67	25	25.167	100.668	
100%	72369.67	50	50.226	100.452	100.373%
150%	107580.3	75	75.005	100.00	

Limit Of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Remogliflozin:

= 2.68μg/ml

Vildagliptin:

= 3.54μg/ml

Limit Of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Remogliflozin:

= 8.04μg/ml

Vildagliptin:

= 10.62μg/ml

Robustness

Table 17: Results for Robustness of Remogliflozin:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1268547	2.345	6587	1.48
Less Flow rate of 0.9 mL/min	1465895	2.911	6452	1.47
More Flow rate of 1.1 mL/min	1234576	2.014	6385	1.46

Less organic phase	1186595	2.361	6458	1.45
More organic phase	1247587	2.038	6268	1.47

Table 18: Results for Robustness of Vildagliptin:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	65842	3.287	8524	1.76
Less Flow rate of 0.9 mL/min	72568	4.075	8825	1.75
More Flow rate of 1.1 mL/min	64258	3.089	8667	1.74
Less organic phase	63597	4.422	8765	1.75
More organic phase	62857	3.015	8396	1.73

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Remogliflozin and Vildagliptin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Remogliflozin was found to be freely soluble in methylene chloride and sparingly soluble in ethanol. Remogliflozin is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF). Vildagliptin is soluble in water. It dissolves in methanol at 50 mg/ml to yield a clear to hazy, colorless solution. It is very slightly soluble in ether and benzene. Methanol: Acetate Buffer pH-5.6 (35:65% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Remogliflozin and Vildagliptin in bulk drug and in Pharmaceutical dosage forms.

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