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Analytical Method Development and Validation of RP-HPLC Method for Estimation of Metformin HCL, Vildagliptin, and Remogliflozin Etabonate in Bulk Drug and its Tablet Dosage Form

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ABSTRACT:

This study reports the Method Development and Validation For Anti Diabetic Drugs By Rp-Hplc. The drug analysis is playing an vital position within the improvement of medicine, their manufacture and therapeutic use For the simultaneous estimation of medicine present in dosage forms, lot, of suitable techniques are adopted like uv – spectrophotometer HPLC. Those techniques are powerful rugged technique .they're additionally extraordinarily specific, specific, correct, linear and speedy. A pharmaceutical industry depends upon quantitative chemical analysis to make sure that the raw material used and the final product obtained meets the required specification. The drugs will occur as a unmarried factor or multi issue dosage paperwork. The later proves to be effective because of its mixed mode of movement at the body.

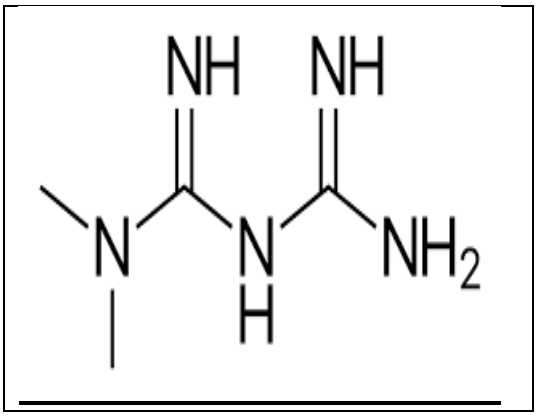
Keywords: RP-HPLC, Metformin (MET), Vildagliptin (VDG) and Remogliflozin (RMG), Diabetes Mellitus.

INTRODUCTION:

In pharmaceutical industry, there is a need for the invention of suitable novel analytical methods from time to time for testing the quality of bulk drugs, excipients and formulations. Method development and validation is an integral part of drug discovery and drug development. UV-visible spectroscopy and HPLC are the most popular techniques used for the identification and estimation of drugs with good accuracy and precision. Simultaneous method development is useful for analysis of combination of drugs.

DRUG PROFILE:

Metformin hydrochloride

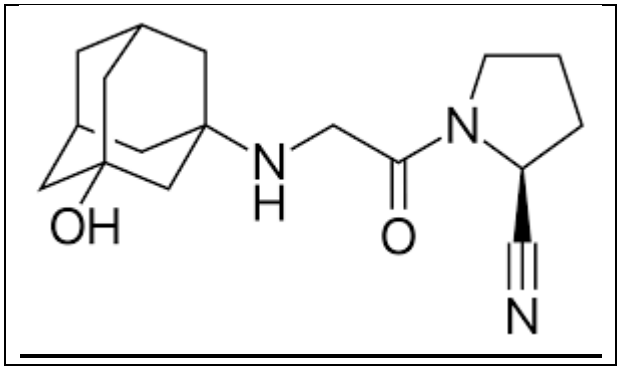


Molecular formula: C4H11N5
Molecular Weight: 129.16
Synonyms: Metfromin.
IUPAC Name: 3-(diaminomethylidiene)-1,1-dimethylguanidine;hydrochloride.
Solubility: Water,Methanol.
Category: Anti-diabetic agent.

Mechanism of action:

Metformin is an antihyperglycemic agent, which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Its pharmacological mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improve insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects and does not cause hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and daylong plasma insulin response may actually decrease.

Vildagliptin

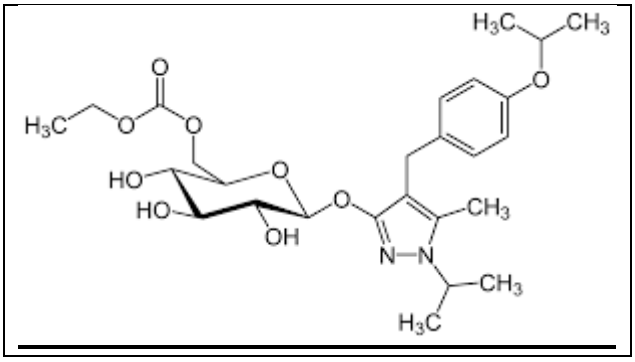


Molecular formula: C17H25N3O2
Molecular Weight: 303.399g/mol
IUPAC Name:(S)-1-[2-(3-Hydroxyadamantan-1-ylamino) acetyl]pyrrolidine-2-carbonitrile
Solubility: Water, Methanol.
Category: Type-2 diabetes mellitus.

Mechanism of action:

Inhibition of dipeptidyl peptidase-4 (DPP-4) by vildagliptin prevents degradation of glucagon-like peptide-1 (GLP-1) and reduces glycaemia in patients with type 2 diabetes mellitus, with low risk for hypoglycaemia and no weight gain. Vildagliptin binds covalently to the catalytic site of DPP-4, eliciting prolonged enzyme inhibition. This raises intact GLP-1 levels, both after meal ingestion and in the fasting state. Vildagliptin has been shown to stimulate insulin secretion and inhibit glucagon secretion in a glucose- dependent manner. At hypoglycaemic levels, the counterregulatory glucagon response is enhanced relative to baseline by vildagliptin. Vildagliptin also inhibits hepatic glucose production, mainly through changes in islet hormone secretion, and improves insulin sensitivity, as determined with a variety of methods. These effects underlie the improved glycaemia with low risk for hypoglycaemia. Vildagliptin also suppresses postprandial triglyceride (TG)-rich lipoprotein levels after ingestion of a fat-rich meal and reduces fasting lipolysis, suggesting inhibition of fat absorption and reduced TG stores in non-fat tissues. The large body of knowledge on vildagliptin regarding enzyme binding, incretin and islet hormone secretion and glucose and lipid metabolism is summarized, with discussion of the integrated mechanisms and comparison with other DPP-4 inhibitors and GLP-1 receptor activators, where appropriate..

Remogliflozin Etabonate



Molecular formula: C26H38N2O9 Molecular
Weight: 522.6
IUPAC Name: 5-Methyl-4-[4-(1-methylethoxy)benzyl]-1-(1-methylethyl)-1H-pyrazol-3-yl 6- O-(ethoxycarbonyl)-β-D-glucopyranoside
Solubility: Methanol
Category: Oral hypoglycemic agent used to treat type-2 diabetes mellitus.

Mechanism of action:

Remogliflozin etabonate is a pro-drug of remogliflozin. Remogliflozin inhibits the sodium-glucose transport proteins (SGLT), which are responsible for glucose reabsorption in the kidney. Blocking this transporter causes blood glucose to be eliminated through the urine.[8] Remogliflozin is selective for SGLT2.

MATERIALS AND INSTRUMENTS:

Procurement of Drug Sample

Sr. No.	Drug sample	Supplier (Gift Sample)
1	**Vildagliptin**	Glenmark Pharmaceuticals, Nashik
2	**Remogliflozin**	Glenmark Pharmaceuticals, Nashik
3	**Metformin HCl**	Glenmark Pharmaceuticals, Nashik

Marketed formulation details:

Remo-Zen MV 500 Tablet (Glenmark Pharmaceuticals)

Label claim:

Each Film coated tablet Contains
Vildagliptin – 50 mg
Remogliflozin – 100 mg
Metformin HCl–500 mg

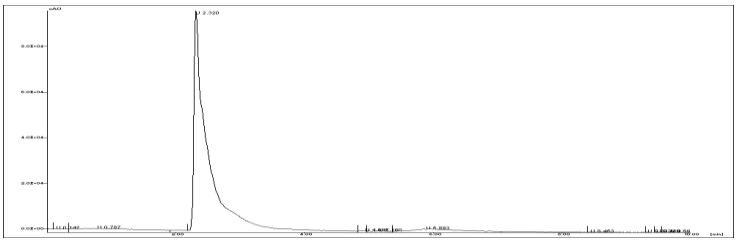
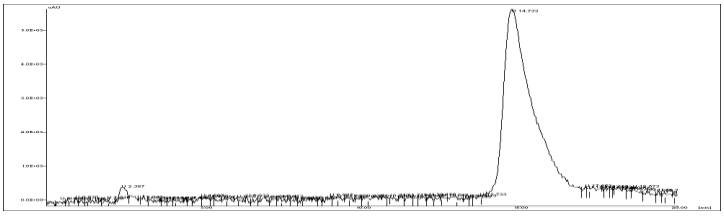
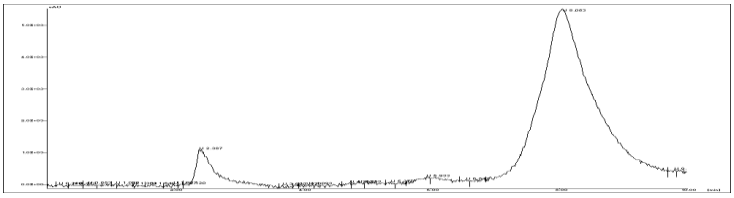
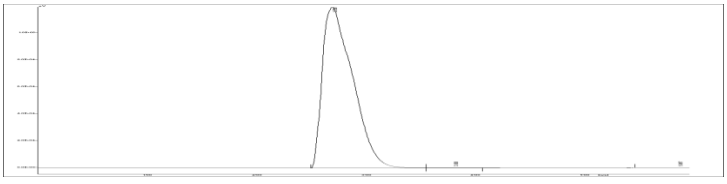
Reagents and chemicals:

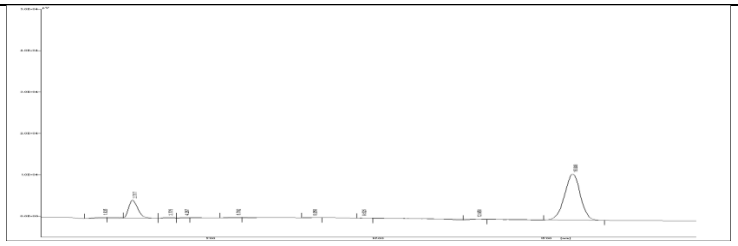
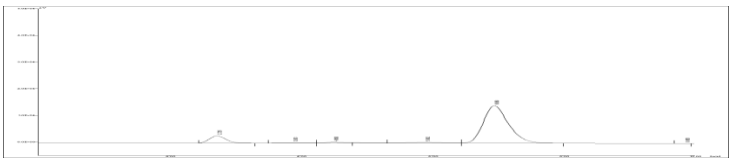
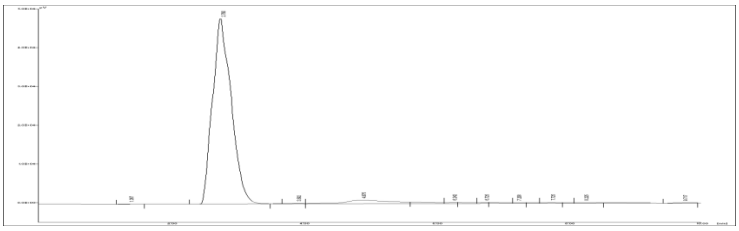
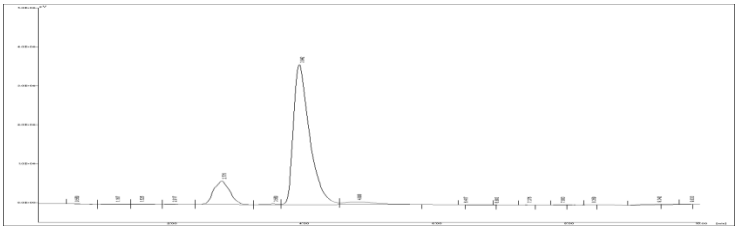
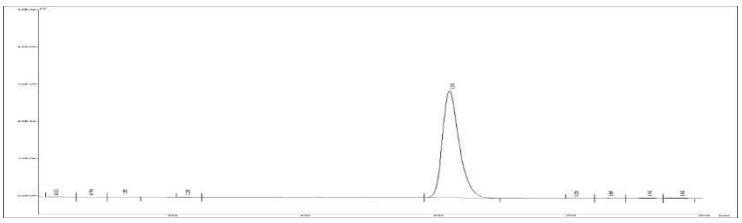
- Methanol (HPLC Grade),
- Potassium dihydrogen Phosphate (AR Grade)
- Ortho phosphoric acid (AR Grade)
- HPLC grade water.
- All chemicals and reagents that is Methanol, Potassium dihydrogen Phosphate,Ortho phosphoric acid were purchased from Merck Ltd., Mumbai.

- **Instruments:**
  1. HPLC:
     - Borwin chromatography software (version 1.50)
     - Model PU 2080 Plus Intelligent HPLC pump
     - Rheodyne sample injection port with 10µl loop
     - Nucleosil C_8 column(250 x 4.6 mm, 5µm)
     - JASCO UV-2075 UV-VIS detector
  2. Double Beam UV-Vis Spectrophotometer (Shimadzu UV-1780)
  3. Shimadzu (model AY-120) Electronic weighing balance
  4. Sonicator: PRAMA solutions for laboratory
  5. Extrapure lab link water purification system
  6. Electronic pH meter
  7. Calibrated Glassware's.

Experimental, Results and Discussion

Table 1 : Trials of mobile phase

Sr.No.	**Mobile phase**	**Observation**	**Densitogram**
1.	10 mM KH_2PO_4 (pH 4 adjusted with OPA): Methanol [40: 60 v/v]	Remogliflozin – 2.320 min Vildagliptin – 14.733 min Metformin HCl – 8.053 min High Retention Times. Broad Peaks. Peak Tailing	Remogliflozin  Vildagliptin  Metformin HCl 
2.	10 mM KH_2PO_4 (pH 4 adjusted with OPA): Methanol [55: 45 v/v]	Remogliflozin – 2.700 min Vildagliptin – 15.808 min Metformin HCl – 6.950 min High Retention Times. Broad Peaks	Remogliflozin  Vildagliptin

			 Metformin HCl 
3.	10 mM KH_2PO_4 (pH 4 adjusted with OPA): Methanol [20: 80 v/v]	Remogliflozin – 2.750 min Broad Peak Vildagliptin – 3.942 min Metformin HCl – 6.183 min Good Peak Shapes, Well Resolved Peaks which pass system suitability parameters	Remogliflozin  Vildagliptin  Metformin HCl 

- **Preparation of Standard stock solutions:**

Standard stock solution of Vildagliptin,Remogliflozinand Metformin HClwere prepared separately by dissolving 10 mg of each drug in 10 ml of methanol separately to get concentration of 1000 µg/ml. From the respective standard stock solution, working standard solution was prepared containing 100 µg/ml of Vildagliptin, RemogliflozinandMetformin HCl, separately in methanol. These solutions were appropriately diluted with methanol to obtain desired solutions.

- **Selection of Detection Wavelength:**

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. It was observed that all drugs showed considerable absorbance at 210 nm (Fig.1)

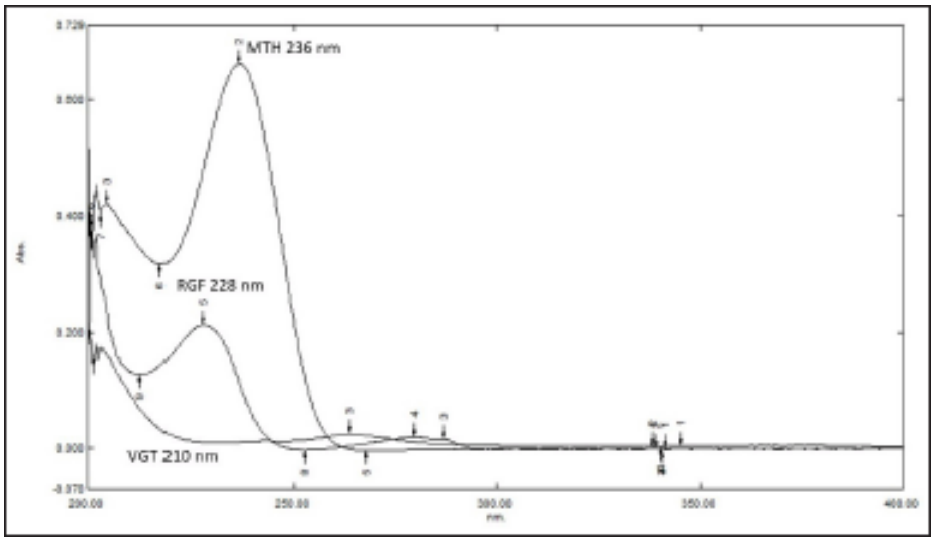


Fig.1: Overlain UV-VIS Spectra of Vildagliptin (10 µg/ml), Remogliflozin (10 µg/ml) and Metformin HCl (10 µg/ml)

- **Selection of mobile phase and chromatographic conditions:**

Chromatographic separation studies were carried out on the working standard solution of Vildagliptin (4 µg/ml) and Remogliflozin (8 µg/ml) and Metformin HCl (40 µg/ml). Initially, trials were carried out using various solvents in different proportions to obtain the desired system suitability parameters. After few trials, 10 mM KH_2PO_4 (pH 4 adjusted with OPA): Methanol [20: 80 v/v], was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

- **Chromatographs of the drug:**

Solution of 4 µg/ml of Vildagliptin, 8 µg/ml of Remogliflozinand 40 µg/ml of Metformin HClwas prepared. Individual Solutions as well as standard mixture were injected on stabilized HPLC system and chromatograph was obtained. Chromatographs were checked for system suitability parameters like number of theoretical plates (N), Asymmetry factor (AF) and resolution between the drugs

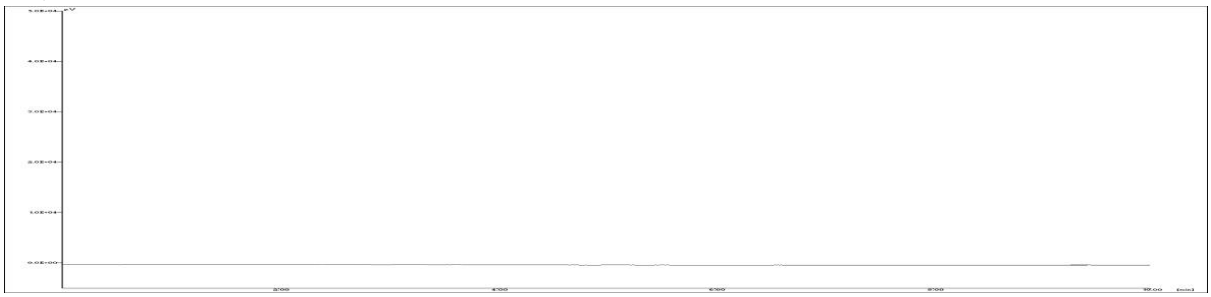
The retention time± % RSD were found to be:

Remogliflozin = 2.771 ± 0.313

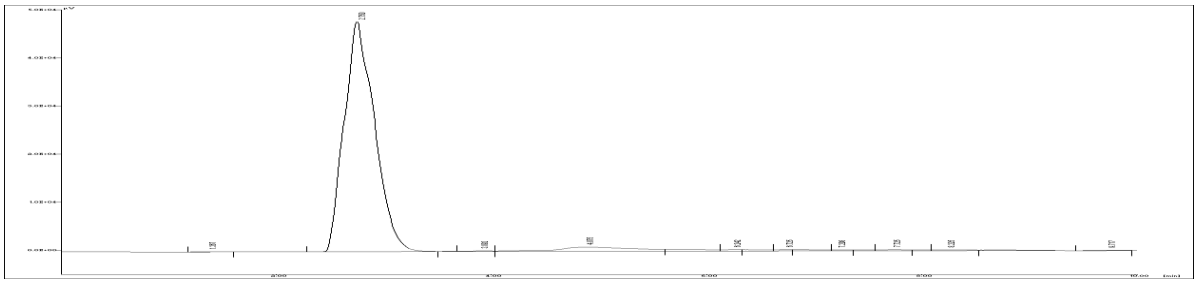
Vildagliptin = 4.103 ± 0.209

Metformin HCl = 6.224 ± 0.052

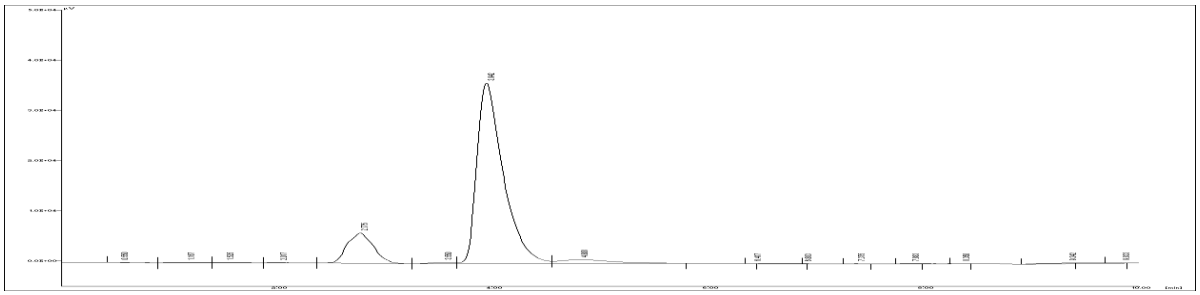
A)



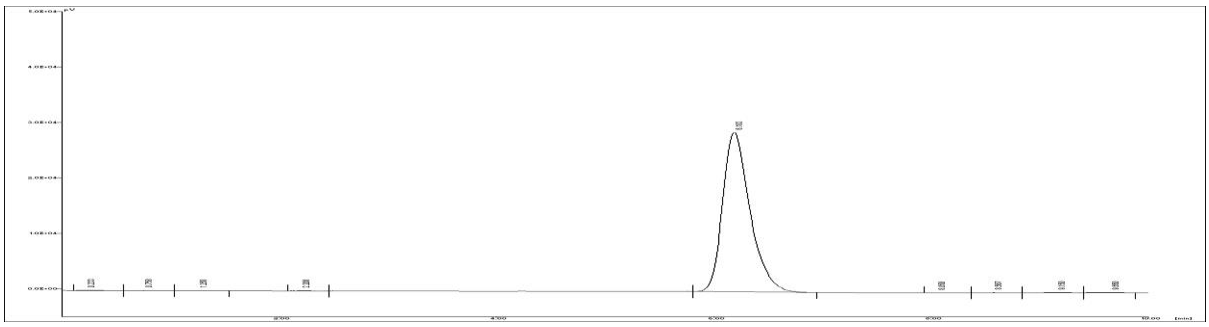
B)



C)



D)



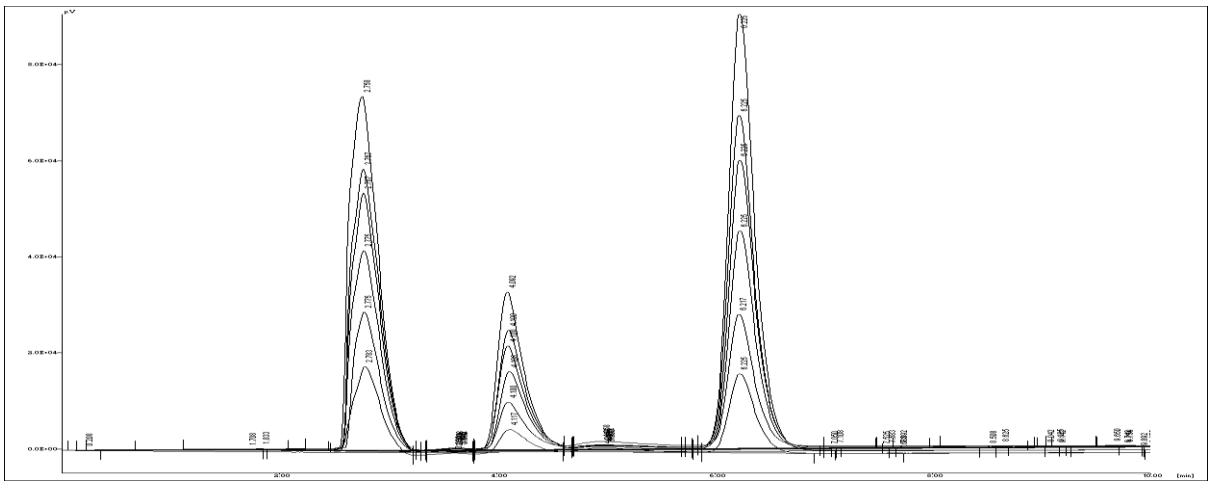


Fig.2 :Chromatograph of A) Blank B) Remogliflozin (8 µg/ml), C) Vildagliptin (4 µg/ml) D) Metformin HCl(40 µg/ml)individuals and E) mixed standard solution of all three drugs

Summary of chromatographic parameters selected:

Chromatographic parameters are summarized in Table 2 ____

Table2: Chromatographic parameters

Sr. No.	Parameter	Conditions used for Analysis
1	Stationary phase(Column)	Nucleosil C_8 column (250 x 4.6 mm, 5µm)
2.	Mobile phase	10 mM KH_2PO_4 (pH 4 adjusted with OPA): Methanol [20: 80 v/v]
3.	Detection Wavelength	210 nm
4.	Injection Volume	10 µl
5.	Temperature	Ambient

Table3: System suitability parameters for Drugs

Drug	Concentration (µg/ml)	RT ± RSD (Min)	Area	Plates	Asymmetry	Rs*
Remogliflozin	8	2.771 ± 0.313	142838.85	2342.71	1.07	-
Vildagliptin	4	4.103 ± 0.209	242698.08	2764.46	1.21	1.69
Metformin HCl	40	6.224 ± 0.052	657594.34	4138.24	1.13	3.15

*** Resolution with respect to previous peak**

- **Preparation of sample solution(Formulation Analysis):**

Ten tablets each containing 50 mg of Vildagliptin, 100 mg of Remogliflozin and 500 mg of Metformin HClwas weighed and powdered. Powder equivalent to 10 mg of Vildagliptin (20 mg of Remogliflozinand 100 mg of Metformin HCl) was transferred to 10 ml volumetric flask and was diluted with methanol, sonicated for 10 min and volume made to 10 ml with methanol. Solution was filtered and further dilutions were made to get the final concentration of 4 µg/ml of Vildagliptin, 8 µg/ml of Remogliflozin and 40 µg/ml of Metformin HCl.

Validation of Analytical Method

Linearity

From the standard stock solution (1000 µg/ml) of Vildagliptin, Remogliflozin and Metformin HCl, further dilutionswere was prepared separately by appropriate dilution with methanol. The linearity (relationship between peak area and concentration)was determined by analyzing six solutions over the concentration range 2 - 12 µg/ml for Vildagliptin, 4 - 24 µg/ml for Remogliflozin and 20 - 120 µg/ml for Metformin HCl.The results obtained are shown in Table_4 for Vildagliptin, Table_5 for Remogliflozin and in Table _6_ for Metformin HCl. Overlain chromatographs of Linearity are shown in Fig.3, 4, 5 and calibration curve shown in Fig. 6,7,8.

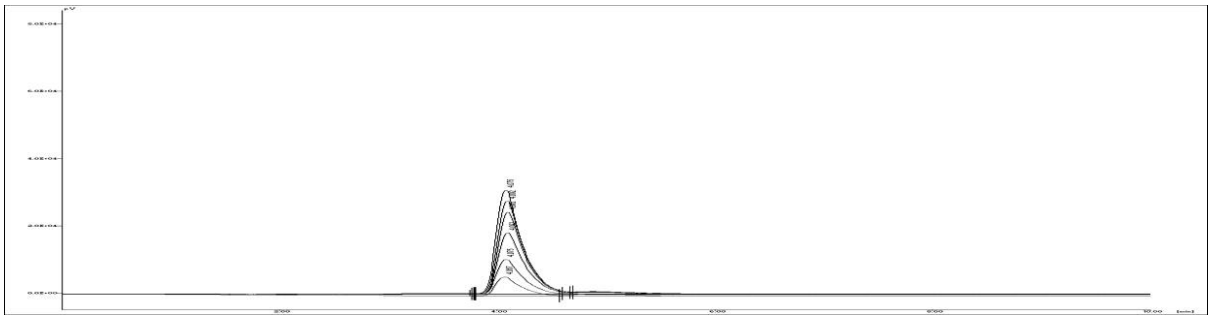


Fig _3__: Overlain chromatographs of Linearity for Vildagliptin (2-12 µg/ml)

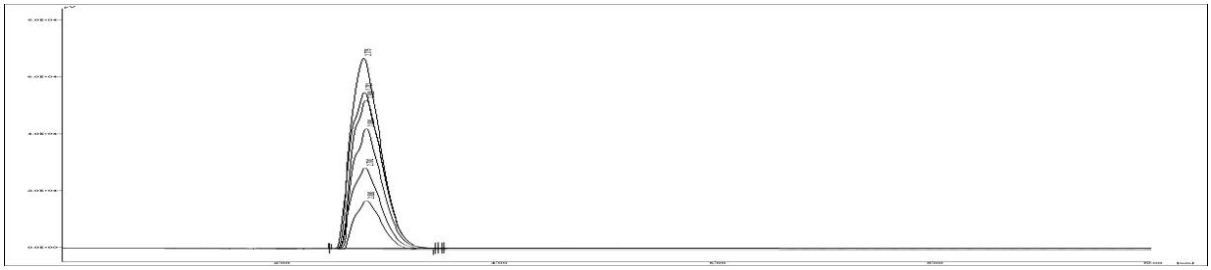


Fig _4___: Overlain chromatographs of Linearity for Remogliflozin (4-24 µg/ml)

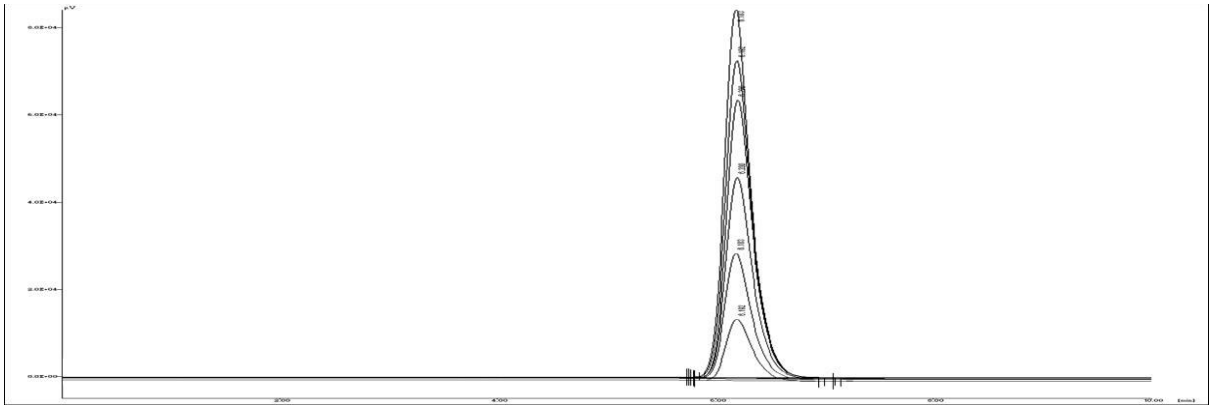


Fig _5___: Overlain chromatographs of Linearity for Metformin HCl (20 - 120 µg/ml)

Table 4___: Linearity study of Vildagliptin

Replicates						
	Concentrations of Vildagliptin(µg/ml)					
	2	**4**	**6**	**8**	**10**	**12**
	Peak Area					
1	72762.69	142233.91	214139.63	302056.76	381370.99	449293.85
2	70699.23	142838.85	208258.05	291566.28	370435.07	434569.14
3	70990.92	144299.05	214994.78	303838.54	390645.26	455535.08
4	69630.56	144632.81	217332.12	304299.00	377902.53	446636.19
5	71475.06	140987.53	214429.22	305219.92	382642.26	455424.35
6	69164.10	139704.64	214078.87	304874.57	377837.46	451155.71
Mean	70787.093	142449.463	213872.109	301975.841	380138.926	448769.050

Std.dev.	1295.935	1900.390	3005.807	5218.408	6672.792	7774.707
%RSD	1.831	1.334	1.405	1.728	1.755	1.732

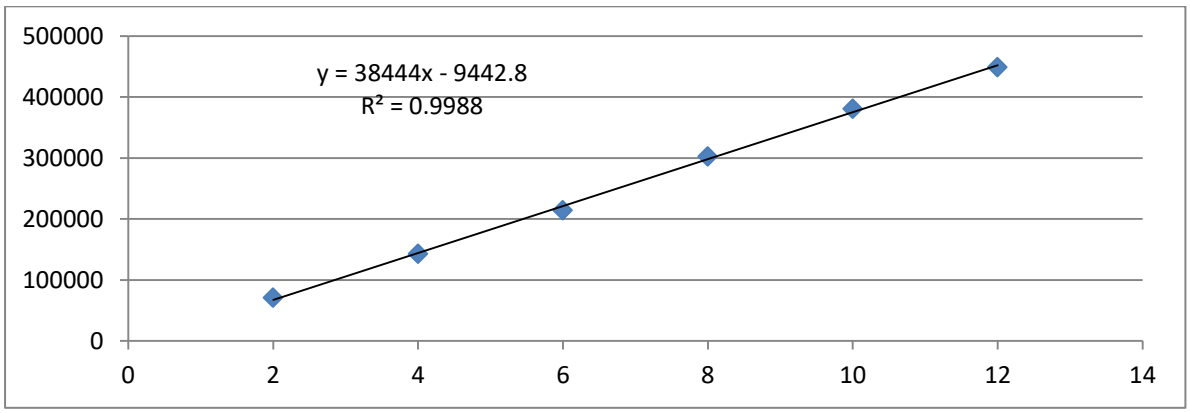


Fig.6: Calibration curve for Vildagliptin

Table 5: Linearity study of Remogliflozin

Replicates	**Concentrations of Remogliflozin(µg/ml)**					
	4	**8**	**12**	**16**	**20**	**24**
	Peak Area					
1	141261.62	239575.48	342987.58	461226.04	571742.66	696297.77
2	143135.18	242698.08	348851.31	461250.66	572852.98	691211.53
3	145262.37	247573.03	347850.69	476367.05	586189.89	693155.60
4	145290.98	249675.89	353039.68	458986.17	578101.35	703683.92
5	141423.76	243754.96	353151.37	465327.45	577560.90	703425.70
6	144882.64	246323.57	352437.80	459412.53	577861.15	674349.49
Mean	143542.756	244933.501	349719.737	463761.647	577384.821	693687.333
Std.dev.	1879.813	3646.480	3993.881	6569.661	5112.185	10788.386
%RSD	1.310	1.489	1.142	1.417	0.885	1.555

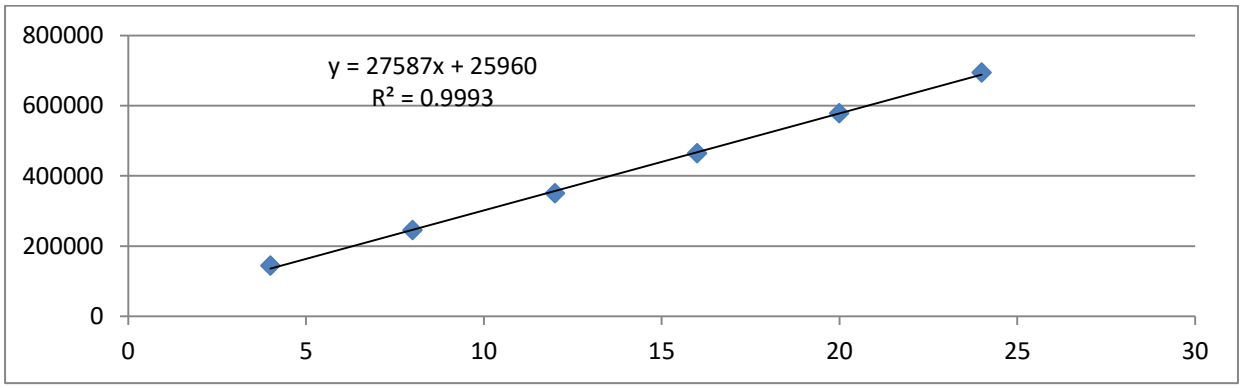


Fig.7: Calibration curve for Remogliflozin

Table 6: Linearity study of Metformin HCl

Replicates	Concentrations of Metformin HCl(µg/ml)					
	20	**40**	**60**	**80**	**100**	**120**
	Peak Area					
1	319759.92	662165.50	1041515.63	1316849.13	1736073.91	2100965.11
2	308450.91	645290.08	1006184.93	1316291.93	1701244.89	2064039.62
3	317799.74	657594.34	1039655.51	1308758.78	1767189.29	2054389.22
4	314703.57	663284.06	1033101.83	1340414.27	1719379.60	2033359.39
5	320755.17	670079.55	1044219.68	1320941.78	1699757.12	2010399.48
6	315749.03	671949.31	1035403.42	1352130.26	1756573.85	2037400.49
Mean	316203.054	661727.138	1033346.833	1325897.689	1730036.443	2050092.215
Std.dev.	4437.855	9634.610	13905.437	16681.302	28214.813	31040.705
%RSD	1.403	1.456	1.346	1.258	1.631	1.514

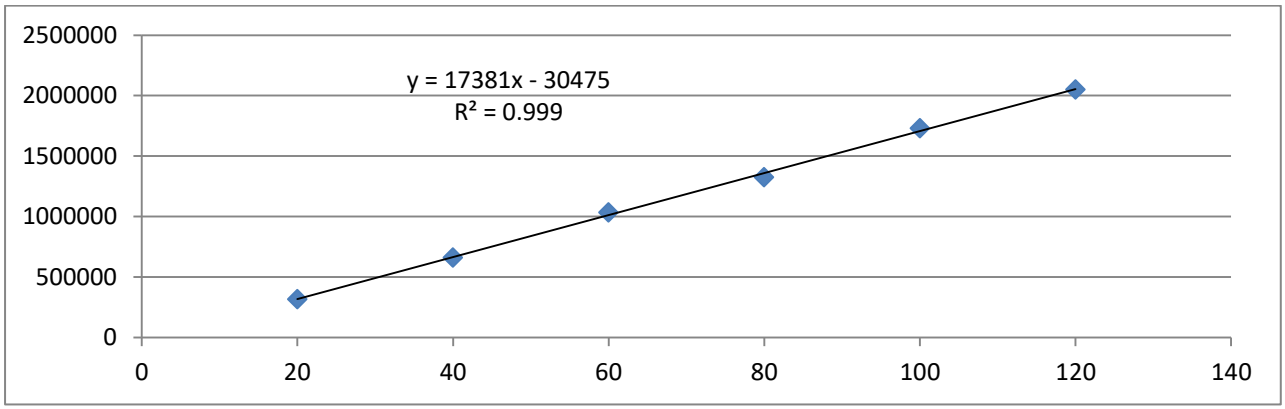


Fig.8: Calibration curve for Metformin HCl

Range

VILDAGLIPTIN = 2 – 12µg/ml

REMOGLIFLOZIN = 4- 24 µg/ml

METFORMIN HCL = 20- 120 µg/ml

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3different concentrations were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD were calculated.The results obtain for intra-day and inter day variations are shown in Table 7, 8, 9 10, 11 and 12_.

Table 7 : Intra-day precision study of Vildagliptin

Concentration (µg/ml)	Area	Practical Concentration (µg/ml)	% Recovery	Mean % Recovery ± RSD
4	144747.65	4.011	100.269	99.838 ± 0.385
	143612.82	3.981	99.531	
	143894.70	3.989	99.714	

6	220806.20	5.989	99.820	100.334 ± 0.488
	222114.66	6.023	100.387	
	223055.35	6.048	100.795	
8	298722.65	8.016	100.199	100.792 ± 0.553
	300795.39	8.070	100.873	
	302121.30	8.104	101.304	

Table 8: Inter-day precision of Vildagliptin

Concentration (µg/ml)	**Area**	**Practical Concentration (µg/ml)**	**% Recovery**	**Mean % Recovery ± RSD**
4	144626.95	4.008	100.191	99.577 ± 0.540
	143080.47	3.967	99.185	
	143344.25	3.974	99.356	
6	223174.65	6.051	100.847	99.989 ± 0.909
	221417.25	6.005	100.085	
	219001.17	5.942	99.037	
8	299858.52	8.045	100.569	100.230 ± 0.322
	298711.23	8.016	100.195	
	297878.70	7.994	99.925	

Table 9 : Intra-day precision study of Remogliflozin

Concentration (µg/ml)	**Area**	**Practical Concentration (µg/ml)**	**% Recovery**	**Mean % Recovery ± RSD**
8	247880.60	8.044	100.555	100.168 ± 0.354
	246854.10	8.007	100.090	
	246345.06	7.989	99.859	
12	356643.12	11.987	99.891	100.344 ± 0.994
	355855.66	11.958	99.653	
	361927.44	12.178	101.487	
16	467110.86	15.991	99.945	99.850 ± 0.132
	466934.75	15.985	99.905	
	466026.12	15.952	99.700	

Table 10: Inter-day precision of Remogliflozin

Concentration (µg/ml)	**Area**	**Practical Concentration (µg/ml)**	**% Recovery**	**Mean % Recovery ± RSD**
8	246019.02	7.977	99.711	100.332 ± 0.537
	248001.60	8.049	100.610	
	248146.15	8.054	100.675	
12	356540.10	11.983	99.860	99.907 ± 0.757

	359276.55	12.082	100.686	
	354273.66	11.901	99.175	
16	468101.67	16.027	100.170	100.380 ± 0.580
	467053.72	15.989	99.932	
	471934.19	16.166	101.038	

Table 11 : Intra-day precision study of Metformin HCl

Concentration (µg/ml)	**Area**	**Practical Concentration (µg/ml)**	**% Recovery**	**Mean % Recovery ± RSD**
40	659590.75	39.702	99.256	99.527 ± 0.433
	659906.75	39.720	99.301	
	664929.85	40.009	100.024	
60	1008198.00	59.759	99.599	99.763 ± 0.359
	1014202.00	60.105	100.174	
	1007348.75	59.710	99.517	
80	1355083.80	79.717	99.646	100.203 ± 0.872
	1356560.10	79.802	99.752	
	1376829.45	80.968	101.210	

Table 12: Inter-day precision of Metformin HCl

Concentration (µg/ml)	**Area**	**Practical Concentration (µg/ml)**	**% Recovery**	**Mean % Recovery ± RSD**
40	664295.10	39.973	99.932	100.364 ± 1.483
	658781.00	39.656	99.139	
	678813.20	40.808	102.021	
60	1008928.75	59.801	99.669	99.715 ± 0.237
	1007230.25	59.703	99.506	
	1012088.75	59.983	99.972	
80	1354385.55	79.677	99.596	99.727 ± 0.406
	1351732.20	79.524	99.405	
	1362525.15	80.145	100.181	

Assay (Formulation Analysis)

Tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was applied and area was recorded. Percentage recovery was determined from linearity equation. Assay results obtained are shown in Table 13, 14, 15 and representative chromatograph in Fig. 9_.

Table 13 : Assay of Marketed Formulation (Vildagliptin)

Sr. No.	Peak area	Amount Recovered (µg/ml)	% Recovery
1	145000.65	4.017	100.434
2	143700.28	3.984	99.588
3	143876.46	3.988	99.702
4	143829.05	3.987	99.672
5	144924.90	4.015	100.384
6	142667.55	3.957	98.916
Mean	143999.814	3.991	99.783
SD	867.927	0.023	0.564
%RSD	0.603	0.566	0.566

Table 14 : Assay of Marketed Formulation (Remogliflozin)

Sr. No.	Peak area	Amount Recovered (µg/ml)	% Recovery
1	248659.98	8.073	100.908
2	244516.80	7.922	99.031
3	247037.04	8.014	100.173
4	247414.75	8.028	100.344
5	246219.88	7.984	99.802
6	247523.65	8.031	100.393
Mean	246895.349	8.009	100.108
SD	1407.999	0.051	0.638
%RSD	0.570	0.637	0.637

Table 15 : Assay of Marketed Formulation (Metformin HCl)

Sr. No.	Peak area	Amount Recovered (µg/ml)	% Recovery
1	660373.67	39.747	99.368
2	659211.07	39.680	99.201
3	658600.22	39.645	99.113
4	657989.36	39.610	99.025
5	662816.60	39.888	99.720
6	666586.61	40.105	100.262
Mean	660929.585	39.779	99.448
SD	3252.565	0.187	0.468
%RSD	0.492	0.470	0.470

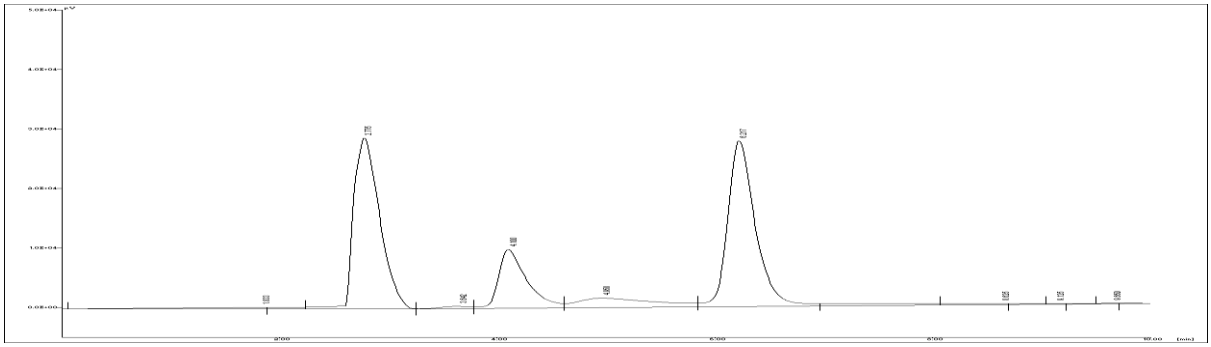


Fig. 9 : Chromatograph of Vildagliptin (4µg/ml), Remogliflozin (8µg/ml) and Metformin HCl (40 µg/ml)

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 50, 100 and 150 %. Basic concentrations of sample chosen were 4 µg/mlof Vildagliptin, 8µg/mlof Remogliflozinand 40 µg/mlof Metformin HClsample solution. These solutions were injected on the system in triplicate to obtain the chromatograph. The drug concentrations of Vildagliptin,Remogliflozinand Metformin HClwere calculated by using linearity equations of each drug. The results obtained are shown in Table _16,17,18_.

Table 16: Recovery studies of Vildagliptin

Level	Conc. (µg/ml)		Area	Concentration (X)	% Recovery	Mean % Recovery ± RSD
	Sample	Std.				
50 %	4	2	220099.20	5.971	99.513	99.963 ± 0.428
			222060.93	6.022	100.364	
			221250.60	6.001	100.012	
100 %	4	4	297854.05	7.993	99.917	100.323 ± 0.357
			299522.85	8.037	100.459	
			299933.87	8.047	100.593	
150 %	4	6	376386.27	10.036	100.361	100.537 ± 0.469
			375684.87	10.018	100.179	
			379113.60	10.107	101.071	

Table 17: Recovery studies of Remogliflozin

Level	Conc. (µg/ml)		Area	Concentration (X)	% Recovery	Mean % Recovery ± RSD
	Sample	Std.				
50 %	8	4	357633.65	12.023	100.190	100.116 ± 0.075
			357394.56	12.014	100.118	
			357133.98	12.005	100.039	
100 %	8	8	469972.40	16.095	100.594	99.993 ± 0.522
			465820.29	15.944	99.653	
			466171.56	15.957	99.733	

150 %	8	12	577197.63	19.982	99.909	99.897 ± 0.164
			576196.73	19.946	99.728	
			577998.26	20.011	100.054	

Table 18: Recovery studies of Metformin HCl

Level	Conc. (µg/ml)		Area	Concentration (X)	% Recovery	Mean % Recovery ± RSD
	Sample	Std.				
50 %	40	20	1012588.20	60.012	100.019	100.162 ± 0.131
			1014374.70	60.114	100.191	
			1015268.50	60.166	100.276	
100 %	40	40	1357457.00	79.853	99.817	99.725 ± 0.106
			1356520.20	79.800	99.749	
			1354585.05	79.688	99.610	
150 %	40	60	1707759.90	100.008	100.008	99.851 ± 0.150
			1704767.40	99.836	99.836	
			1702565.80	99.709	99.709	

Limit of Detection (LOD)

LOD is calculated from the formula: -

$$LOD = \frac{3.3\ \sigma}{S}$$

Where,

σ = standard deviation of response for the lowest conc. in the range

S = slope of the calibration curve.

LOD of Vildagliptin =0.242 µg/ml

LOD of Remogliflozin =0.606µg/ml

LOD of Metformin HCl = 3.315 µg/ml

Limit of Quantification (LOQ)

The Quantitation limit is expressed as:

$$LOQ = \frac{10\ \sigma}{S}$$

LOQ of Vildagliptin=0.732 µg/ml

LOQ of Remogliflozin=1.835µg/ml

LOQ of Metformin HCl =10.046 µg/ml

Specificity:

No peaks were found in blank at retention time of drugs indicating the non interference of any other peak of degradation product or impurity or excipients.

Robustness:

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio,flow rate, pH and detection wavelength were altered and the effect on the area was noted. The results obtained are shown in Table _19 A, B, C.

Table 19A:Robustness Samples - Vildagliptin			
	MP Composition		
	(18:82)	(20:80)	(22:78)
	142646.50	139199.35	140713.45
	144570.80	141419.90	139586.50
	143324.50	141594.10	141254.20
AVG	143513.933	140737.783	140518.050
STD DEV	976.036	1335.166	850.848
% RSD	0.680	0.949	0.606
RSD AVG	0.745		
	Flow Rate		
	0.95	1	1.05
	139266.30	143690.15	140826.75
	137807.05	140862.80	140950.35
	138215.70	141120.30	140620.75
AVG	138429.683	141891.083	140799.283
STD DEV	752.791	1563.348	166.508
% RSD	0.544	1.102	0.118
RSD AVG	0.588		
	pH		
	3.9	4	4.1
	142598.35	143350.25	140826.75
	142242.12	141577.75	139950.35
	141650.75	142835.25	140620.75
AVG	142163.740	142587.750	140465.950
STD DEV	478.638	911.801	458.248
% RSD	0.337	0.639	0.326
RSD AVG	0.434		
	Wavelength (nm)		
	209	210	211
	142588.05	139292.05	137850.05
	141655.49	140837.05	140595.00
	143582.00	141867.05	139631.95
AVG	142608.513	140665.383	139359.000
STD DEV	963.418	1296.055	1392.682
% RSD	0.676	0.921	0.999
RSD AVG	0.865		

Table 19B:Robustness Samples - Remogliflozin			
	MP Composition		
	(18:82)	(20:80)	(22:78)
	249923.75	250450.18	248183.17
	241891.12	249967.85	249981.72
	248261.75	250845.22	248019.75
AVG	246692.207	250421.083	248728.213
STD DEV	4240.093	439.408	1088.639
% RSD	1.719	0.175	0.438
RSD AVG	0.777		
	Flow Rate		
	0.95	1	1.05
	255019.62	251419.85	247794.15
	249189.45	248750.05	248031.85
	253398.21	251655.82	245400.16
AVG	252535.760	250608.573	247075.387
STD DEV	3009.250	1613.847	1455.649
% RSD	1.192	0.644	0.589
RSD AVG	0.808		
	pH		
	3.9	4	4.1
	251456.15	247154.61	247553.92
	252412.05	252269.42	244740.65
	250604.85	244438.15	246216.85
AVG	251491.017	247954.060	246170.473
STD DEV	904.104	3976.372	1407.208
% RSD	0.359	1.604	0.572
RSD AVG	0.845		
	Wavelength (nm)		
	209	210	211
	251208.12	250202.05	248591.47
	247783.86	248219.44	242786.51
	246952.45	248534.36	248352.59
AVG	248648.143	248985.283	246576.857
STD DEV	2255.642	1065.450	3284.709
% RSD	0.907	0.428	1.332
RSD AVG	0.889		

Table 19C :Robustness Samples - Metformin HCl			
	MP Composition		
	(18:82)	(20:80)	(22:78)
	639018.45	650245.25	641955.91

	651841.25	649352.55	652365.54
	654459.75	638380.05	658569.45
AVG	648439.817	645992.617	650963.633
STD DEV	8263.519	6607.769	8395.024
% RSD	1.274	1.023	1.290
RSD AVG	1.196		
	Flow Rate		
	0.95	1	1.05
	656474.72	641053.35	669043.24
	663975.91	651322.55	658838.84
	648135.64	652085.72	657292.65
AVG	656195.423	648153.873	661724.910
STD DEV	7923.828	6161.062	6384.837
% RSD	1.208	0.951	0.965
RSD AVG	1.041		
	pH		
	3.9	4	4.1
	641053.35	653542.05	662873.62
	641322.55	660798.82	652265.25
	652085.7	649711.65	658190.41
AVG	644820.533	654684.173	657776.427
STD DEV	6293.258	5631.134	5316.288
% RSD	0.976	0.860	0.808
RSD AVG	0.881		
	Wavelength (nm)		
	209	210	211
	663746.45	658115.55	655008.37
	651407.42	653402.41	658205.35
	661342.45	654160.53	646160.55
AVG	658832.107	655226.163	653124.757
STD DEV	6541.352	2530.830	6239.415
% RSD	0.993	0.386	0.955
RSD AVG	0.778		

Summary of validation study

The summary of validation parameters aresummarized in Table

Table 20: Summary of validation study

Sr. No.	**Validation Parameter**	**Results**		
		Vildagliptin	**Remogliflozin**	**Metformin HCl**
1.	Linearity	y = 38444x - 9442.8 R^2 = 0.9988	y = 27587x + 25960 R^2 = 0.9993	y = 17381x - 30475 R^2 = 0.9990
2.	Range (µg/ml)	2 – 12	4- 24	20- 120

3.	Precision	%RSD	%RSD	% RSD
	A) Intraday precision	0.385 – 0.553	0.132 – 0.994	0.359 – 0.872
	B) Interday precision	0.322 – 0.909	0.537 – 0.757	0.237 – 1.483
4.	Assay (Mean ± RSD)	99.783±0.566	100.108±0.637	99.448 ±0.470
5.	Accuracy (% Recovery)	**Mean % Recovery ± RSD**		
	50%	99.963 ± 0.428	100.116 ± 0.075	100.162 ± 0.131
	100%	100.323 ± 0.357	99.993 ± 0.522	99.725 ± 0.106
	150%	100.537 ± 0.469	99.897 ± 0.164	99.851 ± 0.150
6.	LOD(µg/ml)	0.242	0.606	3.315
7.	LOQ(µg/ml)	0.732	1.835	10.046
8.	Specificity	Specific	Specific	Specific
9.	Robustness	Robust	Robust	Robust

Conclusion:

The developed method was found to be simple, sensitive, accurate, precise and repeatable for analysis of Vildagliptin, RemogliflozinandMetformin HClinmixture. The method was successfully used for determination of drugs in a pharmaceutical formulationwithout any interference from the excipients.

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