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Analytical Method Development and Validation for Simultaneous Estimation of Remogliflozin Etabonate, Vildagliptin and Metformin Hydrochloride in Combined Dosage Form

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Keywords: Remogliflozin Etabonate, Vildagliptin, Metformin Hydrochloride, Absorbance correction method, UV-spectroscopy, RP-HPLC

ABSTRACT

Two develop simple, precise, accurate and rapid method for simultaneous estimation of Remogliflozin Etabonate, Vildagliptin and Metformin Hydrochloride using spectrophotometric and chromatographic methods. Absorbance Correction Method was developed by using UV spectrophotometry and RP - HPLC was developed. All the two methods were validated as per ICH guideline. For Absorbance Correction Method, three wavelengths were selected 202.75nm for VIL (MET absorbance deducted total absorbance and REM show no interfere because same absorbance) and 226.76-238.65nm for REM (MET show zero absorbance difference and VIL show zero absorbance) and 245.14nm for met (VIL and REM show zero absorbance). RP-HPLC method was developed by selection and optimization of mobile phase. Separation was achieved on Shim- pack solar C18 (250 mm × 4.6 mm, 5 µm). Detection XIX was carried out at 205nm using Acetonitrile: Methanol: Water 60-10-30 (pH 4.5 adjusted with Ortho Phosphoric acid). All UV spectrophotometric method was found to be linear over the concentration range of 1-5 µg/ml 2-10 µg/ml and 10-50 µg/ml for Vildagliptin, Remogliflozin Etabonate and Metformin Hydrochloride respectively. RP-HPLC method was found to be linear over the concentration range of 1-5 µg/ml, 2- 10µg/ml and 10-20 µg/ml for Vildagliptin, Remogliflozin Etabonate and Metformin Hydrochloride respectively. All the methods were validated for linearity, precision, accuracy, LOD and LOQ according to ICH guideline.

INTRODUCTION

Remogliflozin Etabonate IUPAC name 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. It is soluble in methanol. It is an oral hypoglycemic agent; it is used for type 2 diabetes. It is Inhibitor of sodium-glucose cotranspoter-2 (SGLT2) it is expresses in the proximal renal tubules and is responsible for the majority of the reabsorption of filtered glucose from the tubular lumen by inhibiting SGLT2, it also reduces reabsorption of filtered glucose and lowers the renal threshold for glucose and thereby increases urinary glucose excretion. [1-2] Fig.1

Vildagliptin IUPAC name (2S)-1-{2-[(3-hydroxyadamantan-1-yl) amino] acetyl} pyrrolidine-2-carbonitrile. It is Freely soluble in water, methanol. It is used for Type 2 Diabetes Mellitus. It is a dipeptidylpeptidase-4 (DPP-4) inhibitor that improves glycaemic control by preventing DPP-4 from inactivating the incretin hormones glucagon-like peptide-1 and glucose dependent insulinotropic polypeptide, thus prolonging incretin activity in response to ingestion of nutrients. This increases insulin sensitivity, decreases glucagon secretion and improves? -cell function. [3-4] Fig.2

Metformin Hydrochloride IUPAC name 3-(diaminomethylidene)-1,1-dimethylguanidine; hydrochloride. It is Freely Soluble in water, methanol. It is used for the treatment of type 2 diabetes mellitus. It's an antihyperglycemic medication that improves glucose tolerance in type 2 diabetic patients by reducing both basal and postprandial plasma glucose levels. Metformin improves insulin sensitivity by boosting peripheral glucose uptake and utilization. It lowers hepatic glucose production, lowers intestinal glucose absorption, and lowers hepatic glucose production [5-9]. Fig.3

REM, VIL and MET Combination Approved by CDSCO ON 21/09/2021.[10] Fig.4

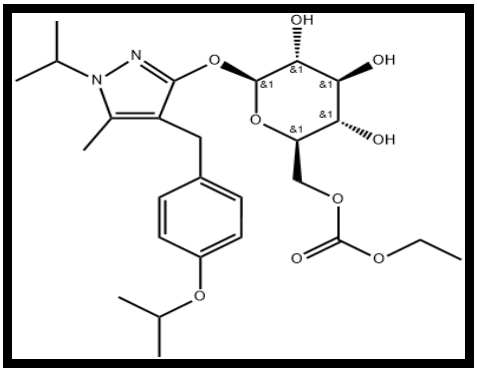

Fig.1 Chemical structure of Remogliflozin Etabonate

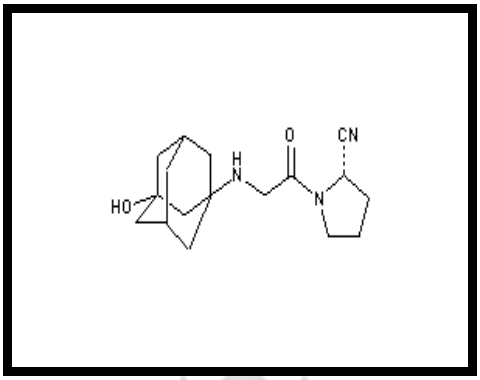

Fig.2 Chemical structure of Vildagliptin

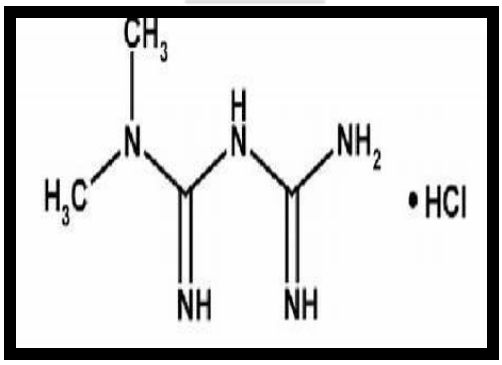

Fig.3 Chemical structure of Metformin Hydrochloride

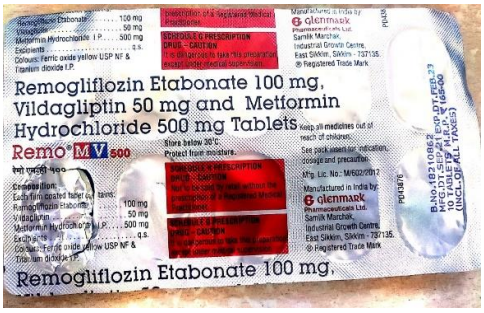

Fig.4 Marketed formulation

Literature review revealed that they were several analytical methods like HPLC, HPTLC, LC-MS, UV and RP-HPLC were reported for individual and along with other drugs. Hence present work aimed at the development and validates a simple, precise, accurate and rapid UV spectrophotometry and RP-HPLC method for the estimation of Remogliflozin Etabonate, Vildagliptin and Metformin Hydrochloride in combined dosage form. Thus, there I have develop two methods of spectrophotometric (Absorbance correction Method**)** and chromatographic methods for combination of Remogliflozin Etabonate, Vildagliptin and Metformin Hydrochloride and validation.

1.1LITERATURE REVIEW

A) LITERATURE REVIEW OF REMOGLIFLOZIN ETABONATE:

REMOGLIFLOZIN ETABONATE is not official drug in any pharmacopoeia.

Table 1. Reported Methods for REMOGLIFLOZIN ETABONATE

<table>
<tr><th>Sr. No.</th><th>Title/Method</th><th>Description</th><th>Ref. No.</th></tr>
<tr><td>1.</td><td>A Validated Stability Indicating High Performance Thin Layer Chromatographic Method for Determination of Remogliflozin Etabonate in Tablet Dosage Form</td><td>Stationary Phase: Silica gel 60 F_{254} (100 mm × 100 mm, 250µm)
Mobile Phase: Toluene: Methanol (8.5:1.5% v/v)
Wavelength: 224nm
Rf value: 0.35±0.03
Linearity: 50-250 ng/band
Forced Degradation Study
<table>
<tr><th>Stress Condition</th><th>% Degradation</th></tr>
<tr><td>Acid Hydrolysis</td><td>18.39</td></tr>
<tr><td>Base Hydrolysis</td><td>18.40</td></tr>
<tr><td>Neutral Hydrolysis</td><td>13.60</td></tr>
<tr><td>Oxidative Hydrolysis</td><td>14.45</td></tr>
<tr><td>Thermal Hydrolysis</td><td>21.61</td></tr>
<tr><td>Photolytic</td><td>18.49</td></tr>
</table></td><td>11</td></tr>
</table>

		hydrolysis	
2.	Method Development and Validation of **UV Spectrophotometric** Estimation of **Remogliflozin Etabonate** in Bulk and Its Tablet Dosage Form	**Model**: Shimadzu 1800 **Solvent**: Methanol **Wavelength**: 229 nm **Linearity**: 2-10 µg/mL	12
3.	Development and Validation of Novel **RP-HPLC** Method for the Simultaneous Determination of **Remogliflozin** and **Vildagliptin** in Bulk and in Synthetic Mixture	**Stationary Phase**: Luna C_{18} (250mm ×4.6mm, 5µm) **Mobile Phase**: Acetate Buffer (pH 5.6): Methanol (30:70%v/v) **Wavelength**: 210 nm **Flow Rate**: 1.0 mL/min **Retention Time**: REM: 4.881 VDG: 6.334 **Linearity**: REM: 10-200µg/mL VDG: 10-200µg/mL	13
4.	Smart **UV Derivative Spectrophotometric** Methods for Simultaneous Determination of **Metformin** and **Remogliflozin** Development Validation and Application to The Formulation	**Model**: Shimadzu 1700 **Solvent**: Methanol, Water **Wavelength:** Third derivative Absorbance Method RGE:234.8nm MFH:240.1nm Zero cross point: RGE: 240.1nm MET: 234 nm Ratio Second derivative Method: Zero crossing point: RGE: 277.2nm MFH: 246.6nm Constant Centre Subtraction Method (Mixture of two analytes spectra into individual zero order	14

		spectra): RGE: 226.2nm MFH: 232.9nm **Linearity**: RGE: 1-24µg/mL MFH: 2.5-30µg/mL	

B) LITERATURE REVIEW OF VILDAGLIPTIN

VILDAGLIPTIN is not official drug in any pharmacopoeia

Table 2. Reported Methods for VILDAGLIPTIN

Sr. No.	Title/Method	Description	Ref. No.
1.	**RP-HPLC** Determination of **Vildagliptin** in Pure and In Tablet Formulation	**Stationary Phase**: Agilent $C_{18,}$ (150mm × 4.6mm ,5µm) **Mobile Phase**: Phosphate Buffer: Acetonitrile (85:15% v/v) **Wavelength**: 210nm **Flow Rate**: 1.0 mL/min **Retention Time**: 3.04 min **Linearity**: 10-150 mg/mL	15
2.	Second Order Derivative **UV Spectrophotometric** and **RP-HPLC** Method for The Analysis of **Vildagliptin** and Application for Study	**UV** **Model**: Shimadzu 1800 **Solvent**: Water **Wavelength**: Zero crossing point: 220 nm **Linearity**: 25-125µg/mL **RP-HPLC** **Stationary Phase**: $C_{8\,(}$150mm × 4.6mm, 5µm) **Mobile Phase**: Potassium Phosphate Buffer (pH 7): Acetonitrile (85:15 %v/v) **Wavelength**: 207nm	16

		Flow Rate: 1mL/ min **Linearity**: 10-90 µg/mL	
3.	Development and Validation of a **RP-HPLC** Method for the Assay of **Vildagliptin**	**Stationary Phase**: Symmetry C_{18} (4.6mm× 150mm, 5µm) **Mobile Phase**: Buffer (pH 8.2): Acetonitrile: Methanol (450: 480:70%v/v) **Wavelength**: 254nm **Flow Rate**: 0.5mL/min **Retention Time**: 3.906 min **Linearity**: 50-90 µg/mL	17
4.	**RP-HPLC** Method Development and Validation of **Vildagliptin** in Bulk and Dosage Form	**Stationary Phase**: Phenomenex C_{18} (250mm× 4.6mm,5µm) **Mobile Phase**: Methanol: Water (At pH 4.5 adjusted with OPA) (60: 40%v/v) **Wavelength**: 207nm **Flow Rate**: 0.8 mL/min **Retention Time**: 3.58 min **Linearity**: 10-60µg/mL	18
5.	Spectrophotometric Method for the Determination of **Vildagliptin** in Bulk and Pharmaceutical Dosage Forms	**Model**: Shimadzu 18001 **Solvent**: 0.5 m HCl **Wavelength**: 202.5nm **Linearity**: 10-40 µg/mL	19
6.	Method Development and Validation of **Vildagliptin** Using **UV Spectrophotometer**	**Model:** Shimadzu 1601 **Solvent**: Water **Wavelength**: 244 nm **Linearity**: 12.5-200 µg/mL	20

C) LITERATURE REVIEW OF METFORMIN HYDROCHLORIDE

Table 3. Official Method for Metformin Hydrochloride

Sr. No.	**Official In**	**Title/Method**	**Description**	**Ref. No.**
1.	**IP 2018**	Chromatographic Methods	**Stationary Phase**: A Stainless-Steel Column 30 cm × 4mm, Packed with Octadecylsilane bonded to porous silica (10 µm) **Mobile Phase**: A Solution Containing 0.087% w/v of Sodium Chloride, adjusted to pH 3.5 using 1% v/v solution of orthophosphoric acid **Flow Rate**: 1 mL/min **Wavelength**: 218nm **Injection Volume**: 20µl	21
2.	BP-2003	Liquid Chromatography	**Stationary Phase**: Irregular, Porous Silica gel to which Benzene sulphonic acid groups have been chemically bonded (0.25m, 4.7mm, 10µm) OR Regular, Porous Silica gel to which Benzene sulphonic acid groups have been chemically bonded (0.11m, 4.7mm, 5µm) **Mobile Phase**: 17g/l solution of ammonium dihydrogen phosphate R adjusted to pH 3.0 with phosphoric acid R. **Flow Rate**: 1mL/min **Wavelength:** 218nm **Injection Volume**: 20µl	22

Table 4. Reported Method for METFORMIN HYDROCHLORIDE

Sr. No.	Title/Method	Description	Ref. No.
1.	Development and Validation of **UV Spectrophotometric** Method for Estimation of **Metformin** in Bulk and Tablet Dosage Form	**Model**: Shimadzu 1800 **Solvent**: Sodium Hydroxide **Wavelength**: 233 nm **Linearity**: 1-25µg/mL	23
2.	**RP-HPLC** Method Development of **Metformin** in Pharmaceutical Dosage Form	**Stationary Phase**: Thermosil C_{18} **Mobile Phase**: Water: Acetonitrile (40: 60%v/v) **Wavelength**: 232nm **Flow Rate**: 1.0mL/min **Retention Time**: 3.25 min **Linearity**: 20-60µg/mL	24
3.	Development and Validation of a New Analytical **HPLC** Method for Simultaneous Determination of the Antidiabetic Drugs **Metformin** and **Gliclazide**	**Stationary Phase**: C_{18} (250mm×4.6mm ×5µm) **Mobile Phase**: Ammonium Formate Buffer (pH 3.5): Acetonitrile (45:55% v/v) **Wavelength**: Metformin: 234nm Gliclazide: 228nm **FlowRate**:1mL/min **Retention Time:** Metformin: 4.101min Gliclazide: 6.964min **Linearity**: Metformin: 2.5-150 µg/mL Gliclazide: 1.25-150 µg/mL	25

2. MATERIAL AND METHOD

2.1. INSTRUMENTS

SHIMADZU double beam UV/Visible Spectrophotometer model UV 1900i, software- Lab solution. REPTECH Electronic balance model and Ultra Sonicator (Athena Technology) were also used during the analysis. The HPLC instrument used that was gradient SHIMADZU HPLC LC-2010 CHT with software LC solution and UV Detector with variable wavelength programme was used for the method development. Shim-pack solar C18 (250 mm × 4.6 mm, 5 µm) column was used for the separation.

2.2.CHEMIACAL AND REAGENTS

Remogliflozin Etabonate pure drug obtained from Glenmark Pharmaceutical Ltd, Mumbai, Vildagliptin pure drug obtained from Exemed Pharmaceutical, Vapi and Metformin Hydrochloride pure drug obtained from Vapi Care Pharma PVT,Ltd, vapi. The marketed formulation of this combination is REMO$_{mv}$ 500 manufactured by Glenmark Pharmaceutical, Himachal Pradesh was purchase from local pharmacy. Acetonitrile HPLC Grade (Ranken Chemicals), HPLC Grade Water (Ranken Chemicals), HPLC Grade Methanol (Ranken Chemicals), OPA (HPLC Grade Fisher Scientific) were used in the research work.

2.3. Chromatographic condition:

Stationary phase: Shim-pack solar C18 (250 mm × 4.6 mm, 5 µm)

Mobile phase: Acetonitrile: Methanol: Water (pH4.5 adjusted with orthophosphoric acid) (60:10:30%v/v/v)

Flow rate :1 ml/min

Wavelength: 205nm.

2.4. Wavelength Selection:

Aliquots of 0.1ml from working solution of VIL (100 µg/ml) and 0.2 ml from working solution of REM (100 µg/ml) and 1ml from working solution of MET were pipette out into three separate 10 ml of volumetric flask and volume was made upto the mark with methanol to get 1µg/ml of VIL and 2 µg/ml of REM and 10 µg/ml of MET. Each Solutions of VIL, REM and MET were scanned between 200-400 nm using UV-Visible Spectrophotometer. Wavelength was selected from the overlay spectra of above solutions. Fig.5

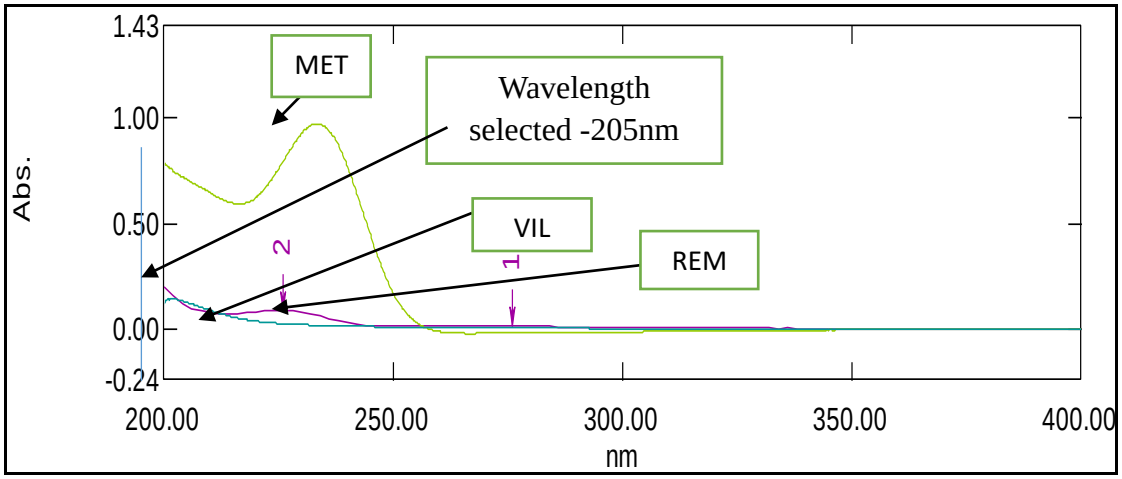

Fig.5 Overlay spectrum of VIL, REM and MET showing selection of wavelength detection

2.5.Preparation of standard stock solution: - Accurately weight 10 mg drug REM, VIL and MET powder and transferred to 10 ml volumetric flask separately and dissolved in methanol and sonicate the flask. The volume was made up to the mark with methanol to give 1000 µg/ml.

2.6.Preparation of working solution: Pipette out aliquots of 1 ml from standard stock in 10ml volumetric flasks for VIL and 1 ml from standard stock in 10 ml volumetric flasks for REM and 1 ml from standard stock in 10ml volumetric flask separately and volume was adjusted to the mark with Methanol to get 100 µg/ml of Working standard solution of VIL, REM and MET.

3. METHOD DEVELOPMENT

3.1.Method:1 Absorbance correction Method

Aliquots of 0.1ml from working stock solution of VIL (100 µg/ml) and 0.2ml from working stock solution of REM (100 µg/ml) and 1ml from working stock solution of MET (100µg/ml) were pipette out and taken into three separate volumetric flasks of 10ml and volume was made up to mark with methanol to give a solution containing 1µg/ml, 2 µg/ml and 10µg/ml of VIL, REM and MET each. Each solution was scanned between 200-400 nm against methanol as blank. Zero order spectra were taken (Fig- 6,7, and 8,9) and it was observed that VIL was estimated at 202.75nm was used where there was interference of REMO which was deducted from absorbance of VIL and MET showed zero absorbance at 202.75nm. For REM 226.76nm and 238.65nm was used at these two-wavelength absorbance

difference of MET was zero and VIL showed zero absorbance.so REM was estimated by taking absorbance difference at 226.76nm and 238.65nm. MET was estimated at 245.14nm where REM and VIL show zero absorbance.Fig.10.

• Estimation of Remogliflozin Etabonate, Vildagliptin and Metformin Hydrochloride by equation.

1. At 245.14nm (METFORMIN HYDROCHLORIDE), Cx = A1 / 0.0339

2.At 202.75nm (VILDAGLIPTIN), Cy = A2 – 0.42589Cx / 0.456004

3. At 226.76-238.65nm (REMOGLIFLOIN ETABONATE), Cz = A3 / 0.01006

• Where A1, A2, A3 are absorbance of mixture at 245.14nm, 202.75nm, 226.76- 238.65nm.

• Cx, Cy, Cz are concentration of METFORMIN HYDROCHLORIDE, VILDAGLIPTIN and REMOGLIFLOZIN.

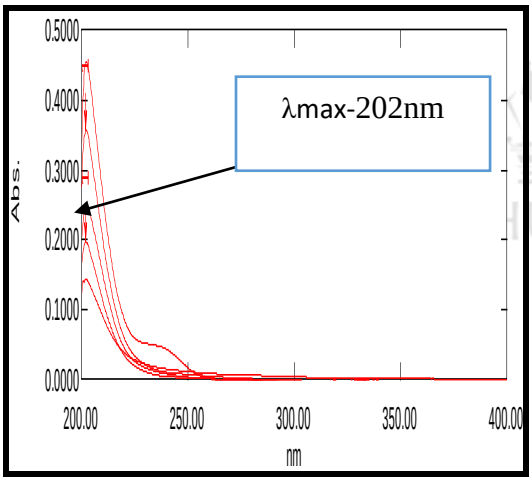

Fig. 6 Linearity of VIL (2-10 µg/ml)

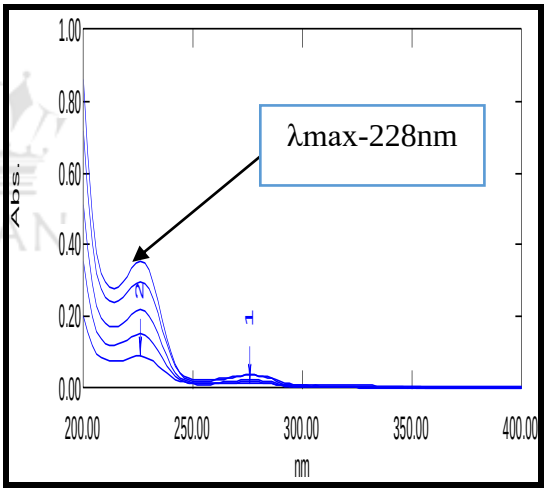

Fig.7 Linearity of REM(1-5µg/ml)

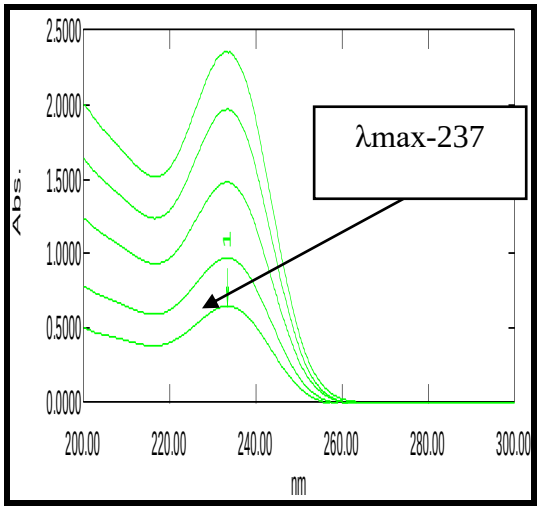

Fig.8 Linearity of MET (10-50 µg/ml)

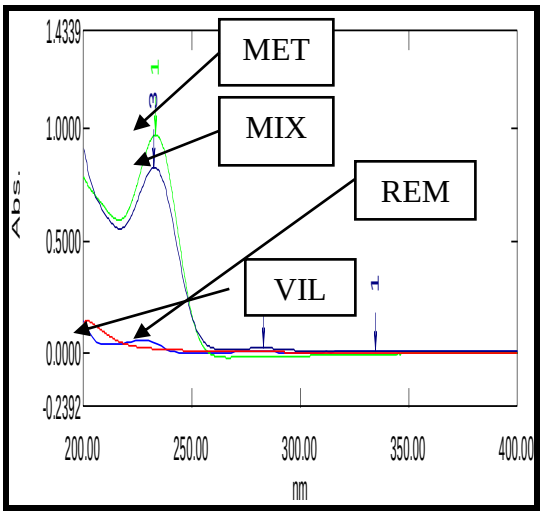

Fig.9 Overlay spectra of Mix, VIL, REM and MET

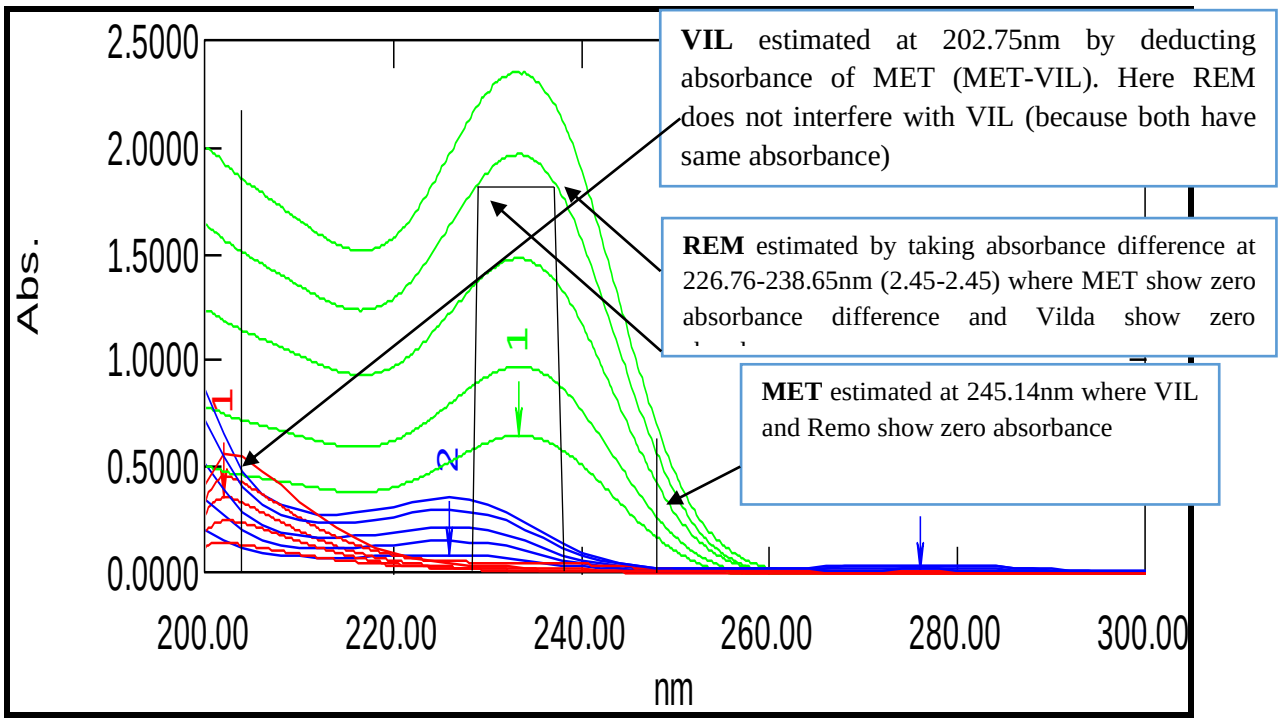

Fig.10 Selection of Wavelength for estimation of Vildagliptin, Remogliflozin Etabonate and Metformin Hydrochloride

3.2.Method 2:- High Performance Liquid Chromatography HPLC

A variety of mobile phases were investigated in the development of a HPLC method for the analysis of Remogliflozin Etabonate, Vildagliptin and Metformin Hydrochloride. A mixture of Acetonitrile, methanol and Water in the ratio of 60: 10:30 was found to be the most suitable mobile phase for ideal separation of Remogliflozin Etabonate, Vildagliptin and

Metformin Hydrochloride. The solvent mixture was filtered through a 0.45µ Membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1ml/min. The detection of the drug was monitored at 205 nm. The run time was set at 20 min. Under the optimized chromatographic condition, the retention time obtained for the drug was 4.497min, 7.304min and 1.735min. A typical chromatogram showing the separation of the drug is as shown in Fig.11.

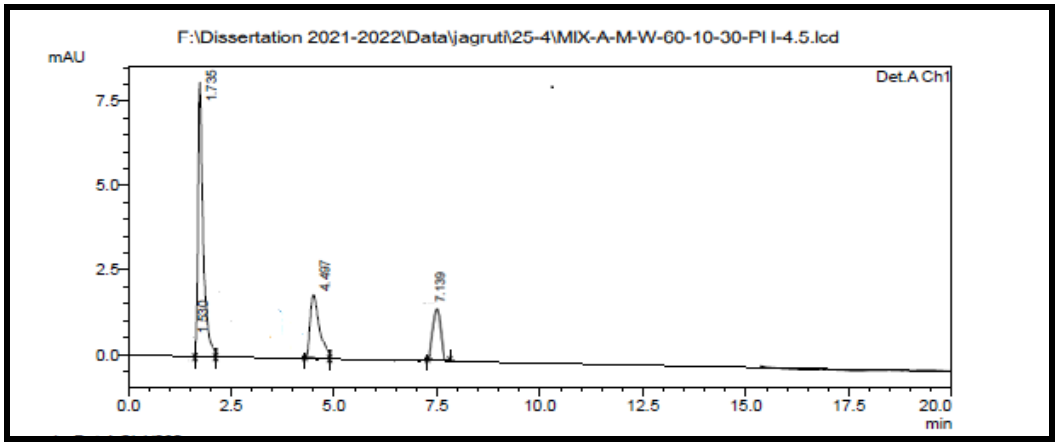

Fig:11 Chromatogram of VIL, REM and MET in Acetonitrile: Methanol: Water (60:10:30% v/v) (pH-4.5 adjusted with 1%OPA)

4. RESULT AND DISCUSSION

Validation of Proposed Method

Method 1: Absorbance correction Method

Result of UV analysis has been shown in Table 5. The standard deviation and %RSD calculated for the method is low, indicating high degree of precision. The %RSD is also less than 2% as required by ICH guidelines. The % recovery was between 98- 102% indicating high degree of accuracy and specificity of the proposed method. The results of the recovery study are shown in Table 5. The developed absorbance correction method was validated for simultaneous estimation of Vildagliptin, Remogliflozin Etabonate and Metformin using linearity, range, accuracy and precision and the results were interpreted in Table 5. The %RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement.

Table No.5 Result of validation parameter

Parameter	VIL	REM	MET
Selected Wavelength Range	202.70nm	226.76-238.65nm	245.14nm
Linearity (µg/ml) (n=5)	1-5(µg/ml)	2-10(µg/ml)	10-50(µg/ml)
Regression Equation (y = mx + c)	Y=0.254x+0.0328	Y=0.0238x+0.0152	Y=0.0223x+0.0223
Regression coefficient (R^2)	0.9986	0.9973	0.9983
Correlation coefficient (r)	0.9992	0.9981	0.9991
Repeatability (%R.S.D.) (n=6)	0.1495	0.2786	0.1252
Intraday Precision (%R.S.D.) (n=3)	0.1030-0.1272	0.1009-0.1813	0.1083-0.1224
Interday Precision (%R.S.D.) (n=3)	0.1243-0.1684	0.1203-0.7289	0.1193-0.7289
LOD (µg/ml) (n=5)	0.04365	0.101576	0.03332
LOQ(µg/ml) (n=5)	0.132271	0.307806	0.10097
% Recovery (n=3)	98.5-99.5	98.25-99.72	98.69-99.70
Assay (%) ± S.D. (n=3)	98.33	98.66	99.16

Method 2:-High Performance Liquid Chromatography (HPLC)

HPLC Data of Optimization of Chromatographic Conditions of VIL, REM and MET in shown Table.6.

Table No.6 Data of Optimization of Chromatographic Conditions of VIL, REM and MET

Parameter	Condition
Mobile Phase	Acetonitrile: Methanol: Water (60:10:30% v/v/v) (pH-4.5 adjusted with 1% OPA)
Flow rate	1.0 mL/min
Run time	20 min
Volume of Injection	10µL
Detection of Wavelength	205nm
Diluent	Methanol
Retention Time	VIL- 7.304 min REM-4.497 min MET-1.735min
Tailing Factor	VIL-1.164 REM-1.5943 MET-1.2656
Theoretical Plate	VIL-2913.2 REM-14436 MET-6884.1
Resolution	4.741 and 3.921

4.1. System Suitability studies

Evaluation of system suitability was done by analyzing six replicates of VIL, REMO and MET in a mixture at concentration of 1 µg/ml of VIL, 2 µg/ml of REM and 10 µg/ml of MET. The column efficiency, peak asymmetry and resolution were calculated for each replicate and data are shows in Table 7.

Table No.7 System Suitability data for VIL, REM and MET

Drugs	Parameters	Mean ± S.D(n=6)	%RSD
VIL	Retention Time	7.144±0.0083	0.1171
	Theoretical Plate	2913.2±4.0198	0.1379
	Tailing Plate	1.164±0.0020	0.1752
REM	Retention Time	4.492±0.0049	0.1105
	Theoretical Plate	14436±40.207	0.2785
	Tailing Plate	1.5943±0.0040	0.2560
MET	Retention Time	1.735±0.0025	0.1458
	Theoretical Plate	6884.1±7.8799	0.1144
	Tailing Plate	1.2656±0.004	0.3225

4.2. Specificity

Specificity involves quantitative detection of analyte in the presence of those components that may be expected to be part of sample matrix. Specificity of developed method was established by spiking of VIL, REM and MET in hypothetical placebo (i.e., might be expected to be present) and expressing that analytes peak were not interfered from excipients.Fig.12,13.

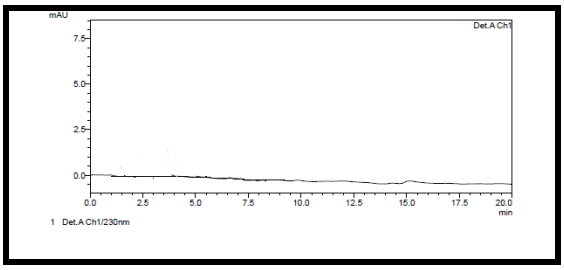

Fig.12 Chromatogram of Placebo

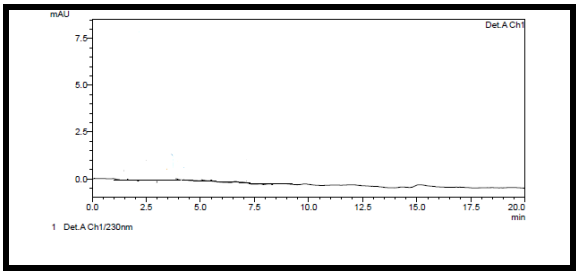

Fig.13. Chromatogram of Mobile Phase

4.3.Linearity

The linearity response was determined by analyzing 5 independent levels of concentration in the range of 1-5 µg/ml, 2-10 µg/ml and 10-50 µg/ml for VIL, REM and MET respectively given in Table.8.(Fig.14)

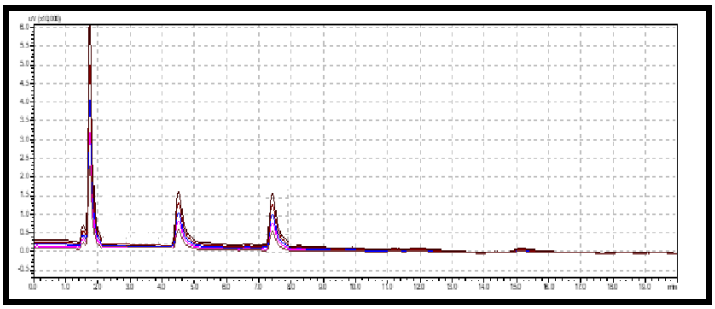

Fig.14 Linearity graph for VIL, REM and MET

Table No.8 Linearity data of VIL, REM and MET

Sr. No.	Concentration (µg/ml)			Mean Peak Area ± S.D. (n=5)			%RSD		
	VIL	REM	MET	VIL	REM	MET	VIL	REM	MET
1.	1	2	10	51209±94.4394	30661±42.4264	71964±108.28	0.1844	0.1383	0.1504
2.	2	4	20	109374±110.995	75711±134.164	97834±110.77	0.1014	0.1772	0.1132
3.	3	6	30	160612±439.454	113679±163.15	138358±314.35	0.2736	0.1435	0.2272
4.	4	8	40	211033±421.900	149336±447.26	178644±579.32	0.1999	0.2995	0.3242
5.	5	10	50	259642±402.492	185601±447.21	218759±899.06	0.1550	0.2409	0.4109

4.3.1. Preparation of Calibration Curves

I. Calibration curve for VIL

Calibration curve for VIL consisted of five different concentrations solution ranging from 1-5 µg/ml. Calibration curve of Peak area vs Conc. was plotted and regression equation was determined.Fig.15

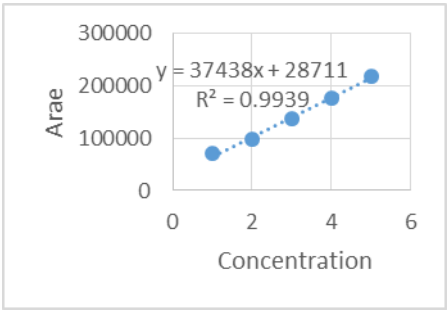

Fig.15 Calibration curve for VIL

II. Calibration curve for REM

Calibration curve for REM consisted of five different concentrations solution ranging from 2-10 µg/ml. Calibration curve of Peak area vs Conc. was plotted and regression equation was determined.Fig.16

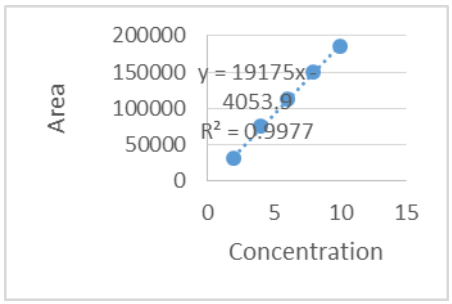

Fig.16 Calibration curve for REM

III. Calibration curve for MET

Calibration curve for MET consisted of five different concentrations solution ranging from 10-50 µg/ml. Calibration curve of Peak area vs Conc. was plotted and regression equation was determined. Fig.17

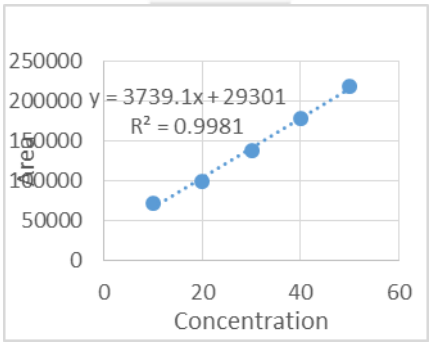

Fig.17 Calibration Curve for MET

4.4.Precision

a) Repeatability

Repeatability of the developed method was assessed by analyzing samples from the same batch 6 times with standard solutions containing concentrations 3 µg/ml for VIL, 6 µg/ml for REM and 30 µg/ml for MET and % R.S.D. was calculated. The results were shown in Table.9.

Table No.9 Repeatability data of VIL, REM and MET

Drugs	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=6)	%RSD
VIL	2	10937±123.57	0.1129
REM	4	75884.3±116.2	0.1531
MET	20	97907±115.56	0.1180

b) Intraday precision

It was assessed by analyzing samples from the same batch with three standard solutions containing concentrations 2,3 and 4 µg/ml for VIL and 4, 6 and 8 µg/ml for REM and 20,30 and 40 µg/ml for MET. Solutions were analyzed thrice (n=3) on the same day within short interval of time and % R.S.D. was calculated. The results were shown in Table.10.

Table No.10 Intraday precision data of VIL, REM and MET

Drugs	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=5)	%RSD
VIL	2	10943±110.15	0.1006
	3	16062±318.60	0.1983
	4	21090±228.10	0.1081
REM	4	75884.3±115.47	0.1521
	6	113617.7±148.4	0.1306
	8	149402±152.7	0.1022
MET	20	97797±101.79	0.1040
	30	137892±516.75	0.3747
	40	178249±577.35	0.3239

c) Interday precision

It was assessed by analyzing samples from the same batch with three standard solutions containing concentrations 2,3 and 4 µg/ml for VIL and 4,6 and 8 µg/ml for REM and 20,30 and 40 µg/ml for MET. Solutions were analyzed thrice (n=3) on the three different day and % R.S.D. was calculated. The results were shown in Table.11.

Table No.11 Interday precision data of VIL, REM and MET

Drugs	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=5)	%RSD
VIL	2	10949±288.67	0.2636
	3	16079±695.72	0.4326
	4	21113±550.75	0.2608
REM	4	75814.3±158.2	0.2086
	6	113747±208.1	0.1830
	8	149236±510.9	0.3423
MET	20	97886±110	0.1123
	30	137862±577.35	0.4187
	40	178019±849.72	0.4773

4.5.Accuracy

For accuracy study data from nine determination over three concentrations at 80%, 100% and 120% of expected sample concentration covering the specified range was determined and expressed as recovery values. The results were shown in Table.12.

Table No.12 Accuracy data for VIL, REM and MET

Drugs	Level	Amount of sample (µg/ml)	Amount of sample spiked (µg/ml)	Total amount	Mean Peak Area±S.D. (n=3)	Amount of sample found (µg/ml)	Mean % Recovery ±S.D. (n=3)
VIL	0	2	0	2	10930±51.9615	1.98	99
	80	2	1.6	3.6	18972±57.7350	3.57	98.31
	100	2	2	4	21077±5.7735	3.95	98.75
	120	2	2.4	4.4	22864±3.4641	3.98	99.45
REM	0	4	0	4	75954.3±5.773	3.97	98.5
	80	4	3.2	7.2	134584±4.0414	7.56	98.88
	100	4	4	8	149539±5.7735	7.96	99
	120	4	4.8	8.8	163185±57.735	8.36	99.09
MET	0	20	0	20	97882±5.773	19.97	99.85
	80	20	16	36	160127±5.773	35.94	99.83
	100	20	20	40	177919±5.773	39.98	99.95
	120	20	24	44	192855±5.773	43.98	99.95

LOD and LOQ

The LOD (Limit of Detection) was assessed from the set of 5 calibration curves that were used to determine linearity of the method. The LOD was calculated by using the formula:

$$LOD = 3.3 \times S.D./Slope$$

Where, S.D. = Standard deviation of the Y – intercepts of 5 calibration curves

Slope = Mean slope of 5 calibration curves

The LOQ (Limit of Quantitation) was assessed from the set of 5 calibration curves that were used to determine linearity of the method. The LOQ was calculated by using the formula:

$$LOQ = 10 \times S.D./Slope$$

Where, S.D. = Standard deviation of the Y – intercepts of 5 calibration curves

Slope = Mean slope of 5 calibration curves

The LOD for VIL, REM and MET were found to be 0.262954 µg/ml, 0.199752 µg/ml and 0.0815 µg/ml respectively. The LOQ for VIL, REM and MET were found to be 0.796831 µg/ml, 0.605308 µg/ml and 0.2471µg/ml respectively.

4.6. Robustness

Robustness of the method was determined by subjecting the method to slight change in the method condition like,

- Mobile Phase Ratio
- Flow rate

Three replicates were prepared for the same of concentration 1 µg/ml for VIL and 2 µg/ml for REM and10 µg/ml MET and % R.S.D. was calculated. The results were shown in Table.13.

Table No.13 Robustness data for VIL, REM and MET

Drug	Parameters	Level	Mean Peak area ±S.D. (n=3)	%RSD	Rt±S.D. (n=3)	%RSD
VIL	Mobile Phase	60-12-28	4689±6.0827	0.1297	7.149±0.0100	0.1398
		58-10-32	4928±6.3508	0.1288	7.834±0.0152	0.1949
	Flow Rate	0.8 ml/min	3757±5.7735	0.1536	5.718±0.0110	0.1926
		1.2 ml/min	5634±5.7735	0.1024	8.574±0.0107	0.1257
REM	Mobile Phase	60-12-28	30724±57.735	0.1879	4.4923±0.0098	0.2196
		58-10-32	34964±43.588	0.1246	5.1243±0.0057	0.1126
	Flow Rate	0.8 ml/min	24602±78.102	0.3174	3.5963±0.0046	0.1284
		1.2 ml/min	37216±55.075	0.1479	5.3876±0.0076	0.1417
MET	Mobile Phase	60-12-28	72229±105.03	0.1454	1.731±0.0057	0.3334
		58-10-32	75526±112.69	0.1492	2.124±0.0057	0.2717
	Flow Rate	0.8 ml/min	67689±101.00	0.1492	1.6843±0.0057	0.3427
		1.2 ml/min	86621±112.69	0.1301	2.2223±0.0057	0.2597

4.7.Analysis of tablet formulation

For the estimation of drugs in the commercial formulation, twenty tablets were weighed accurately. The average weight was calculated and then crushed to obtain fine powder. A quantity of tablet powder equivalent to about 50mg VIL, 100mg REM and 500mg of MET was transferred to 100ml volumetric flask; 50 ml methanol was added and sonicate for 10-15 min, volume was than make up to the mark with methanol (500µg/ml VIL, 1000 µg/ml REM, 5000 µg/ml MET) and the solution filtered through Whatman filter paper No.41. This solution was used at stock solution 0.3 ml of aliquot solution was pipetted out and transferred to a 50ml volumetric flask. Then the volume made up to the mark with methanol to get sample solution containing 3 µg/ml of VIL and 6 µg/ml of REM and 30 µg/ml of MET respectively. The results were shown in Table.14.

Table No.14 Determination of Assay VIL, REM and MET

REMO mv 500 Tablet	Amount taken (µg/ml)			Amount obtained (µg/ml)			VIL ±S.D. (n=3)	REM ±S.D. (n=3)	MET ±S.D. (n=3)
	VIL	REM	MET	VIL	REM	MET			
	3	6	30	2.96± 0.0005	5.95± 0.0012	29.48± 0.0002	98.33	98.66	99.16

Table No.15 Summary of Validation Parameter for Proposed Method

Parameter	VIL	REM	MET
Linearity (µg/ml) (n=5)	1-5(µg/ml)	2-10(µg/ml)	10-50(µg/ml)
Regression Equation (y = mx + c)	Y=37438x+28711	Y=19175x-4053.9	Y=3739.1x+29301
Regression coefficient (R^2)	0.9989	0.9977	0.9981
Correlation coefficient (r)	0.9996	0.9982	0.9990
Repeatability (%R.S.D.) (n=6)	0.1129	0.1531	0.1180
Intraday Precision (%R.S.D.) (n=3)	0.1006-0.1983	0.1022-0.1521	0.1040-0.3747
Interday Precision (%R.S.D.) (n=3)	0.2608-0.4326	0.1830-0.3423	0.1123-0.4773
LOD (µg/ml) (n=5)	0.262954	0.199752	0.0815
LOQ(µg/ml) (n=5)	0.796831	0.605308	0.2471
% Recovery (n=3)	98.31-99.45	98.5-99.05	99.83-99.95
Assay (%) ± S.D. (n=3)	98.33	98.66	99.16

5. CONCLUSION

The proposed UV and HPLC methods are new, simple, provide a rapid, accurate, precise result for Remogliflozin Etabonate, Vildagliptin and Metformin Hydrochloride in Tablet can be successfully employed in the routine analysis. In conclusion the developed method in

good recommended for the assay of Remogliflozin Etabonate, Vildagliptin and Metformin Hydrochloride in marketed pharmaceutical dosage form Tablet.

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