ANALYTICAL DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF REMOGLIFLOZIN ETABONATE AND METFORMIN HCL BY RP-HPLC

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ABSTRACT

A simple, rapid, economical, precise and accurate RP-HPLC method for simultaneous estimation of Remogliflozin Etabonate and Metformin HClhas for the simultaneous estimation of Remogliflozin Etabonate and Metformin HCl has been developed. The separation was achievedby Cosmosil C_{18} (250mm x 4.6mm, 5µm) column and Buffer (pH 4.0): methanol (60:40) as mobile phase, at a flow rate of 1 ml/min. Detection wascarriedoutat241 nm.RetentiontimeofRemogliflozin etabonateand Metformin HClwere found to be 5.493 min and 3.183 minrespectively. The method hasbeenvalidatedforlinearity,accuracyandprecision.LinearityobservedforRemogliflozin etabonate 5-15 µg/mlandforMetformin HCl 20-60 µg/ml.Theproposedmethodwassuccessfullyappliedforthesimultaneousestimationofboth thedrugsin marketed formulation.

KEYWORD: 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. It is prodrug of Remogliflozin, with benzyl pyrazoleglucoside-based inhibitor of renal SGLT2 with antihyperglycemic activity. [01,02]

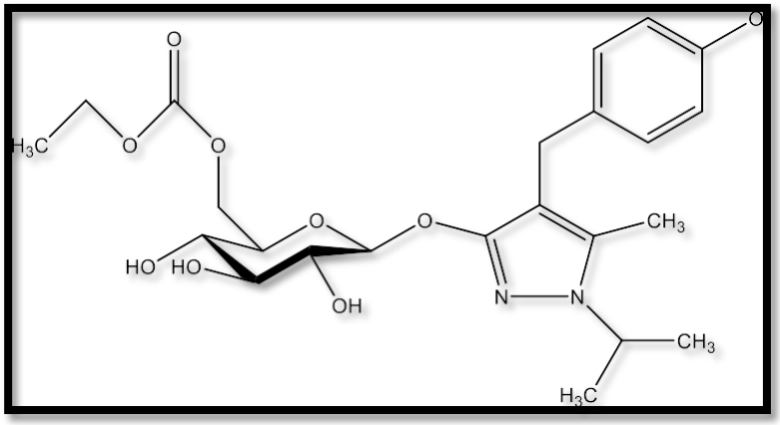

Figure 1: Structure of Remogliflozin etabonate

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Metformin is a first line agent for the treatment of type 2 diabetes thatcan be used alone or in combination with sulfonylureas,thiazolidinedione, incretin-based drugs, sodium glucose co-transporter-2inhibitors,orotherhypoglycemicagents.Metformin hasnotbeen linkedtoserumenzymeelevationsduringtherapy and is an exceeding rare cause of idiosyncratic clinicallyapparentacute liverinjury.

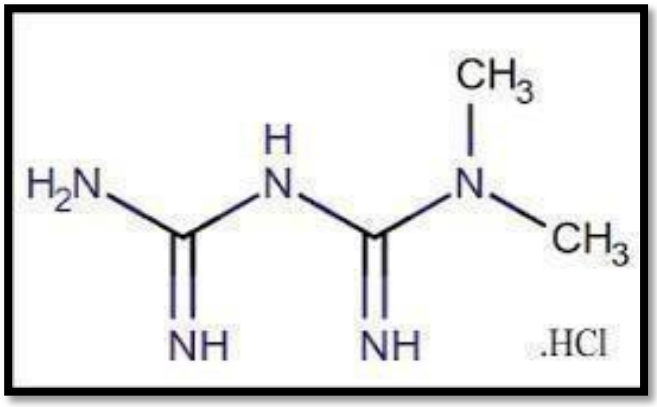

Figure 2: Structure of Metformin HCl

Literature review reveals that few methods are reported for determination of Remogliflozin etabonate and metformin HCl by UV spectroscopy,[03] LC-MS/MS. [04] But no RP-HPLC method has been reported for development and validation for the Estimation Remogliflozin Etabonate and metformin HCl in its API or pharmaceutical dosage form. Therefore, the aim of the present work was to develop RP-HPLC Method for the Estimation of Remogliflozin Etabonate and metformin HCl in its dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed RP- HPLC detection method was validated in accordance with international conference in Harmonization (ICH) [05] guidelines, by assessing its selectivity, linearity, accuracy, and precision, limit of detection and limit of quantification in this method.

MATERIALS AND METHODS

Chemicals and Reagents

Remogliflozin Etabonate and metformin HCl were procured from Glyra Healthcare, Ahmedabad, Gujarat, India. HPLC grade reagents methanol, acetonitrile (Finar, Ahmedabad) was used for study. The entire reagent prepared by carbon dioxide free water and whereas the sample solution prepared in double Distilled water for HPLC Purpose.

Apparatus

RP-HPLC method development and validation was done on a HPLC instrument (LC- 10AT, 20µL fixed loop.Spinchrom) UV Detector, Stationary Phase used was Cosmosil C_{18} (25cm × 0.46cm), 5µm column particle size and mobile phase consisting of Buffer, (phosphate Buffer (pH 4.2): Acetonitrile (60:40) was used. The flow rate was 1.0 ml/min and the effluents were monitored at 241nm. Injection volume was 20 µL. All weighing were done on analytical balance(Shimadzu).

Preparation of Standard Stock Solution

Preparationofstandardsolutionofmixturesof Metforminhydrochloride(100ppm)andRemogliflozin(100ppm)

(A) Metforminhydrochloridestandardstocksolution:(500µg/mL)

A50mgofMetforminhydrochloridewasweighed andtransferredtoa100 mL volumetricflask.Volume wasmadeuptothemarkwithmobile phase.

(B) Remogliflozinstandardstocksolution:(500 µg/mL)

A50mgofRemogliflozinwasweighedandtransferredtoa100mLvolumetricflask. Volumewas madeuptothemark withmobilephase.

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Preparation of working standard solution

Preparation of standard solution of binary mixtures of Metformin hydrochloride (50 µg/mL) and Remogliflozin (50 µg/mL)

Take 1 mL from the Metformin hydrochloride stock solution and 1mL from Remogliflozin stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials

RESULT AND DISCUSSION

Standardized Chromatographic conditions

Standard solutions of 50µg/ml of Remogliflozin and 50 µg/ml of Metformin hydrochloridewere injected in column with 20 µL micro syringe. The chromatogram was run for appropriateminutes with mobile phase Buffer (pH 4.0): Methanol (60:40). The detection was carried out atwavelength 241 nm.

Figure 3: HPLCChromatogramofMetforminhydrochloride(50ppm) andRemogliflozin(50ppm)inBuffer,pH4.0:Methanol(60:40)

System Suitability

System suitability tests were carried out on freshly prepared standard solution of Remogliflozin etabonate and metformin HCl under optimized chromatographic condition and parameters were studied to evaluate the suitability of the system. **(Table 1).**

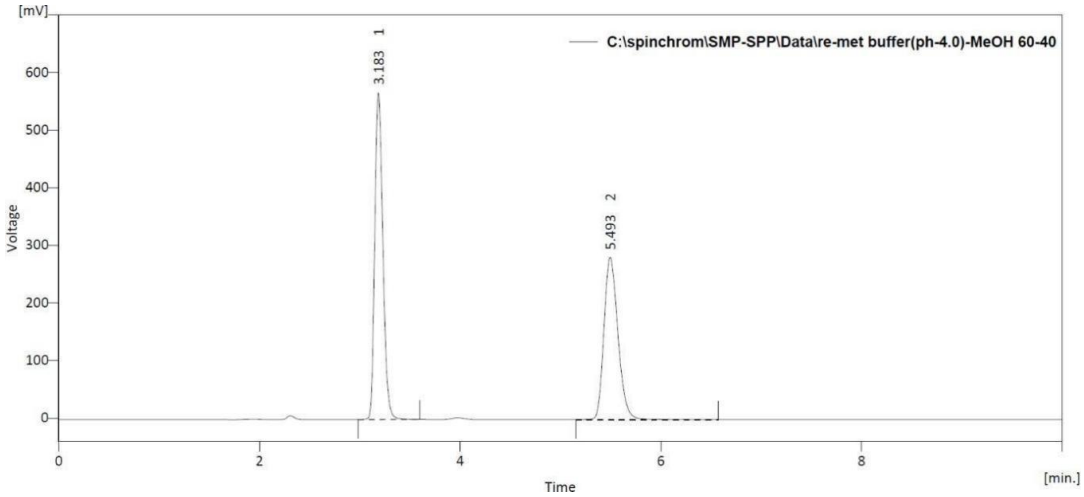

Table 1: System suitability testing

Parameter	Result(remo)	Result (met)
Retention Time	5.493	3.183
Theoretical plate	7430	6931
Asymmetry	1.400	1.450
Resolution	-	-

METHOD VALIDATION

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures.[05]

Linearity

The linearity for Remogliflozin and Metformin hydrochloride were assessed by analysis of combined standard solution in range of 5-15 µg/ml and 20-60 µg/ml respectively, 0.5,0.75,1,1.25,1.5 ml solutions were pipette out from the Stock solution of Remogliflozin (500 µg/ml) and Metformin hydrochloride (500 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 5,7.5,10,12.5,15 µg/ml and 20,30,40,50,60 µg/ml for Remogliflozin and Metformin hydrochloride respectively.

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In term of slope, intercept and correlation co-efficient value, the graph of peak area obtained verses respective concentration was plotted.

Table 2: Linearity data for Remogliflozin etabonate

Sr. No	Concentration (µg/ml)	Area
1	5	1363.447
2	7.5	2044.602
3	10	2746.260
4	12.5	3488.236
5	15	4116.664

Table 3: Linearity data for metformin HCl

Sr. No	Concentration(µg/ml)	Area
1	20	1592.772
2	30	2386.066
3	40	3204.900
4	50	4015.007
5	60	4802.524

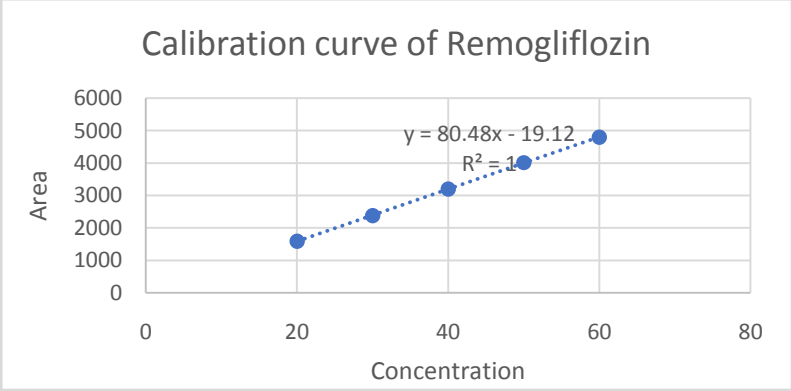

Figure 4: CalibrationCurveofremogliflozinetabonate (5-15µg/ml)

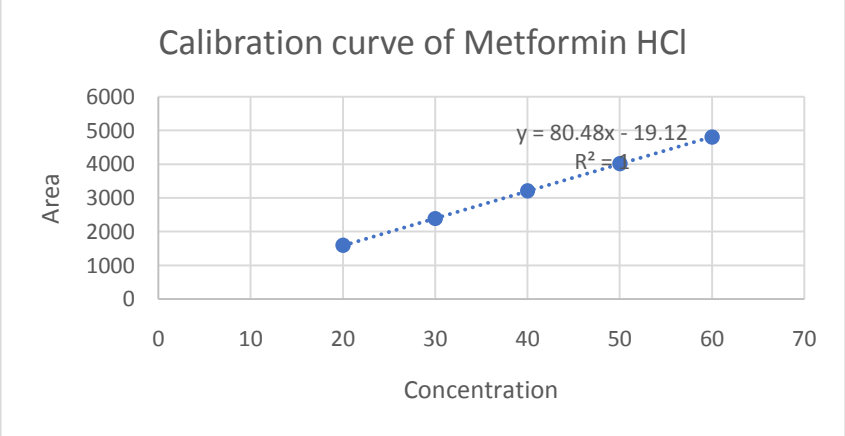

Figure 5: CalibrationCurveofMetforminHCl(20-60µg/ml)

Precision

Intraday precision

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Standard solution containing (5,10,15 µg/ml) of Remogliflozin and (20,40,60 µg/ml) of Metformin hydrochloride were analyzed three times on the same day and % R.S.D was calculated.

Interday precision

Standard solution containing (5,10,15 µg/ml) of Remogliflozin and (20,40,60 µg/ml) ofMetformin hydrochloride were analyzed three times on the different days and % R.S.D wascalculated.

Table 4: Intraday precision data for estimation of remogliflozin etabonate and metformin HCl.

SR. NO.	Remogliflozin etabonate			Metformin HCl		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD
1	5	1489.90 ± 6.865	0.46	20	2600.03 ± 14.15	0.55
2	10	3006.73 ± 35.77	1.19	40	5241.82 ± 54.52	1.04
3	15	4470.67 ± 50.81	1.14	60	7783.30± 103.62	1.33

Table 5: Interday precision data for estimation of Remogliflozin etabonate and Metformin HCl.

SR. NO.	Remogliflozin etabonate			Metformin HCl		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% RSD
1	5	1539.46± 15.49	1.00	20	2678.01± 37.28	1.39
2	10	3066.45± 31.76	1.03	40	5332.45± 42.05	0.79
3	15	4439.31± 31.23	0.70	60	7728.91± 32.81	0.42

Accuracy

For Remogliflozin

5 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 247 nm. The amount of Remogliflozin was calculated at each level and % recoveries were computed.

For Metformin hydrochloride

20 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 241 nm. The amount of Metformin hydrochloride was calculated at each level and % recoveries were computed.

Table 6: Recovery dataforRemogliflozin

SR.NO.	Conc.Level(%)	Sample amount(µg/ml)	Amount Added(µg/ml)	Amountrecovered(µg/ml)	% Recovery	% MeanRecovery±S.D
1	80%	10	8	7.95	99.42	100.57±1.20
2		10	8	8.15	101.81	
3		10	8	8.04	100.46	
4	100%	10	10	10.01	100.09	100.49±0.52
5		10	10	10.11	101.08	
6		10	10	10.03	100.29	
7	120%	10	12	12.12	101.01	100.02±1.11
8		10	12	12.03	100.22	
9		10	12	11.86	98.82	

Table 7: RecoverydataforMetforminhydrochloride

SR.NO.	Conc.Level(%)	SampleAmount	Amount Added	Amountrecovered(µg/ml)	% Recovery	% MeanRecovery±SD
1	80%	10	8	7.98	99.74	99.95±0.76
2		10	8	7.95	99.32	
3		10	8	8.06	100.79	
4	100%	10	10	10.04	100.37	100.77±0.53
5		10	10	10.14	101.37	
6		10	10	10.06	100.57	
7	120%	10	12	12.15	101.25	100.24±0.89
8		10	12	11.99	99.89	
9		10	12	11.95	99.59	

LOD and LOQ

The LOD was estimated from the set of 3 calibration curves used to determine Method linearity. The LOD may be calculated as,

LOD = 3.3 × (SD/Slope)

Where, SD= Standard deviation of Y-intercepts of 3 calibration curves.

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Slope = Mean slope ofthe 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method linearity.

The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of Y-intercepts of 3 calibration curves.

Table 8: Limit of Detection data for Remogliflozin etabonate and Metformin HCl

Remogliflozin etabonate	Metformin HCl
LOD = 3.3 x (SD / Slope) = 3.3 x (34.679/149.832) = 0.764 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (62.163/261.183) = 0.785 µg/ml

Limit of Quantitation:

Table 9: Limit of Quantitation data for Remogliflozin etabonate and Metformin HCl

Remogliflozin etabonate	Metformin HCl
LOQ = 10 x (SD / Slope) = 10 x (34.679/149.832) = 2.314 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (62.163/261.183) = 2.380 µg/ml

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1.Flow rateof mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.

2.Ratio of Mobile phase was changed (±2) Buffer: Acetonitrile (58:42) and Buffer: Acetonitrile (62:38)

3.pH of buffer was changed (±0.2) pH 4.2 and pH 3.8

Table 10: Robustness data for Remogliflozin etabonate

SR NO.	Area at Flow rate (-0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at Mobile phase (-2)	Area at Mobile phase (+2)	Area at pH (-0.2)	Area at pH (+0.2)
1	3093.562	2828.682	3086.743	2803.617	2926.147	2935.305
2	3123.658	2808.151	2971.756	2776.426	2954.735	2964.807
3	3076.551	2801.72	3039.4	2734.32	2914.962	2994.237
% RSD	0.770	0.501	1.906	1.260	0.700	0.994

Table 11: Robustness data for Metformin HCl

SR NO.	Area at Flow rate (-0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at Mobile phase (-2)	Area at Mobile phase (+2)	Area at pH (-0.2)	Area at pH (+0.2)
1	5359.257	4909.374	5325.678	4850.847	5054.13	5123.496
2	5420.908	4874.379	5389.099	4767.736	5150.686	5158.945

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3	5361.944	4825.483	5294.13	4797.421	5026.97	5135.115
% RSD	0.648	0.865	0.906	0.876	1.281	0.352

Analysis of marketed formulation

Taken sample equivalent to 50 mg of Remogliflozin and 50 mg of Metformin hydrochloride was transferred to a 100 ml volumetric flask, add 60 ml of Mobile phase and shake for 15 minutes and made-up volume up to the mark with mobile phase. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 1 ml of this solution was diluted to 10 ml with mobile phase. The solution was injected 20 µl. The areas of resulting peak were measured at 247 nm.

Applicability of the proposed method was tested by analyzing the commercially available formulation.

Table 12: Analysis of marketed formulation

Formulation	Label claim		Assay (% of label claim) Mean ± S. D.	
	Remogliflozin etabonate	Metformin HCl	%Remogliflozin etabonate	%Metformin HCl
Tablet	50 mg	50 mg	99.344 ± 0.422	100.025 ± 0.142

*Average of three determinations

CONCLUSION

Hence, we can conclude that the developed RP-HPLC method is simple and rapid as it separates components with good chromatographic criteria. Method has short run time. The method was successfully validated for all the validation parameters as per ICH guidelines. The method can be conveniently used for assay of Remoglifilozin Etabonate and metformin HCl in API and tablet dosage form.

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