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Research Article

Bioanalytical Method Development And Validation For The Simultaneous Estimation Of Remogliflozin Etabonate, Vildagliptin And Metformin In Tablet Dosage Form

Sanket G. Kadam*[1], Madhuri D. Game[2], Vaibhav V. Narwade[3]

[1]*Department of Quality Assurance, Vidya Bharti College of Pharmacy, Amravati, Maharashtra*
[2]*Department of Pharmaceutical Chemistry, Vidya Bharti College of Pharmacy, Amravati, Maharashtra*
[3]*Department of Pharmaceutical Quality Assurance, Vidya Bharti College of Pharmacy*

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ABSTRACT

An HPLC method was developed and validated for the simultaneous estimation of Remogliflozin Etabonate, Vildagliptin, and Metformin in tablet dosage form. These drugs are commonly used in the treatment of type 2 diabetes mellitus. The method development involved optimization of chromatographic conditions, selection of suitable solvents and wavelengths, and preparation of standard solutions. The chromatographic conditions were established using a Younglin-HPLC system with a C18 analytical column and a mobile phase consisting of Buffer: Methanol (85:15). The method exhibited good accuracy, linearity, system suitability, and robustness. Assay results of marketed formulations indicated the method's suitability for routine analysis in pharmaceutical laboratories. Overall, the developed HPLC method provides a reliable means for the simultaneous estimation of these antidiabetic drugs in tablet dosage form.

INTRODUCTION

Antidiabetic drugs are medicines developed to stabilise and control blood glucose levels amongst people with diabetes. Type 1 diabetes is a disease in which the body does not make enough insulin to control blood sugar levels. Type 1 diabetes was previously called insulin-dependent diabetes or juvenile diabetes. Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone. Low levels of insulin to achieve adequate response and/or insulin resistance of target tissues, mainly skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes are responsible for these metabolic abnormalities. The severity of

***Corresponding Author:** Sanket G. Kadam
Address: *Department of Quality Assurance, Vidya Bharti College of Pharmacy, Amravati, Maharashtra*
Email : shivsainiksk@gmail.com

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symptoms is due to the type and duration of diabetes. Some of the diabetes patients are asymptomatic especially those with type 2 diabetes during the early years of the disease, others with marked hyperglycemia and especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Uncontrolled diabetes may lead to stupor, coma and if not treated death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome.(1,2,3) Vildagliptin (VGT) [(S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile], Fig. 1,is a new oral anti-diabetic drug belonging to the class of dipeptidyl peptidase-4 inhibitor (reduces glucose-induced glucagon-like peptide 1 and gastric inhibitory polypeptide secretion) 3 and is used as mono therapy in adults with type 2 diabetes mellitus treatment especially in patients inadequately controlled by diet and exercise alone(4,5).

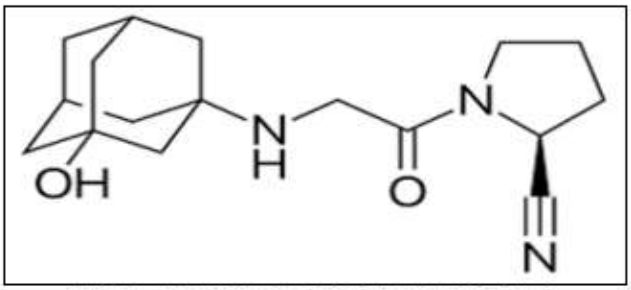

FIG. 1: STRUCTURE OF VILDAGLIPTIN

Metformin Hydrochloride (MTF) is chemically known as [1-carbamimidamido-N, N dimethylmethanimidamide] (Fig. 2) is an oral anti-diabetic drug in the class of biguanides. It is used as the first-line drug for noninsulin-dependent diabetes mellitus treatment 11. It works as improving glycemic control factor through decreasing hepatic glucose production, decreasing glucose absorption, and increasing the insulin-mediated uptake of glucose.

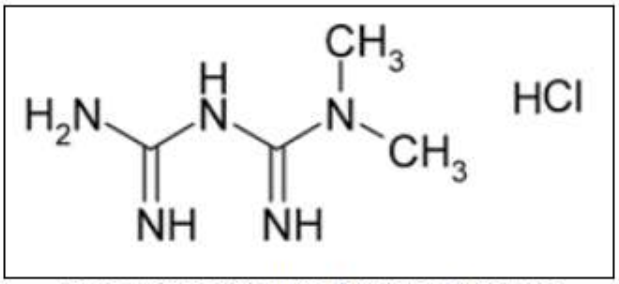

FIG. 2: STRUCTURE OF METFORMIN HCl

Therapeautic indications of metformin competent is indicated as second line treatment of type 2 diabetes mellitus adult patients, particularly overweight patients, who are unable to achieve sufficient glycaemic control at their maximally tolerated dose of oral metformin alone 12 . The mechanism through which metformin HCl decreases blood glucose and lipid concentrations is by activation of the enzyme AMP-activated protein kinase (AMK) and the Peutz-Jeghers protein, LKB1, to regulate AMPK 13. Remogliflozin Etabonate is chemically 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 Fig.1.Vildagliptin Chemically (2S)-1-[2-[(3-hydroxy1adamantyl)amino] acetyl]pyrolidine-2-carbonitrile Remogliflozin Etabonate reduces glucose concentration in type 2 diabetes by blocking renal glucose reabsorption as Vildagliptin prevents the degradation of GLP-1 and GIP, Which are incretion hormones that promote insulin secretion and control blood glucose levels.

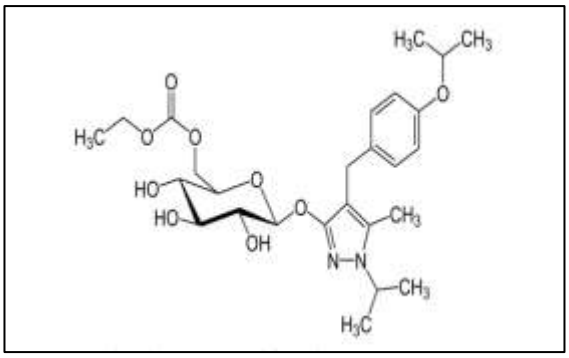

Fig. 3 Remogliflozin Etabonate

MATERIALS AND EQUIPMENTS

The drugs, chemicals, reagents, instruments and filters used during the experiment. Metformine Hydrochloride, Remogliflozin Etabonate and Vildagliptin. API Aarti Drugs Limited, Mumbai Maharshtra, Laurus Labs Limited, Vishakhapatnam, Aandhra Prasdesh

Instruments Used:

Table No 01 Instruments Used In Method Development

Sr. No	Name of instrument	Model
1	HPLC System	Younglin-HPLC system
2	Detector System	Detector – UV detector (730D)
3	Analytical Column	C18 (Hypersil BDS) (4.6 × 250mm, 5µm)
4	Software	Autochrom 3000
5	Ph Meter	M Lab
6	Injector	Manual
7	Analytical Balance	Shimadzu Model-ATX224
8	UV Spectrophotometer	Shimadzu UV1800 Spectrophotometer Japan Corporation)

Solvents And Chemicals:

- Methanol (gradient grade)
- Ammonium Dihydrogen Phosphate Buffer
- Water (HPLC grade)
- Human plasma

EXPERIMENTAL WORK

Optimization of chromatographic condition for the estimation of Metformin Hydrochloride, Vildagliptin and Remogliflozin

Solubility Studies:

As a first step of method development solubility of drugs was tasted in different solvents to obtain a suitable solvent which can be used for method development.

Selection of wavelength

By scanning between 200 and 400 nm, UV spectrum of 10 µg/ml Metformine Hydrochloride, Remogliflozin Etabonate And Vildagliptin in method was captured, a wavelength that provide a favorable reaction for the drug selection. 233 nm was chosen as the wavelength from the UV spectrum. At this Wavelength, drugs exhibited excellent absorption.

Selection of Mobile Phase

The prepare homogenous mixture of 850 ml HPLC grade methanol, 150 ml of buffer pH 3.0 shake well and through membrane filter paper. And sonicated the mobile phase for 5 min in sonicator. After trials Methanol: Buffer, 85:15 was found to be most satisfactory since it gave sharp peak with symmetry within limits and significant reproducible retention time.

Optimization of Chromatographic Condition:

The following chromatographic conditions were established by trial by error and were kept constant throughout the experimentation.

Mobile phase	MeOH : Buffer (85: 15)
Column	Hypersil BDS, C18 column, (250mm × 4.6mm, Particle size 5µm)
Flow Rate	1.0 ml/min
Injection Volume	20µl

Temperature	Ambient
Wavelength	233nm
Run Time	15 min
Elution Mode	Isocratic
Diluent	Mobile phase

Preparation of stock solution

Weight accurately 125 mg MET, 25mg REMO and 12.5 mg VILDA and transferred into 100 ml volumetric flask then dissolved and diluted with diluent and make volume up to mark with help of diluent.

Preparation of Buffer

Ammonium Dihydrogen Phosphate Buffer: Weight Accurately 1.15 gm Ammonium Dihydrogen Orthophosphate dissolve with 900 ml HPLC grade water, add 1ml of Triethylamine shake well sonicate for 5min, adjust pH to 3.0 with diluted Orthophosphoric acid make up volume to 1000 ml with HPLC grade water.

Preparation of Standard solution

a. Metformine Hydrochloride standard Stock solution:

Accurately weighed quantity 125 mg of MET was dissolved in diluent and volume was made up to 100 ml mark (1250 µg/ml). The stock standard solution was diluted further with diluent to get final concentration of about 125µg/ml of MET.

b. Remogliflozin Etabonate standard stock solution:

Accurately weighed quantity 25 mg of REMO was dissolved in diluent and volume was made up to 100 ml mark (250 µg/ml). The stock standard solution was diluted further with diluent to get final concentration of about 25 µg/ml of REMO.

c. Vildagliptin standard stock solution:

Accurately weighed quantity 12.5 mg of VILDA was dissolved in diluent and volume was made up to 100 ml mark (125 µg/ml). The stock standard solution was diluted further with diluent to get final concentration of about 12.5 µg/ml of VILD.

RESULT AND DISCUSSION

For method optimization various mobile phases were tried in different ratios, such as ACN: H2O: TFA (50: 50: 0.1) , MeOH: H2O (90: 10), Buffer: ACN (50: 50) . All these mobile phases were unacceptable due to tailing, fronting and no sharpness in the peak. After various trials mobile phase consisting of Buffer: MeOH (85: 15) was selected which gave sharp peaks with no tailing and fronting. The chromatogram of standard was shown in fig.

Accuracy:

The concentration used were 80 %, 100 %, and 120% to analyte the recovery studies using the standard method. The procedure involved combining 0.8, 1.0, and 1.2 ml, of standard solution with 0.2 ml of solution having 10 µg/ml concentration.

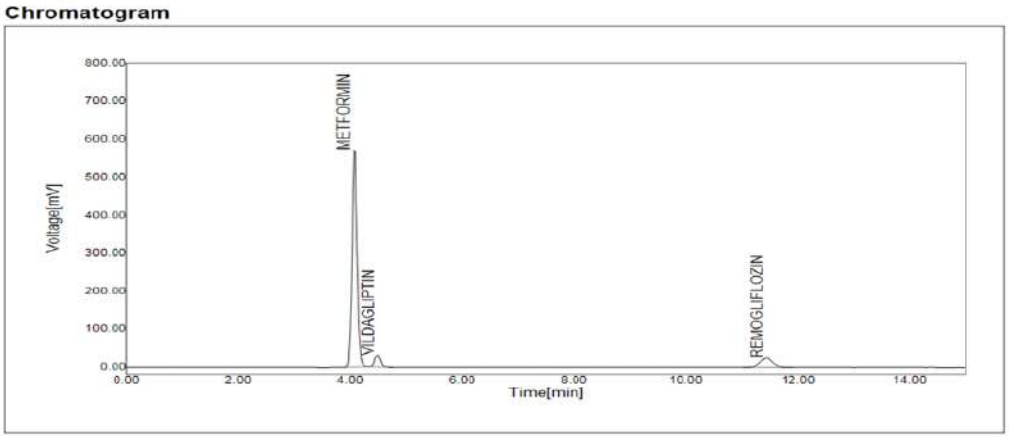

Fig. 4:- Typical chromatogram of standard solution.

Table no 2 Accuracy data of MET by HPLC method

Accuracy	MEAN % recovery	SD	%RSD (NMT 2)
Accuracy at 80 %	101.19	0.7587	**0.75**
Accuracy at 100 %	99.79	1.3642	**1.37**
Accuracy at 120 %	99.52	1.1095	**1.11**

Table no 3 Accuracy data of REMO by HPLC method

Accuracy	MEAN % recovery	SD	%RSD (NMT 2)
Accuracy at 80 %	98.85	1.0393	**1.05**
Accuracy at 100 %	98.99	1.3464	**1.36**
Accuracy at 120 %	100.06	0.8268	**0.83**

Table no 4 Accuracy Data of VILDA by HPLC method

Accuracy	MEAN % recovery	SD	%RSD (NMT 2)
Accuracy at 80 %	100.23	1.3311	1.33
Accuracy at 100 %	101.43	0.7264	0.72
Accuracy at 120 %	100.19	1.2714	1.27

Linearity

1. The linearity for Metformin was determined in the range of 25-75µg/ml. The regression equation was found to be y = 27.33x -5.7783 R^2 = 0.9991. Data for calibration curve was shown in Table 3 and the calibration curve was shown in Fig

Table no 6 Calibration Standard Peak Area

Con. (ppm or ug/ml)	Area
62.50	1725.1638
93.75	2566.6743
125.00	3374.3809
156.25	4222.0439
187.50	5169.0234
Correlation coefficient (r) (NLT 0.995)	0.9996
Intercept	5.7783
Slope	27.338

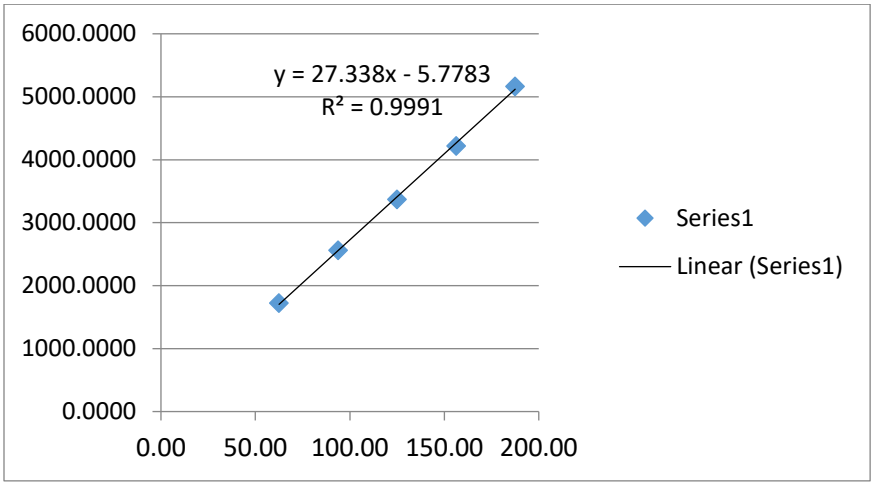

Fig 5 Linearity and range of Metformin

2. The linearity for Remogliflozin etabonate was determined in the range of 25-75µg/ml. The regression equation was found to be y = 28.807x -25.635 R^2 = 0.999. Data for calibration curve was shown in Table 3 and the calibration curve was shown in Fig

Table no 7 Calibration Standard Peak Area

Con. (ppm or µg/ml)	Area
12.50	159.5447
18.75	237.2726
25	334.4115
31.25	425.4406
37.50	515.5632
Correlation coefficient (r) (NLT 0.995)	0.9995
Intercept	25.6355
Slope	28.8066

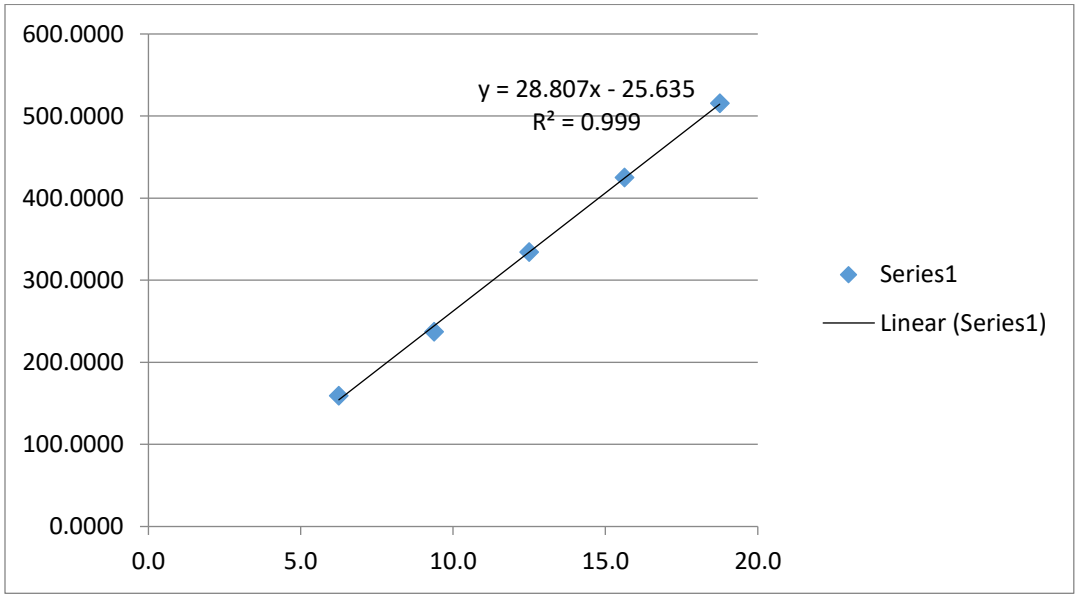

Fig 6 Linearity and range of Remogliflozin Etabonate

3. The linearity for Vildagliptin was determined in the range of 25-75µg/ml. The regression equation was found to be y = 17.22x -2.2601 R^2 = 0.999. Data for calibration curve was shown in Table 3 and the calibration curve was shown in Fig.

Table no 8 Calibration Standard Peak Area

Con. (ppm or µg/ml)	**Area**
6.25	108.5816
9.38	157.4274
12.50	209.6895
15.63	265.7746
18.75	323.4686
Correlation coefficient (r) (NLT 0.995)	0.9994
Intercept	-2.2601
Slope	17.2199

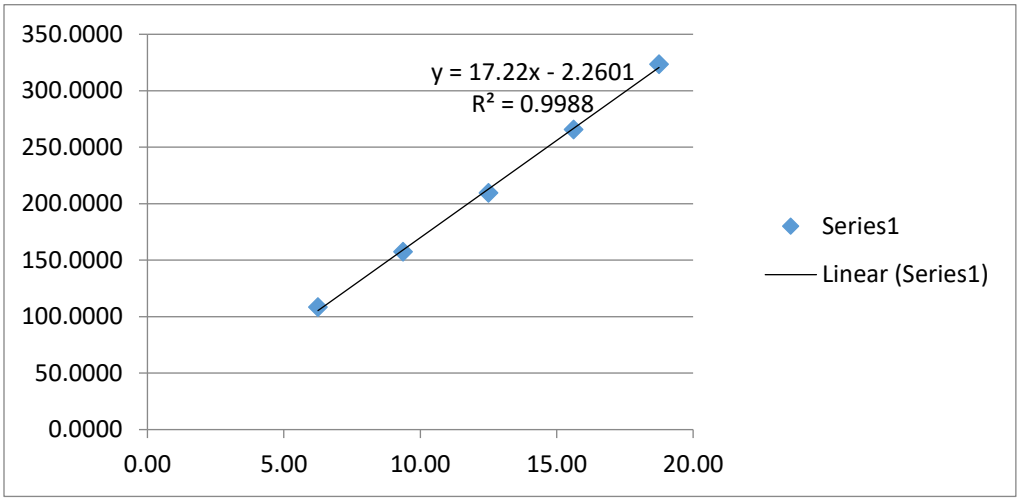

Fig 7 Linearity and range of Vildagliptin

System Sutability

System Suitability parameter were shown to be within specified limits. Column efficiency (theoretical plates), resolution factor and peak asymmetry factor, tailing factor, LOQ and LOD are the system suitability parameter.

Table no 9 System Suitability Studies for MET

Name	**Area**	**RT(min)**	**TP (NLT 2000)**	**TF (NMT 2.0)**	**Resolution (NLT 2.0)**
Standard _Inj_01	353.4425	11.23	19038	0.98	28.6
Standard_Inj_02	352.6442	11.17	14152	1.01	24.86
Standard_Inj_03	343.7445	11.13	14244	0.97	25.79
Standard_Inj_04	344.6841	11.08	17004	0.93	27.64
Standard_Inj_05	349.0739	11.02	16961	0.94	28.66

Mean	3424.4874	4.10
SD	64.3084	0.0421
%RSD (NMT 2)	**1.88**	**1.03**

Table no 10 System Suitability Studies for REMO

Name	Area	RT(min)	TP (NLT 2000)	TF (NMT 2.0)	Resolution (NLT 2.0)
Standard _Inj_01	3338.3801	4.13	17888	1.08	3.00
Standard_Inj_02	3498.8247	4.12	12743	1.12	2.62
Standard_Inj_03	3466.7207	4.13	17251	1.07	3.02
Standard_Inj_04	3435.2761	4.03	12463	1.11	2.80
Standard_Inj_05	3383.2354	4.10	16397	1.14	3.12

Mean	348.7178	11.13
SD	4.4405	0.0808
%RSD (NMT 2)	**1.27**	**0.73**

Table no 11 System Suitability Studies for VILDA

Name	Area	RT(min)	TP (NLT 2000)	TF (NMT 2.0)	Resolution (NLT 2.0)
Standard _Inj_01	208.8208	4.53	16106	1.03	3.00
Standard_Inj_02	201.2381	4.52	12828	1.01	2.62
Standard_Inj_03	205.2859	4.53	17093	0.99	3.02
Standard_Inj_04	207.7938	4.43	15788	1.00	2.80
Standard_Inj_05	204.9947	4.48	23278	1.04	3.12

Mean	205.6267	4.50
SD	2.9442	0.0432
%RSD (NMT 2)	**1.43**	**0.96**

Limit of Detection (LOD) and Limit of Quantification

Limit of Detection (LOD)

The limit of detection is the lowest concentration of an analyte that can be detected in a sample but not necessary quantitated, under the given experimental conditions.

Limit of Quantification (LOQ)

It is the lowest concentration of analyte in a sample that can be accurately and precisely identified under the given experimental condition.

LOD and LOQ were determined using the following formulas

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

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

Where, SD = Standard deviation

S = Slope

Table no 12 LOD and LOQ data of MET

Con.(ppm or µg/ml)	Area
62.50	1725.1638
93.75	2566.6743
125.00	3374.3809
156.25	4222.0439
187.50	5169.0234

Correlation coefficient (r)	0.9996
STEYX	45.7214
SLOPE	27.3379
LOD (µg/ml)	5.52
LOQ (µg/ml)	16.72

Table no 13 LOD and LOQ data of REMO

Con. (ppm or µg/ml)	**Area**
12.50	159.5447
18.75	237.2726
25	334.4115
31.25	425.4406
37.50	515.5632
Correlation coefficient (r)	0.9995
STEYX	5.1539
SLOPE	28.8066
LOD (µg/ml)	0.59
LOQ (µg/ml)	1.79

Table no 14 LOD and LOQ data of VILDA

Con. (ppm or µg/ml)	**Area**
6.25	108.5816
9.38	157.4274
12.50	209.6895
15.63	265.7746
18.75	323.4686
Correlation coefficient (r)	0.9994
STEYX	3.3418
SLOPE	17.2199
LOD (µg/ml)	0.64
LOQ (µg/ml)	1.94

ROBUSTNESS:

For the parameter like flow rate, wavelength and the chosen solution was used for a robustness assessment. % RSD (NMT 2) should not be present in the variation. The percent assay should also fall between 98- 102 %

Table no 15 Robustness Changes in Method Parameter for REMO

Name	**Preparations**	**%Assay**
Original method parameters	Test prep-1	98.21
Original method parameters	Test prep-2	98.27
Flow rate 0.97 ml/min	Test prep	98.15
Flow rate 1.03 ml/min	Test prep	99.56
Wavelength 231 nm	Test prep	98.22
Wavelength 235 nm	Test prep	99.06
Mean		98.58
SD		0.5896
%RSD (NMT 2)		**0.60**

Table no 16 Robustness Changes in Method Parameter for MET

Name	Preparations	%Assay
Original method parameters	Test prep-1	99.09
Original method parameters	Test prep-2	98.32
Flow rate 0.97 ml/min	Test prep	102.51
Flow rate 1.03 ml/min	Test prep	99.55
Wavelength 231 nm	Test prep	101.16
Wavelength 235 nm	Test prep	102.53
Mean		100.53
SD		1.8020
%RSD (NMT 2)		**1.79**

Table no 17 Robustness Changes in Method Parameter for VILDA

Name	Preparations	%Assay
Original method parameters	Test prep-1	99.27
Original method parameters	Test prep-2	100.46
Flow rate 0.97 ml/min	Test prep	98.60
Flow rate 1.03 ml/min	Test prep	101.91
Wavelength 231 nm	Test prep	98.99
Wavelength 235 nm	Test prep	101.61
Mean		100.14
SD		1.4034
%RSD (NMT 2)		**1.40**

ASSAY:

%Assay determination of marketed formulation.

Table no 18 Assay of Metformin Hydrochloride

Name	Area	RT(min)	Metformin obs. In mg	Label claim in mg	% Assay
Test solutions-1	3390.6221	4.12	493.79	500	98.76
Test solutions-2	3361.5525	4.03	488.95	500	97.79

Table no 19 Assay of Remogliflozin Etabonate

Name	Area	RT(min)	Remo obs. In mg	Lable claim in mg	% Assay
Test solutions-1	341.1055	10.95	97.57	100	97.57
Test solutions-2	339.9330	10.83	97.11	100	97.11

Table no 20 Assay of Vildagliptin

Name	Area	RT(min)	Vilda obs in mg	Lable claim
Test solutions-1	204.1355	4.50	49.51	50
Test solutions-2	213.1782	4.42	51.64	50

CONCLUSION:

The developed HPLC method showed good accuracy, linearity, system suitability, and robustness for the simultaneous estimation of Remogliflozin Etabonate, Vildagliptin, and Metformin in tablet dosage form. The assay results

indicated that the method is suitable for analyzing the marketed formulation. Overall, the method can be considered reliable for routine analysis in pharmaceutical laboratories.

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