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BIOANALYTICAL METHOD DEVELOPMENT & VALIDATION FOR SIMULTANEOUS ESTIMATION OF VILDAGLIPTIN AND REMOGLIFLOZIN ETABONATE IN PLASMA

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ABSTRACT

The Bioanalytical Method was Developed and Validated for the Simultaneous Estimation of Remogliflozin Etabonate and Vildagliptin in Tablet formulation. Both drug was Extracted by Protein Precipitation Method. Internal standard used as a Metformin. The estimation was achieved on the C18 column with Isocratic mixture Phosphate Buffer: Acetonitrile pH 3 adjusted with 0.1 % Orthophosphoric acid in the ratio of 30:70 v/v was used as Mobile Phase at flow rate 0f 1.0 ml/min. injection volume 20µl. and wavelength of detection was kept at 229 nm. The Retention time of Remogliflozin was 3.1 min and Vildagliptin was 6.1 min. Metformin 1.5 mint. The Linearity of the proposed method was investigated in the range of 10 µg/ml- 160 µg/ml for the formulation. The correlation coefficient for Remogliflozin was 0.999 & for vildagliptin was 0.997. The proposed method was validated as per ICH guidelines for linearity, accuracy, precision, and robustness for the estimation of the formulation.

Keywords: Bioanalytical, Validation, High Performance liquid chromatography, ICH

INTRODUCTION

BIOANALYTICAL METHOD:

Bioanalysis is a method for determining the concentration of drugs, their metabolites, and/or endogenous substances in biological specimens such as blood plasma, serum, cerebrospinal fluid, urine, and saliva. Bioanalytical methods are widely used to quantify drugs and their metabolites in physiological matrices, and the methods could be applied to study in human clinical pharmacology and non-human pharmacology / toxicology. It supports the conduct of studies such as pharmacodynamics, toxicology, pharmacokinetics, bioequivalence, therapeutic drug monitoring (TDM), and clinical trials. In the early stages, these studies are only carried out to identify overdosage conditions and in toxicological studies. When the concentration of a drug in a biological matrix is known, pharmacokinetic parameters can be calculated. Bioanalytical studies are essential in the discovery and development of new drugs. As a result, these studies are expertly carried out.

Bioanalysis can determine the therapeutic efficacy of a specific drug. Bioanalysis is important in the pharmaceutical industry. The following steps are involved in bioanalysis.

- Selection and collection of biological fluid
- Preparation of sample – Analyte extraction from biological matrix.
- Analyte detection done by various methods.

After selection of biological fluid, the required analyte should be extracted from it.

This step in bioanalytical method is more important because sample preparation can be done by different methods of extraction.

So, there are following types of extraction techniques:

1. Dilution followed by injection
2. Solid Phase extraction (off line/online)
3. Protein precipitation
4. Filtration
5. Liquid-liquid extraction
6. Protein removal by equilibrium dialysis or ultrafiltration
7. Restricted access media
8. Solid-supported liquid-liquid extraction
9. Monolithic columns
10. Immunoaffinity extraction

Out of all these methods, only three of them are most prominently used.

1. Protein precipitation method
2. Liquid-liquid extraction method (LLE)
3. Solid-phase extraction method (SPE)

1. **Protein precipitation method:** Protein precipitation is based on the interaction between the precipitation reagent and protein groups. When a volume of solvent (usually acetonitrile) is added to serum, the proteins precipitate, leaving the analyte of interest in the solvent, which can then be injected directly or dried down and reconstituted in a smaller volume.

2. Liquid-liquid extraction method: Liquid-liquid extraction is a common technique used to extract analytes from liquid matrices. LLE typically has one aqueous phase (often the denser or heavier phase) and one organic solvent phase (usually the lighter phase). The polar aqueous phase is preferred by hydrophilic compounds, whereas the organic solvent is preferred by hydrophobic compounds. The basic principle is the tendency of an analyte to prefer one solvent over another immiscible solvent. The process is based on the Nernst distribution law, which states that any species will distribute between two immiscible solvents so that ratio of the concentrations remain constant.

3. Solid-phase extraction method: Solid-phase extraction (SPE) is a sample preparation method that separates compounds that are dissolved or suspended in a liquid mixture based on their physical and chemical properties from other compounds in the mixture. SPE separates a mixture into desired and undesired components by utilizing the affinity of solutes dissolved or suspended in a liquid for a solid through which the sample is passed. As a result, the stationary phase retains either the desired analytes of interest or the undesired impurities in the sample. If the portion retained on the stationary phase includes the desired analytes, they can then be removed from the stationary phase by washing the stationary phase with an appropriate eluent.The leading cause of death worldwide is diabetes mellitus **[1-3]**. Numerous drugs are used to minimise the impact of diabetes on its side effects, including renal failure, retinopathy, neuropathy, and cardiovascular issues **[4]**. According to its chemical formula, Remogliflozin etabonate is 5-Methyl4-[4-(1-methyl ethoxy) benzyl]. 1H-pyrazol-3-yl-6-O-(ethoxycarbonyl)-Dglucopyranoside is a chemical that contains 1-(1-methylethyl). It belongs to the group of SGLT 2 inhibitors. The reabsorption of glucose by the kidney is controlled by these transporters. Through blockage of transporters, blood glucose is eliminated through the urine. The structural formula is shown in **Figure 1** [Vildagliptin is an incretion hormone that maintains glucose homeostasis and regulates blood sugar levels. It is an analogue of the peptides glucosesubordinate insulin tropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). According to estimation GLP-1 and GIP action, estimates 0 % of the insulin response to an oral glucose challenge is produced.

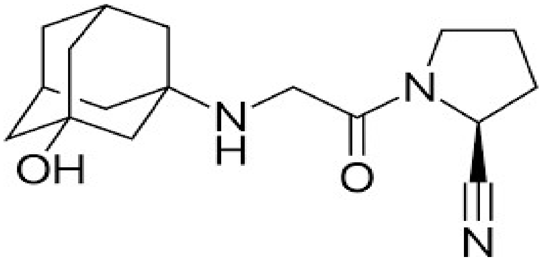

Figure 1: Chemical structure of vildagliptin

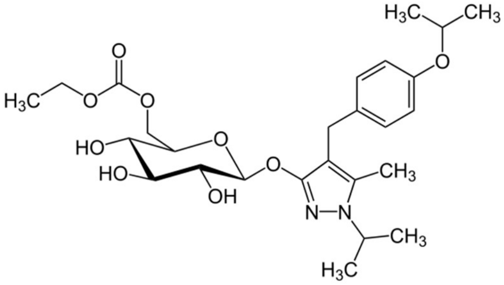

Figure 2: Chemical structure of Remogliflozin

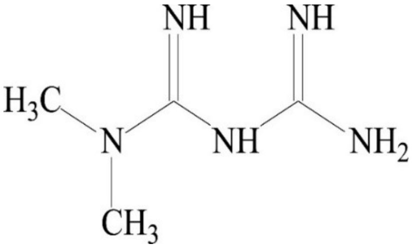

Figure 3: Chemical structure of Metformin (Internal Standard)

Vildagliptin, Remogliflozin, and Etabonate have no published literature, whereas Dapagliflozin, Empagliflozin, and canagliflozin do, according to a review of the literature. Using analytical techniques including spectrophotometric HPLC and LC-MS Remogliflozin Etabonate and vildagliptin were quantified in pharmaceutical dosage form and biological samples. Vildagliptin is estimated using HPLC together with Canagliflozin and empagliflozin. In the current work, a simple, exact, and accurate HPLC method is being developed to estimate Remogliflozin Etabonate and vildagliptin in bulk and pharmaceutical dose form. The procedure has received Approval in line with ICH M10 requirements.

MATERIAL AND METHOD

Chemicals and Reagents

Vildagliptin and remogliflozin etabonate were gifts from the companies. Acetonitrile, methanol, distilled water, and ethanol of HPLC-grade were utilised. In this study, tablets were used as a Medical dosage form and were purchased from a local pharmacy in Vadodara, India (Trade name is Remozen v containing 100 mg Remogliflozin and 50 mg

Vildagliptin manufactured by Glenmark Pharmaceuticals).

The HPLC was employed (Isocratic, Shimadzu, Japan). The pump, injector, column, detector, and data processor are some of its parts. The HPLC C18 column was used.

Solubility Study:

50mg of Powder was weighed and transferred in a clean and dry test tube and then the solubility of this sample was checked in above solvent.

Table: 1 Solubility Study of Remogliflozin Etabonate

Solvent	Vildagliptin
Water	Freely soluble
Methanol	Freely soluble
Acetonitrile	Freely soluble

Table: 2 Solubility Study of Vildagliptin

Solvent	Remogliflozin etabonate
Water	Not soluble
Methanol	Freely soluble
Ethanol	Freely soluble
Acetonitrile	Freely soluble
DMSO	Freely soluble

Melting point study

Melting Point of Remogliflozin Etabonate: A small quantity of Glimepiride powder was weighed and then the powder was placed in a glass capillary tube. Then the glass capillary tube is placed inside the aluminum block inside the sample chamber. Further, the block is heated by increasing the temperature and the sample is observed through the magnifying lens on the viewing tube. Finally, the temperature is noted at which the powder melts.

The reported range for Remogliflozin Etabonate is 151 ºC -153 ºC and the observed range of temperature is152 ºC - 154˚ ºC

Melting Point of Vildagliptin: A small quantity of Metformin powder was weighed and then the powder was placed in a glass capillary tube. Then the glass capillary tube is placed inside the aluminum block inside the sample chamber. Further, the block is heated by increasing the temperature and the sample is observed through the magnifying lens on the viewing tube. Finally, the temperature is noted at which the powder melts.

The reported range for Vildagliptin is 149 ºC -152ºC and the observed range of temperature is 150ºC - 152ºC

FTIR Spectra Characterization of Remogliflozin Etabonate

Figure 4: IR Spectra of Remogliflozin Etabonate

Table: 3 FTIR Interpretation of Remogliflozin Etabonate

Functional group	Wave number (cm-1)
O-H (Alcohol)	1265
N–H bend (Amine)	1591
C=C Stretch (Alkenes)	1642
C=O Stretch (Ketones)	1746
Hydrogen bonded asymmetric (primary Amide)	3343
$(N=O)_2$ (Nitro compound)	1506
C-H Bending (Alkanes)	1464,1422
C – H Bending (Aldehyde)	1383

FTIR Spectra Charactrization of Metformin

Figure 5: FTIR Spectra of Metformin

Table 4: FTIR Interpretation of Vildagliptin

Functional group	Wavenumber (cm-1)
-N – H (primary amine)	3371, 3294
-N – H (secondary amine)	3174
-C – H (Aliphatic)	2814
-C=N (Imine)	1625
C – N (amine)	2814
C – C	1254
O – H (carboxylic acid)	2691

Selection of wavelength

The UV spectra of 10 g/ml of remogliflozin etabonate, vildagliptin, and metformin in methanol were recorded and were scannable between 200 and 400 nm. Remogliflozin's maximum absorbance was recorded at 228 nm. At 210 nm, vildagliptin's greatest absorbance was noted. Metformin showed the maximum absorption at 233 nm. The isosbestic point was found to be 229 nm away. The UV wavelength of 229 nm, which was picked from the UV spectrum, showed good absorption for the drug.

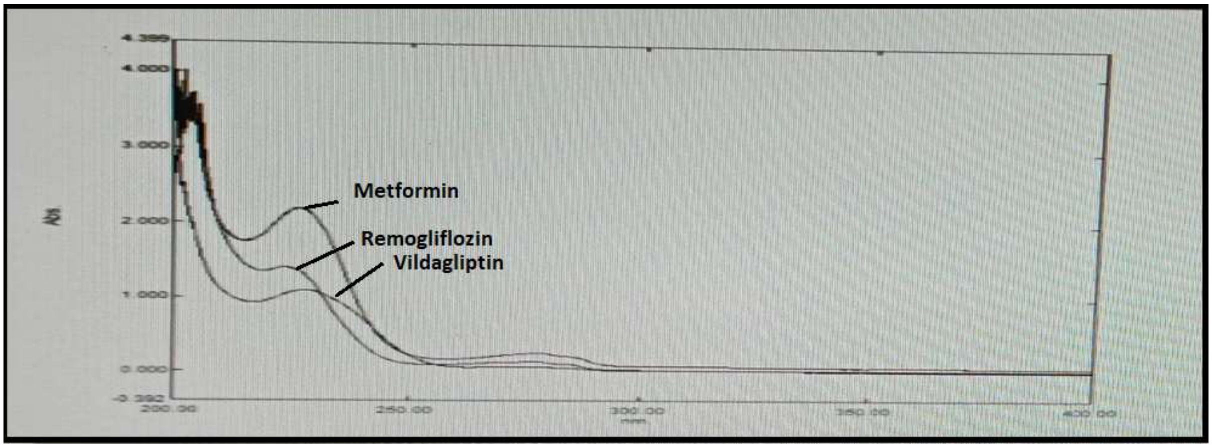

Figure 5: Wavelength selection by UV spectra of synthetic mixture

HPLC Trial 1(Synthetic Mixture)

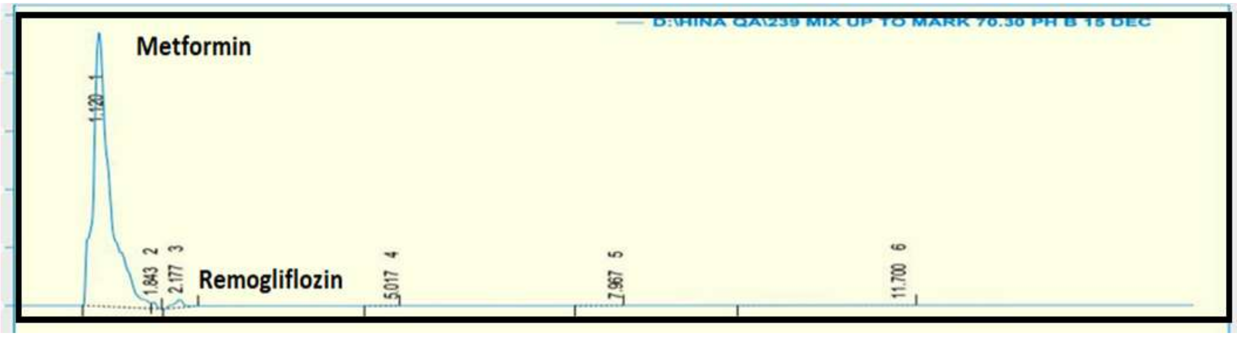

Figure 6: HPLC Trail 1

Table 5: HPLC Trail 1(Synthetic Mixture)

Solvent	Mobile phase	Ratio	pH	Retention time	Area	Height	Wavelength
Remogliflozin	**Phosphate buffer: acetonitrile**	**70:30**	**5**	**2.1min**	**121.0**	**13.5**	**229**
Vildagliptin	**Phosphate buffer: acetonitrile**	**70:30**	**5**	**-**	**-**	**-**	**-**
Metformin(IS)	**Phosphate buffer: acetonitrile**	**70:30**	**5**	**1.1 min**	**7267.3**	**470.4**	**229**

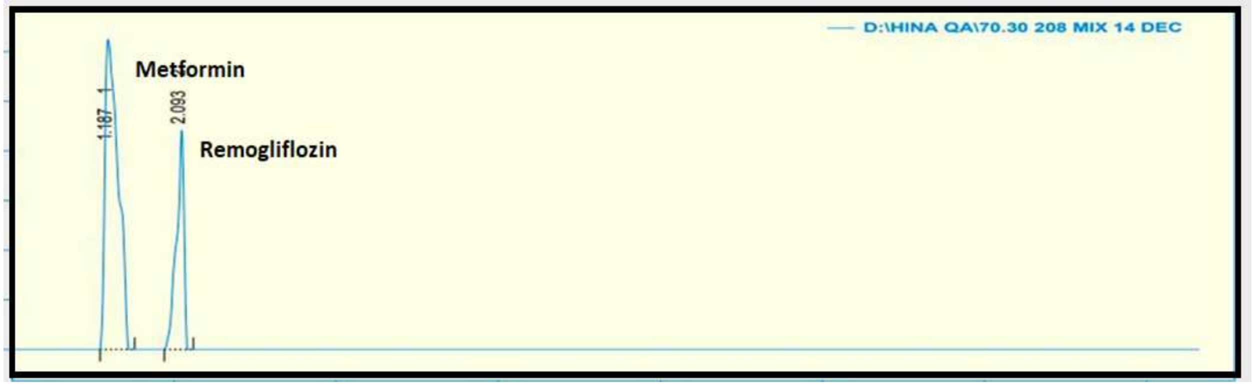

Figure 7: HPLC Trail 2

Table 6: HPLC Trail 2(Synthetic Mixture)

Solvent	Mobile phase	Ratio	pH	Retention time	Area	Height	Wavelength
Remogliflozin	**Phosphate buffer: acetonitrile**	**70:30**	**3**	**2.0min**	**569.4**	**88.1**	**229**
Vildagliptin	**Phosphate buffer: acetonitrile**	**70:30**	**3**	**-**	**-**	**-**	**-**
Metformin(IS)	**Phosphate buffer: acetonitrile**	**70:30**	**3**	**1.1 min**	**1409.6**	**124.8**	**229**

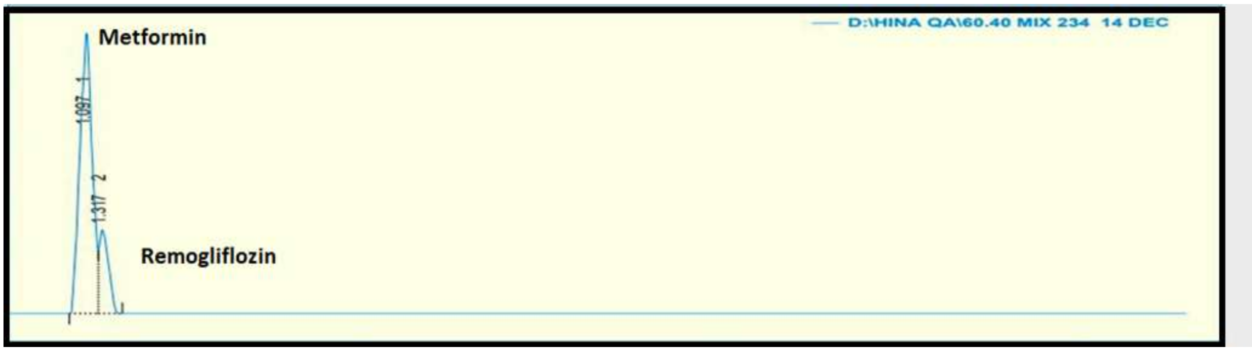

Figure 8: HPLC Trail 3

Table.7: HPLC Trail 3(Synthetic Mixture)

Solvent	Mobile phase	Ratio	pH	Retention time	Area	Height	Wavelength
Remogliflozin	**Phosphate buffer: acetonitrile**	**60:40**	**4.5**	**1.2 min**	**662.6**	**72.4**	**229**
Vildagliptin	**Phosphate buffer: acetonitrile**	**60:40**	**4.5**	**-**	**-**	**-**	**-**
Metformin(IS)	**Phosphate buffer: acetonitrile**	**60:40**	**4.5**	**1.0 min**	**2948.2**	**243.0**	**229**

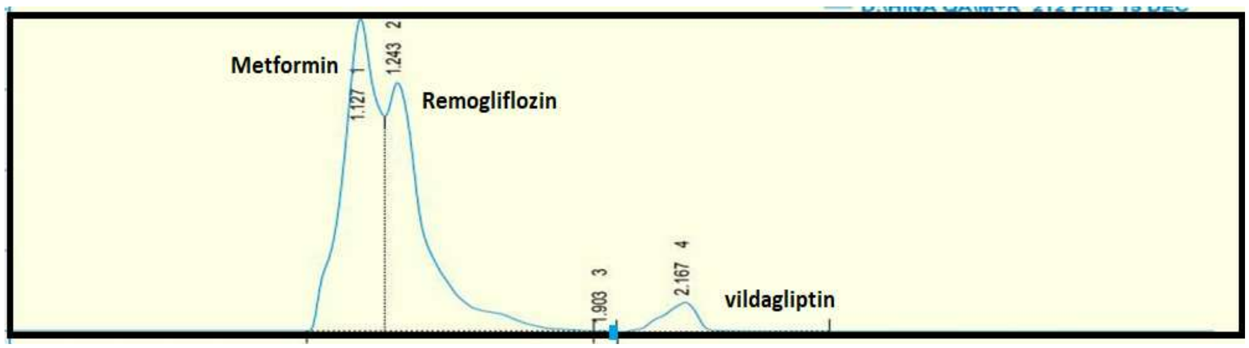

Figure 9: HPLC Trail 4

Table 8: HPLC Trail 4(Synthetic Mixture)

Solvent	Mobile phase	Ratio	pH	Retention time	Area	Height	Wavelength
Remogliflozin	**Phosphate buffer: acetonitrile**	**60:40**	**4.5**	**1.2 min**	**5455.8**	**617.2**	**229**
Vildagliptin	**Phosphate buffer: acetonitrile**	**60:40**	**4.5**	**2.1 min**	**581.6**	**73.3**	**229**
Metformin(IS)	**Phosphate buffer: acetonitrile**	**60:40**	**4.5**	**1.1 min**	**6053.8**	**775.2**	**229**

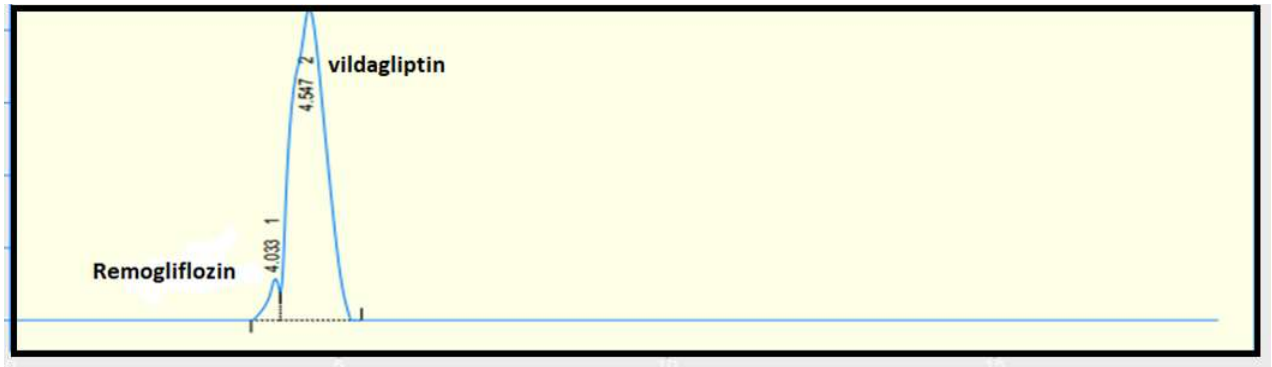

Figure 10: HPLC Trail 5

Table.9: HPLC Trail 5(Synthetic Mixture)

Solvent	Mobile phase	Ratio	pH	Retention time	Area	Height	Wavelength
Remogliflozin	**Acetate buffer: methanol**	**60:40**	**4.5**	**4.0min**	**136.3**	**11.436**	**229**
Vildagliptin	**Acetate buffer: methanol**	**60:40**	**4.5**	**4.5**	**3045.3**	**85.8**	**229**
Metformin(IS)	-	-	-	-	-	-	-

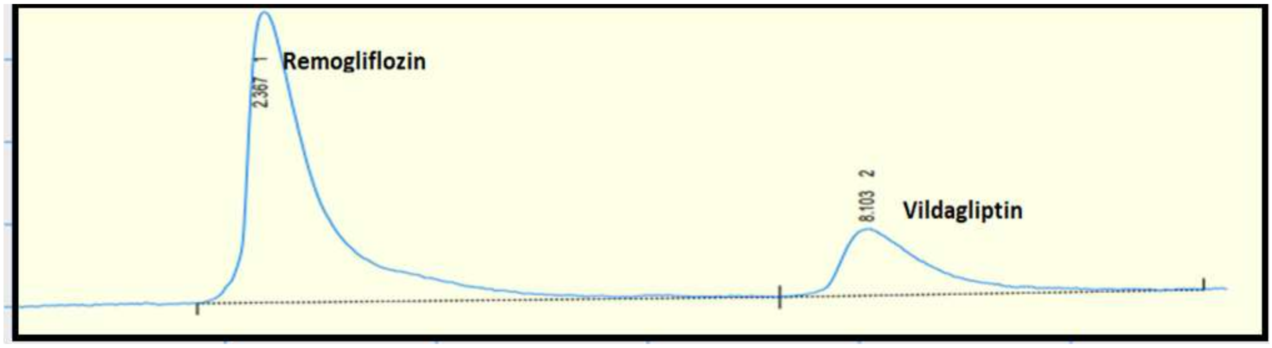

Figure 11: HPLC Trail 6

Table 10: HPLC Trail 6(Synthetic Mixture)

Solvent	Mobile phase	Ratio	pH	Retention time	Area	Height	Wavelength
Remogliflozin	**Acetate buffer: methanol**	**40:60**	**4.5**	**2.3 min**	**1609.0**	**35.2**	**229**
Vildagliptin	**Acetate buffer: methanol**	**40:60**	**4.5**	**8.1**	**462**	**8.1**	**229**
Metformin (IS)	**-**	**-**	**-**	**-**	**-**	**-**	**-**

Preparation of mobile phase

The process of making the phosphate buffer (pH 3). The solution is made by combining 0.477 moles of monosodium phosphate and 0.523 moles of disodium phosphate in little water. Phosphate buffer was then added to acetonitrile. (30:70)

Preparation of standards solution of Remogliflozin

100 mg of Remogliflozin are precisely weighed and put into a 100 ml volumetric flask, where they are subsequently dissolved with the addition of distilled water to produce the final 1000 µg/ml standard stock solution. The same 0.1 ml should be divided into appropriate aliquots, and the water should be diluted to 10 µg/ml before being added to a volumetric flask (working stock solution).

Preparation of standards solution of vildagliptin

A 50 ml volumetric flask is filled with 50 mg of vildagliptin after the volume has been correctly measured, weighed, and transferred to generate the final 1000 µg/ml standard stock solution. The same 0.1 ml should be divided into appropriate aliquots, and the water should be diluted to 10 µg/ml before being added to a volumetric flask (working stock solution).

Preparation of standards solution of

metformin

Once the volume has been precisely weighed and 100 mg of metformin has been placed into the 100 ml volumetric flask, the capacity has been filled to the mark with distilled water to make the final 1000 µg/ml standard stock solution. The same 0.1 ml should be divided into appropriate aliquots, and the water should be diluted to 10 µg/ml before being added to a volumetric flask (working stock solution).

Preparation of working standards:

Remogliflozin, vildagliptin, and metformin stock solutions taking 1 ml each were blended with the stock solutions of each other. Afterward, injection into HPLC was done.

Blank Plasma spiking procedure:

A hundred microlitres of both drug and internal standard were added to 0.5 ml plasma and vortexed for 2 min. To this 1 ml, methanol was added and again vortexed for 2-3 min. It was then centrifuged at 3000 rpm for 5 min. and the supernatant was collected and refrigerated until analysis.

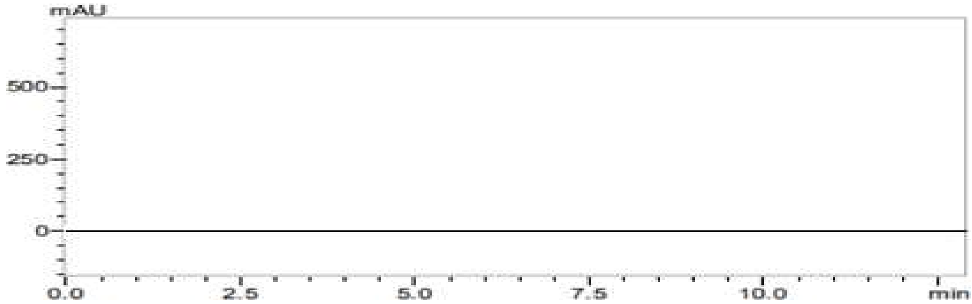

Figure 12: Optimized chromatogram of blank plasma

Extraction by protein precipitation

A 10 ml centrifuge tube was filled with 100 µl of human plasma, 50 µl of internal standard, 20 µl of Remogliflozin, and 20 µl of Vildagliptin. Then, 2 ml of methanol was added. A brief mixing process was followed by 5 minutes of room temperature rest. Next, a 2-minute vortex. After 10 minutes, the mixture was centrifuged at 3000 rpm. Ten milliliters of the supernatant layer were collected and added to the HPLC after Centrifugation.

Preparation of Standard Graph:

100 µl of human plasma, 50 µl of internal standard 20 µl of Remogliflozin, and 20 µl of vildagliptin were pipetted out into 10 ml centrifuge tube and 2 ml of methanol was added. The mixture was mixed shortly, standing for 5 minutes at room temperature and then vortex for 2 minutes. Finally, the mixture was centrifuged at 3000 rpm for 10 min. After the centrifugation, the supernatant layer was collected and injected into HPLC. For the calibration curve 10 µg/ml, 40 µg/ml, 80/ml µg/ml 120µg/ml, 160 µg/ml, concentration was taken

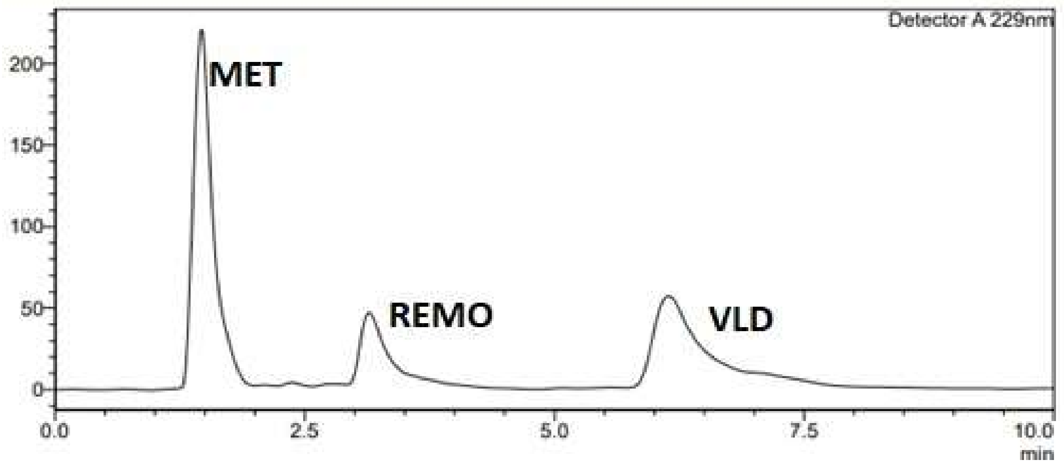

Figure 13: optimized chromatogram of synthetic mixture

Linearity: Linear responses were given for Remogliflozin, vildagliptin, and IS between 10 and 160 µg/ml. For Remogliflozin and vildagliptin, the correlation coefficient value was found to be 0.999 and 0.997, respectively.

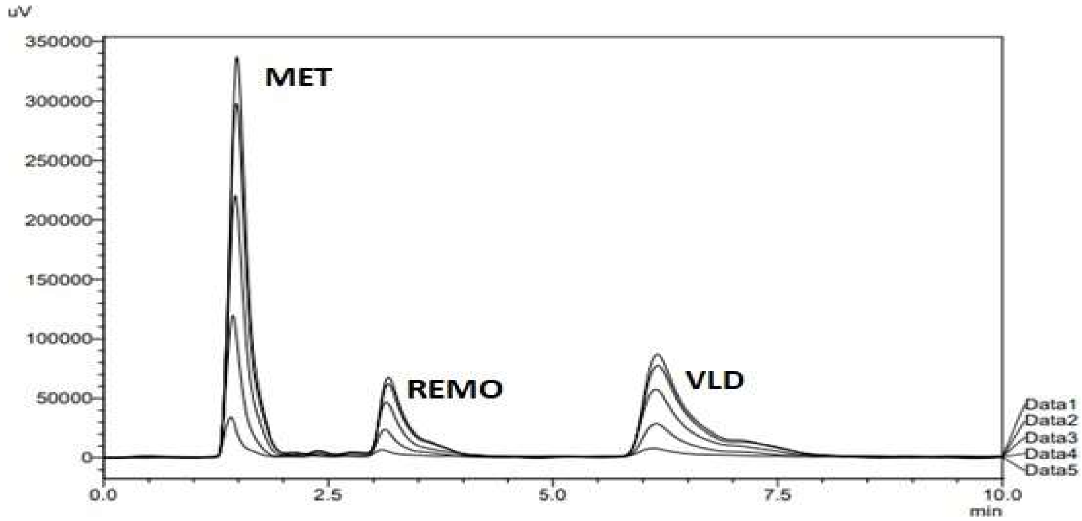

Figure 14: HPLC Overlay Chromatogram (synthetic Mixture)

Linear responses were given for Remogliflozin, vildagliptin, and IS between 10 and 160 µg/ml. For Remogliflozin and vildagliptin, the correlation coefficient value was found to be 0.999 and 0.997, respectively.

Precision & accuracy

Three injections of 10-160 µg/ml into this plasma concentration were made; the mean peak area for each concentration was then determined independently, and the accuracy and precision percentage RSD values were calculated and displayed in the table.

Interday precision & accuracy

In this, the plasma concentrations of 10-160 µg/ml were injected into the HPLC three times on three different days, and mean peak areas were calculated from that accuracy and precision percentage RSD was calculated and shown in the table. The percentage relative to the standard deviation of precision for Remogliflozin & Vildagliptin for the bioanalytical study was less than 2%. The outcomes were within acceptable limits.

Acceptance standards: For a bioanalytical investigation, the percentage RSD value should be less than 2%

Recovery

The percentage recovery of remogliflozin & vildagliptin for the bioanalytical investigation was 95% when plasma concentrations of 40-120 µg/ml were injected into HPLC three times on three distinct days. Mean peak areas were calculated from that area calculated and presented in the table. The outcomes were within acceptable bounds.

Robustness

The method's resilience was verified by purposefully varying the wavelength (nm) and mobile phase (pH). The percentage RSD was discovered to be less than 2%, which shows that no substantial differences in the findings from deliberate alterations exist. So, we can state that the development process is reliable.

RESULTS

Vildagliptin and Remogliflozin Etabonate can both be estimated simultaneously using an accurate and precise HPLC approach that has been designed and validated. All vildagliptin and Remogliflozin correlation coefficients were discovered to be higher than 0.9997. Remogliflozin's linearity range was discovered to be between 10-160 g/ml, as well as vildagliptin's range of 10-160 µg/ml. This number demonstrates that the procedure is precise because the impact of %RSD for intraday and interday precision was determined to be less than 2. The fact that this method's value of % Recovery is more than 98 but less than 102 indicates that it is accurate and free of excipients used in formulation interfering with results. Remogliflozin's coefficient value was discovered to be 0.999, whereas vildagliptin was 0.9997.

Table 11: Optimised chromatographic conditions for HPLC analysis of Vildagliptin and Remogliflozin.

Mobile phase	Phosphate buffer (pH 3): acetonitrile (30:70)
wavelength	229 nm
software	HPLC (shimadzu, Japan)
column	C18 5µm
Run time	10 min

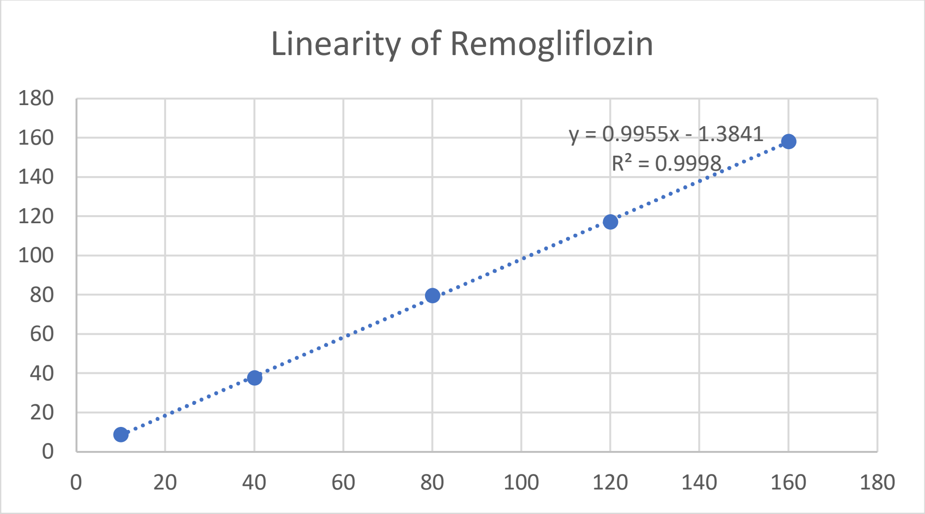

Figure 15: Linearity graph of Remogliflozin

Table 12: The Linearity Data of Remogliflozin

concentration	Calculated concentration			Average	%cv
	1	2	3		
10	8.687	7.897	8.100	8.909	0.0095923
40	37.678	37.687	37.234	37.987	0.0004751
80	79.657	79.689	78.113	78.911	0.0003347
120	117.122	118.115	118.213	159.214	0.0002051
160	159.100	159.120	158.113	159.199	0.0003039
R2 value	0.9997	0.9997	0.9997		

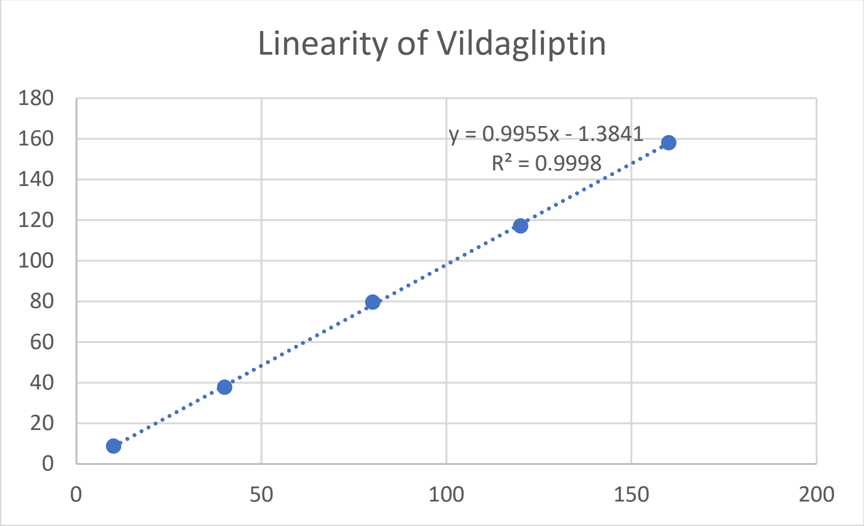

Figure 16: Linearity Curve of Vildagliptin

Table 13: The Linearity Data for Vildagliptin

concentration	Calculated concentration			Average	%cv
	1	2	3		
10	8.687	7.897	8.190	8.909	0.0095923
40	37.678	37.687	37.294	37.987	0.0004751
80	79.657	79.689	78.213	78.911	0.0003347
120	117.122	118.115	118.203	159.214	0.0002051
160	158.100	159.120	158.123	159.199	0.0003039
R2 value	0.9997	0.9997	0.9997		

Table 14: Intraday Precision & Accuracy Data

Concn.	Remogliflozin				Vildagliptin			
	LLOQ	LOQ	MQC	HQC	LLOQ	LOQ	MQC	HQC
Concn.	10	40	80	120	10	40	80	120
	7.672	38.239	78.452	119.445	7.248	37.139	79.193	115.258
	7.676	38.255	78.499	119.442	7.529	38.571	79.248	119.705
	7.678	38.219	78.411	119.342	7.516	38.503	79.102	119.493
Average	7.679	38.239	78.459	119.443	7.432	38.071	81.181	118.152
%RSD	0.206014	0.906014	0.996014	0.916014	0.956014	0.986014	0.916014	0.956014
recovery	76.887	94.863	98.027	95.143	76.887	94.883	98.027	95.122
%cv	0.002212	0.009042	0.000878	0.002926	0.021226	0.021226	0.021229	0.021228

Table 15: Interday Precision & Accuracy Data

Concntration	Remogliflozin				Vildagliptin			
	LLOQ	LOQ	MQC	HQC	LLOQ	LOQ	MQC	HQC
Concn.	10	40	80	120	10	40	80	120
	7.672	38.239	78.452	119.45	7.248	37.139	79.193	115.258
	7.676	38.255	78.499	119.442	7.529	38.571	79.248	119.705
	7.678	38.219	78.411	119.342	7.516	38.503	79.102	119.493
Average	7.679	38.239	78.459	119.443	7.432	38.071	81.181	118.152
%RSD	0.206014	0.906014	0.996014	0.916014	0.956014	0.986014	0.916014	0.956014
Recovery	76.887	94.863	98.027	95.143	76.887	94.883	98.027	95.122
%cv	0.002212	0.009042	0.000878	0.002926	0.021226	0.021226	0.021229	0.021228

Table 16: Recovery studies of Remogliflozin and Vildagliptin

	Remogliflozin			Vildagliptin		
	Unex. Peak area	Exec. Peak area	(%) recovery	Unex. Peak area	Exec Peak area	(%) recovery
LQC	637467	647567	94.86	813187	803187	95.86
	647889	637589	98.03	801319	803319	99.03
	638886	637686	99.70	882189	802189	98.70
Mean		644347.7			802898.3	
% cv		0.006283			0.021227	
QMC	1321478	1321278	94.88	1812722	1712722	95.88
	1341259	1321259	98.05	1718744	1711744	99.05
	1423234	1323234	99.80	1811784	1711744	99.10
Mean		1321946			1712070	
%cv		0.000744			0.021227	
HQC	2019037	2009037	94.80	2493455	2492455	94.88
	2012135	2009135	98.99	2481432	2491432	98.90
	2045288	2019288	99.89	2591456	2491456	98.89
Mean		2012511			2491781	
%cv		0.002635			0.021227	

Table 17: Robustness Data of Remogliflozin

Parameter change		arget conc. µg/ml	Mean peak area ± SD	% RSD
Change in wavelength	229	160 µg/ml	2728089 ± 28.19034	0.991443
	228		2729908 ± 29.19034	0.914443
Change in M.P pH of phosphate buffer	3.5		2730908 ± 28.19844	0.991449
	4.5		2729898 ± 29.19944	0.951443

Table 18: Robustness Data of Vildagliptin

Parameter change		Target conc. µg/ml	Mean peak area ± SD	% RSD
Change in wavelength	229	160 µg/ml	3130980 ± 45.19034	0.991443
	228		3130987 ± 46.19034	0.914543
Change in M.P PH Of phosphate buffer	3.5		3130999 ± 46.19944	0.998449
	4.5		3130999 ± 46.19944	0.961443

CONCLUSION

Vildagliptin and Remogliflozin Etabonate can both be estimated simultaneously using an accurate and precise HPLC approach that has been designed and validated. All vildagliptin and Remogliflozin correlation coefficients were discovered to be higher than 0.9997. Remogliflozin's linearity range was discovered to be between 10-160 µg/ml, as well as vildagliptin range of 10-160 µg/ml. This number demonstrates that the procedure is precise because the impact of %RSD for intraday and interday precision was determined to be less than 2. The value of % Recovery for this method is greater than 98 but less than 102, which indicates that the procedure is accurate and free from excipient interference.

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CONFLICT OF INTERESTS

The authors say they have no competing interests

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