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HPLC Method Development And Validation Of Simultaneous Determination Of Remogliflozin And Metformin In Tablets Dosage Form

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ABSTRACT

A simple, rapid, economical, precise and accurate RP-HPLC method for simultaneous estimation of Remogliflozin Etabonate and Metformin HCl in their tablet dosage form has been developed. The separation was achieved by C18 (4.6 mm X 10 cm) column containing packing L1 and HPLC Grade water: methanol (60:40) as mobile phase, at a flow rate of 0.7 ml/min. Detection was carried out at 230 nm. Retention time of Remogliflozin etabonate and Metformin HCl were found to be 4.351 min and 3.294 min respectively. The method has been validated as per ICH guidlines for linearity, accuracy, repeatability, precision, and robustness. Linearity observed for Remogliflozin etabonate 20-100 µg/ml and for Metformin HCl100-500 µg/ml with correlation coefficient greater than 0.999. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Remogliflozin etabonate and Metformin HCl in their Synthetic Mixture.

Keywords: Remogliflozin Etabonate, metformin HCl, RP-HPLC, validation.

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INTRODUCTION

Remogliflozin etabonate (ethyl[(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6[5-methyl1--propan-2-yl-4-[(4- propan-2- yloxyphenyl) methyl]pyrazol-3-yl]oxyoxan-2-yl]methyl carbonate) (Figure 1) is an antidiabetic agent that resulting from complete or relative in insulin excretion and or insulin action. The low-affinity sodium glucose cotransporter (SGLT2) plays an important role in renal glucose reabsorption and is a remarkable transporter as a molecular target for the treatment of diabetes. We have discovered Remogliflozin etabonate, which is a novel category of selective SGLT2 inhibitors. Remogliflozin etabonate is a prodrug based on benzylpyrazole glucoside and is metabolized to its active form. [1, 2]

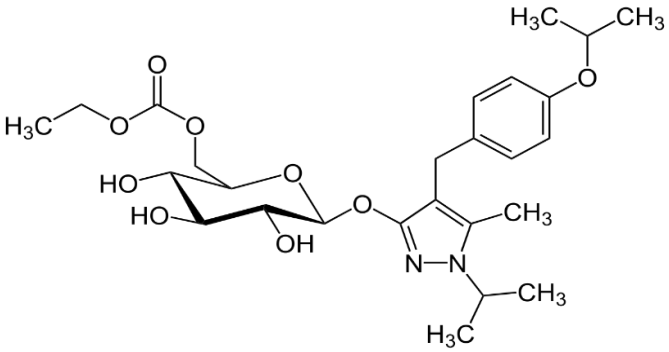

Figure 1: Structure of Remogliflozin etabonate.

Metformin **(**N, N-Dimethyl imidodicarbonimidic diamide) (Figure 2) is a first line agent for the treatment of type 2 diabetes that can be used alone or in combination with sulfonylureas, thiazolidinediones, incertin-based drugs, sodium glucose cotransporter-2 inhibitors, or other hypoglycemic agents. Metformin has not been linked to serum enzyme elevations during therapy and is an exceeding rare cause of idiosyncratic clinically apparent acute liver injury. [3,4]

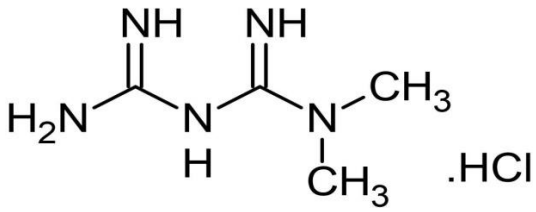

Figure 2: Structure of Metformin HCl.

Reverse phase High Performance Liquid Chromatograph

Reverse phase mode is the most popular mode for analytical and preparative separation of compounds of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non-polar hydrophobic packing with octyl and octadecyl functional group boded to silica gel and the mobile phase is a polar solvent, often a partially or

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fully aqueous mobile phase. Polar substances prefer the mobile phase and elute first. As the hydrophobic character of the solutes increases, retention increases. Generally, the lower the polarity of the mobile phase, the higher is its eluent strength. The elution order of the classes of compounds is reversed (thus the name reverse-phase chromatography). Hydrocarbons are retained more strongly than alcohols. Thus water is the weakest eluent. Methanol and Acetonitrile are popular solvents because they have low viscosity and are readily available with excellent purity. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity. The polar compound gets eluted first in this mode and non-polar compounds are retained for loner time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster. [5, 6, 7]

MATERIALS AND METHODS

Chemical and reagent:

Sr. no	Name of API	Supplier
1	Remogliflozin	Intas pharmaceuticals Ltd
2	Metformin	Ali-Allana college of pharmacy, Akkalkuwa
3	HPLC Grade Methanol	Thermosil fine chem industry
4	HPLC Grade Water	Thermosil fine chem industry

Marketed formulation

Formulation	Brand Name	Strength	Name of company	Mfg. Date	Exp. Date
Remogliflozin etabonate and metformin HCL	Remo-M 500	Remogliflozin etabonate 100 + Metformin HCl 500	Glenmark pharmaceutical Ltd	DEC. 2020	NOV. 2022

Apparatus

RP-HPLC method development and validation was done on a HPLC instrument (LC- Solution, 20µL fixed loop. Shimadzu) UV Detector, Stationary Phase used was C18 (4.6 mm X 10 cm), mobile phase consisting of Methanol and water (40:60) was used. The flow rate was 0.7 ml/min and the effluents were seen at 230nm. Injection volume was 20 µl. Every weight was measured using an analytical balance (Shimadzu).

Preparation of Standard Stock Solution

A) Preparation of std.Remogliflozin solution:

From the freshly prepared standard stock solution (1000ug/ml), 0.1ml stock solution was pipette out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration of 10 µg/ml.

B) Preparation of std. Metformin solution:

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From the freshly prepared standard stock solution (10000ug/ml), 0.1 ml stock solution was pipette out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 100 µg/ml.

C) Preparation of working std. Remogliflozin and Metformin solution:

From the freshly prepared standard stock solution (1000 + 10000ug/ml), 0.1 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 10 +100ug/ml which was used in particular trials.

Standardized Chromatographic conditions

Standard solutions of 10µg/ml of Remogliflozin and 100 µg/ml of Metformin hydrochloride were injected in column with 20 µl micro syringe. The chromatogram was run for appropriate minutes with mobile phase Methanol and water (40:60). The detection was done at wavelength 230 nm.

Figure 3: Remogliflozin water: methanol (60:40); flow rate 0.7

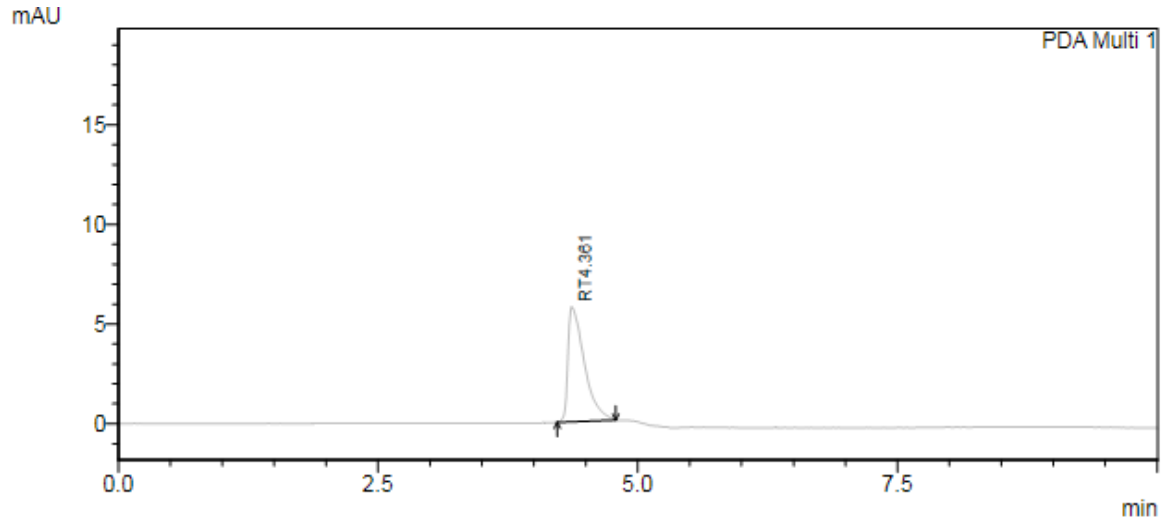

Figure 4: Metformin water: methanol (60:40); flow rate 0.7

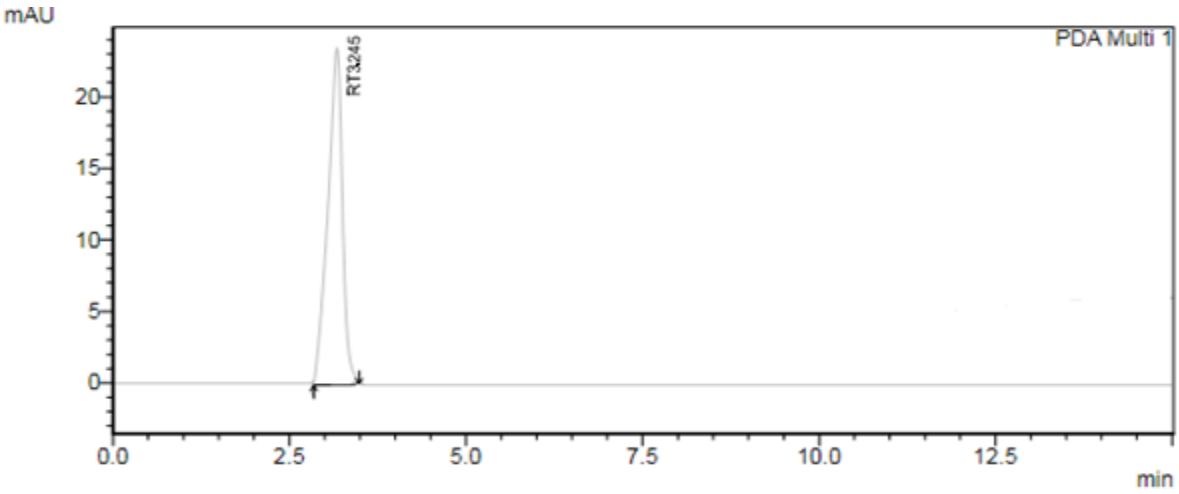

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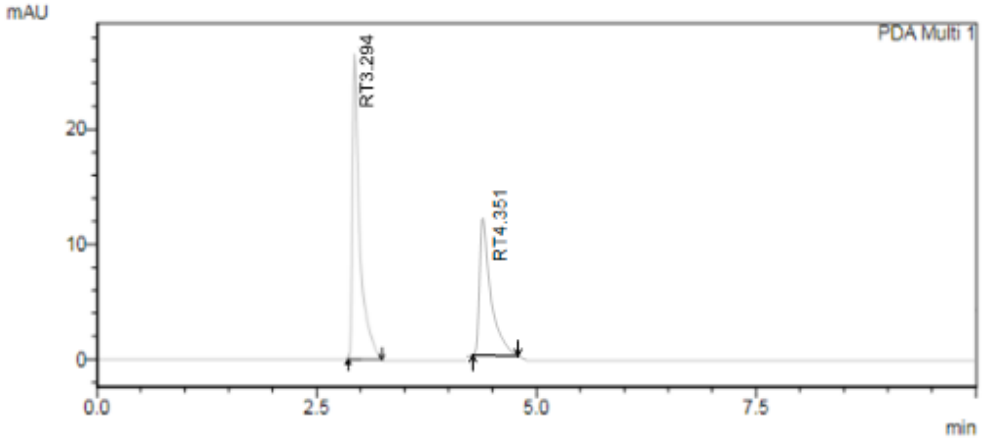
Figure 5: combination (Remogliflozin and Metformin) water: methanol (60:40); flow rate 0.7



Sr. no	concentration µg/ml	Area	Retention time	Height	% Area
1	50	1125.7	4.351	11678	16.006
2	500	34123.54	3.294	79571	83.994

Table 1: Chromatographic condition

Parameters	Chromatographic Condition
Mobile phase	methanol: Water(40:60)
Flow rate	0.7ml/min
Wavelength	230nm
Column	C18
Software	LC solution
Detector	PDA detector
Injector	20µl fixed loop
Run time	10 min
Diluents	methanol: water

Method Validation

The method was validated as per the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures [8]

Linearity:

"The linearity of an analytical procedure is its ability (within given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample" Linearity

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is mathematical expression through graph plotted for average against concentration. the linearity proves that sample would prepare in any given concentration is useful to analysis. the linearity study is generally selected on the type of experiment.

Acceptance Criteria:

The plot should be linear passing through the origin.

Correlation Coefficient should not be less than 0.999.

Preparation of standard stock solution

The linearity for Remogliflozin and Metformin were assessed by preparing the solution in the range of 100-500µg/ml respectively. Take 2, 3,4,5,6 ml of sample stock solution and transferred into 10ml volumetric flask and volume was made up to the mark with diluents. Correlation co-efficient for calibration curve Remogliflozin and Metformin was found to be 0.999.

Table 2: Linearity data for Remogliflozin etabonate

Sr no.	concentration	Area
1	20	468.47
2	40	912.07
3	60	1346.79
4	80	1822.46
5	100	2204.2

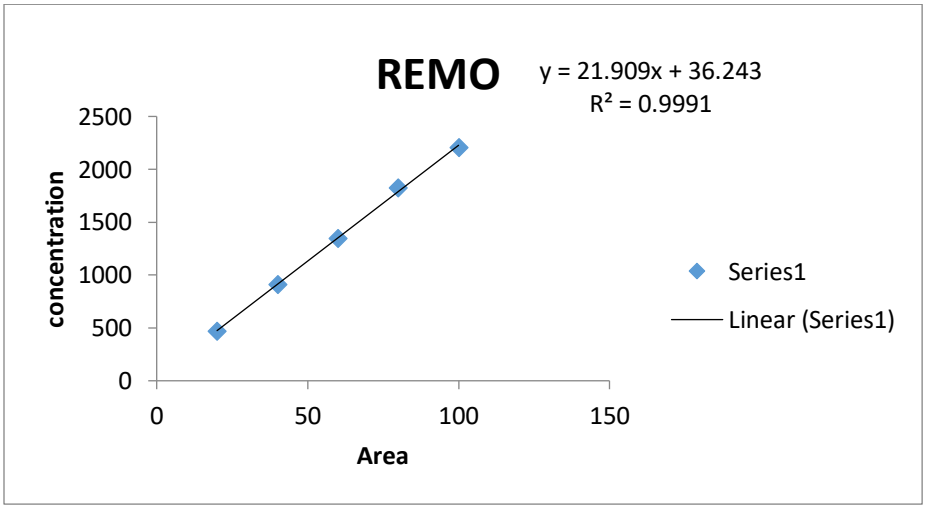

Table 3: Linearity data for Metformin HCL

Sr no.	concentration	Area
1	100	7578.33
2	200	13528

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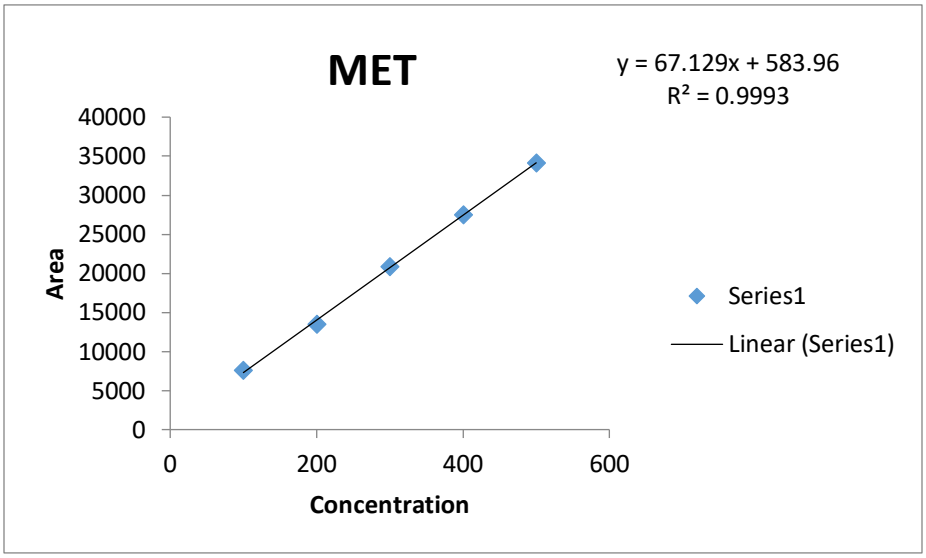
3	300	20831.66
4	400	27537.3
5	500	34138.27



Accuracy:

The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness"

The accuracy is carried out by adding of drugs with placebo at lower level to higher level of the test concentration for assay and with limit concentration for related substances. Accuracy also calculated by spiking of sample through a stock. This parameter is useful to prove the accuracy of sample from 40.0 -% to 120.0-%

Acceptance Criteria:

Mean recovery should be in the range of 98-102%.

The Relative Standard Deviation should not be more than 2.0%.

For Remogliflozin

10 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10 ml. The area of each solution peak was measured at 230 nm. At each level, the amount of Remogliflozin was determined, and percent recoveries were computed.

For Metformin hydrochloride

100 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10 ml. The area of each solution peak was

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measured at 230 nm. At each level, the amount of Metformin was determined, and percent recoveries were computed.

Table 4: Recovery data for Remogliflozin

SR NO.	Conc. Level(%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	40%	10	4	4.02	100.47	100.37 ± 0.15
2		10	4	4.01	100.26	
3	80%	10	8	8.026017	100.33	100.58± 0.36
4		10	8	8.066867	100.84	
5	120%	10	12	12.03601	100.30	100.53± 0.32
6		10	12	12.09078	100.76	

Table 5: Recovery data for Metformin

SR NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	40%	10	4	4.02	100.47	100.37 ± 0.15
2		10	4	4.01	100.26	
3	80%	10	8	8.026017	100.33	100.58± 0.36
4		10	8	8.066867	100.84	
5	120%	10	12	12.03601	100.30	100.53± 0.32
6		10	12	12.09078	100.76	

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions"

In method precision multiple sample is prepare by same method the criteria are to achieve % RSD below than2.0 %. Precision is a parameter that measures the degree of repeatability of method from the sample population under the normal operating circumstance. Relative standard deviation (RSD) in percent is generally used to represent the precision of the method for sample between two sets of experiments.

- **Acceptance criteria:**

The Relative Standard Deviation should not be more than 2% for test

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Intraday precision

Standard solution containing (20, 40 and 60 µg/ml) of Remogliflozin and (100,200 and 300 µg/ml) of Metformin hydrochloride were analysed on the same day and % R.S.D was calculated.

Table 6.1: Intraday Precision for Remogliflozin

Sr.no	Concentrations µg/ml(Remo and metformin	Remogliflozin		Mean	S.D	%SD
		Retention time	Area			
1	20	4.233	467.18			
	20	4.191	471.34	469.26	2.94	0.63
2	40	4.192	913.17			
	40	4.218	917.86	915.52	3.32	0.36
3	60	4.192	1350.53			
	60	4.247	1348.98	1349.76	1.10	0.08

Table 6.2: Intraday Precision for Metformin

Sr.no	Concentrations µg/ml(Remo and metformin	Metformin		Mean	S.D	%SD
		Retention time	Area			
1	100	3.017	7581.601			
	100	2.990	7588.31	7584.96	4.74	0.06
2	200	3.154	13524.7			
	200	3.176	13526.4	13525.55	1.20	0.01
3	300	3.276	20836.12			
	300	3.281	20832.22	20834.17	2.76	0.01

Interday precision

Standard solution containing (20,40 and 60 µg/ml) of Remogliflozin and (100,200 and 300 µg/ml) of Metformin hydrochloride were analysed on the different day and % R.S.D was calculated.

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Table 6.3: Interday Precision for Remogliflozin

Sr.no	Concentrations µg/ml(Remo and metformin	Remogliflozin		Mean	S.D	%SD
		Retention time	Area			
1	20	5.340	465.525			
	20	4.373	467.363	466.44	1.30	0.28
2	40	3.906	914.824			
	40	3.805	915.178	915.00	0.25	0.03
3	60	4.520	1315.526			
	60	4.008	1318.256	1316.89	1.93	0.15

Table 6.4: Interday Precision for Metformin

Sr.no	Concentrations µg/ml(Remo and metformin	Metformin		Mean	S.D	%SD
		Retention time	Area			
1	20	3.597	7584.2			
	20	2.425	7587.1	7585.65	2.05	0.03
2	40	2.527	13527.64			
	40	2.309	13525.21	13526.43	1.72	0.01
3	60	2.856	20833.35			
	60	2.440	20837.5	20835.43	2.93	0.01

7.2.5 Robustness

"The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage"

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

The mobile phase composition was changed in (±1 ml/min^{-1}**)** proportion and the flow rate was varied by (±1ml/min^{-1}**)**, and wavelength change (±1 ml/min^{-1}**)** of optimized chromatographic

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condition. The results of robustness studies are shown in **(Table No.** 7). Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

Table 7: Robustness data for estimation of Remogliflozin and metformin

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	%RSD	Amount of detected (mean ±SD)	%RSD
		For Metformin		For Remogliflozin	
Chromatogram of flow change 0.8 ml	40+400	1777.28±2.95	0.17	27117.50±4.24	0.02
Chromatogram of flow change 0.6 ml	40+400	1868.89±1.03	0.05	27728±4.95	0.02
Chromatogram of mobile phase change 41 + 59 ml	40+400	1806±2.28	0.13	27279.7±8.37	0.03
Chromatogram of mobile phase change 39 + 61 ml	40+400	1849.49±7.30	0.39	27101.06±4.76	0.02
Chromatogram of comp change wavelength change 232 nm	40+400	27932.63±4.34	0.02	1766.23±14.26	0.81
Chromatogram of comp change wavelength change 228 nm	40+400	27603.2±1.89	0.01	1832.3±3.56	0.19

Detection Limit

"The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantifiable under stated experimental conditions"

Based on the S.D. of the response and the slope of calibration curve, the detection limit (DL) was calculated as,

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

Where,

σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

The result is shown in table

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Table 8.1: Limit of Detection (LOD)

Remogliflozin	Metformin
Formula LOD = 3.3×avg S.D/Slope Avg.SD = 2.20 Slope = 21.909 LOD = 3.3x 2.20/21.909 =0.33137	Formula LOD = 3.3×avg S.D/Slope Avg.SD = 19.69 Slope = 67.129 LOD = 3.3x19.69/67.129 =0.968

Quantitation Limit

"The Quantitation limit of an individual analytical procedure is defined as the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy"

Based on the S.D. of the response and the slope of calibration curve, the quantitation limit (QL) was calculated as,

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

Where,

σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

The result is shown in table

Table 8.2: Limit of Quantitation (LOQ)

Remogliflozin	Metformin
Formula LOQ = 10×avg S.D/Slope Avg.SD = 2.20 Slope = 21.909 LOD = 10x2.20/21.909 =1.004154	Formula LOQ = 10 ×avg S.D/Slope Avg.SD = 19.69 Slope = 67.129 LOD = 10x19.69/67.129 =0.2933

Result and Discussion

Analysis of marketed formulation

To determine the content of Remogliflozin and Metformin in marketed tablets (label claim 10 mg of Canagliflozin and 100mg Metformin), 20 tablets powder weighed in 17.831 gm sand

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average weight of powder was calculated in 891. 55mg.Tablets were triturated and powder equivalent to weigh in 90 mg the drug was extracted from the tablet powder with 10 mL MEOH. To ensure complete extraction it was sonicated for 15 min. 0.1mL of supernatant was then diluted up to 10 mL with mobile phase. The resulting solution was injected in HPLC and drug peak area was noted**.**

Table 9: Analysis of marketed formulation.

	Sr no.	**Concentration (µg/ml)**	**Area**	**Mean**	**S.D**	**% RSD**	**ASSAY (% of label claim)**
Remogliflozin Etabonate	1	40.00	914.71				
	2	40.00	917.01	915.86	1.626	0.178	100.37
	Sr no.	**Concentration (µg/ml)**	**Area**	**Mean**	**S.D**	**%RSD**	**ASSAY (% of label claim)**
Metformin HCL	1	200	14023.32	14020.37	4.172	0.030	100.08
	2	200	14017.42				

CONCLUSION

Simple, rapid, accurate and precise RP-HPLC as well as chromatographic methods have been developed and validated for the routine analysis of Metformin and Remogliflozin in API and tablet dosage forms. Both methods are suitable for the simultaneous determination of Metformin and Remogliflozin in multi-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low.

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Conflict of Interest

The authors have declared no conflict of interest.

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Abbreviations: RP-HPLC: Reverse Phase High performance liquid chromatography; REMO: Remogliflozin; MET: Metformin; ICH: International conference on harmonization; RSD: Relative standard deviation; SD: Standard deviation; LOD: Limit of detection; LOQ: Limit of Quantitation; Rf: Retention factor; SGLT2: Sodium-glucose co-transporter 2; API: Active pharmaceutical ingredient. Conc: Concentration.

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