WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 10, 981-993. Research Article ISSN 2277–7105

STABILTY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF REMOGLIFLOZIN ETABONATE AND METFORMIN HCL IN SYNTHETIC MIXTURE AND TABLET DOSAGE FORM

Shivani V. Trivedi*

*M.Pharm Student, Sharda School of Pharmacy, Gandhinagar, Gujarat- 382610.

Article Received on
15 June 2021,

Revised on 05 July 2021,
Accepted on 25 July 2021

DOI: 10.20959/wjpr202110-21237

***Corresponding Author**
Shivani V. Trivedi
M.Pharm Student, Sharda School of Pharmacy, Gandhinagar, Gujarat- 382610.

ABSTRACT

A simple, rapid, economical, precise and accurate Stability indicating RP- HPLC method for simultaneous estimation of Remogliflozin Etabonate and Metformin Hcl in their Synthetic Mixture and tablet dosage form has been developed. A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of remogliflozin etabonate and metformin hcl in their Synthetic Mixture has been developed. The separation was achievedby Cosmosil C18 (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 was carried out at 241 nm. Retention time of remogliflozin etabonate and Metformin Hcl were found to be 5.493 min and 3.183 min respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for remogliflozin etabonate 5-15 µg/ml and for Metformin Hcl 20-60 µg/ml. Developed method was found to be accurate, precise and rapid for simultaneous estimation of remogliflozin etabonate and Metformin Hcl in their Synthetic Mixture. The drug was subjected to stress condition of hydrolysis, oxidation, photolysisand Thermal degradation, the proposed method was successfully applied for thesimultaneous estimation of both the drugs in commercial Synthetic mixture.

KEYWORD: Remogliflozin Etabonate, metformin hcl, RP-HPLC, validation.

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 benzylpyrazole glucoside based inhibitor of renal SGLT2 with antihyperglycemic activity.[01,02]

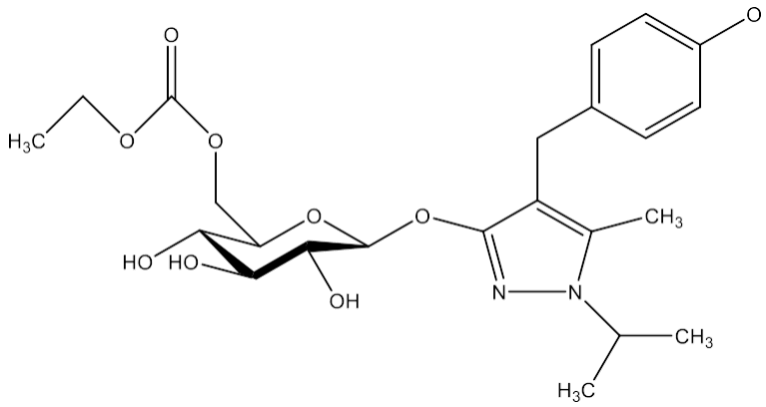


Figure 1: Structure of Remogliflozin etabonate.

Metformin is a first line agent for the treatment of type 2 diabetes that can be used alone or in combination with sulfonylureas, thiazolidinediones, incretin-based drugs, sodium glucose cotransporter-2 inhibitors, or other hypoglycemic agents. Metformin has not been linked to serumenzyme elevations duringtherapy and is an exceeding rare cause of idiosyncratic clinically apparent acute liver injury.

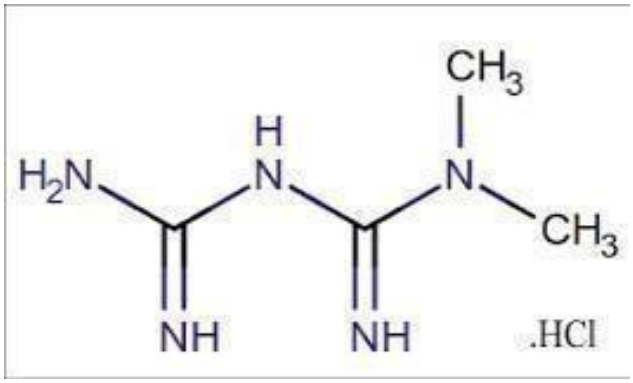


Figure 2: Structure of Metformin Hcl.

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 stability indicating analytical method 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 stability indicating RP-HPLC

Method for the Estimation of Remogliflozin Etabonate and metformin hcl in its dosage forms. Because analytical methods must be validate 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) were 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 C18 (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

Preparation of standard solution of mixtures of Metformin hydrochloride (100 ppm) and Remogliflozin (100 ppm)

(A) Metformin hydrochloride standard stock solution: (500 µg/mL)

A 50 mg of Metformin hydrochloride was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with mobile phase.

(B) Remogliflozin standard stock solution: (500 µg/mL)

A 50 mg of Remogliflozin was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with mobile phase.

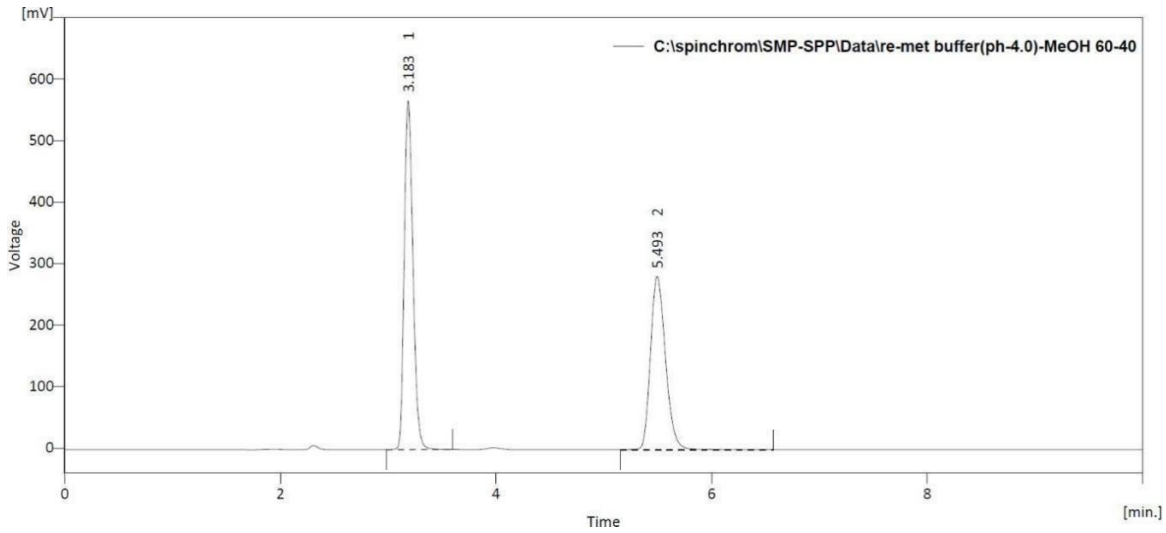
Preparation of working standard solution

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.

Standardized Chromatographic conditions

Standard solutions of 50µg/ml of Remogliflozin and 50 µg/ml of Metformin hydrochloride were injected in column with 20 µl micro syringe. The chromatogram was run for appropriate minutes with mobile phase Buffer (pH 4.0): Methanol (60:40). The detection was carried out at wavelength 241 nm.



HPLC Chromatogram of Metformin hydrochloride (50 ppm) and Remogliflozin (50 ppm) in Buffer, pH 4.0: 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. Results are shown **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.

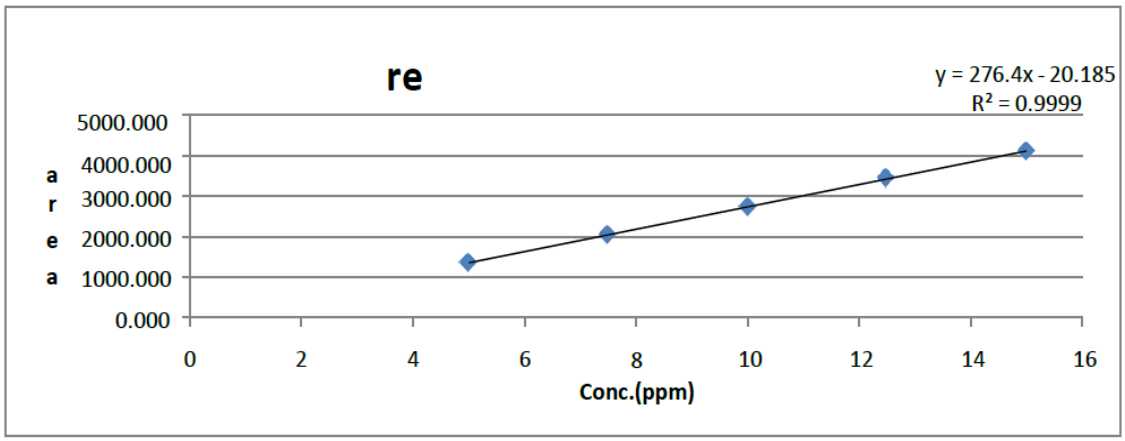
In termof slope, intercept and correlation co-efficient value, the graph of peak area obtained verses respective concentration was plotted.

Table 6.18: Linearity data for remoglflozin etabonate.

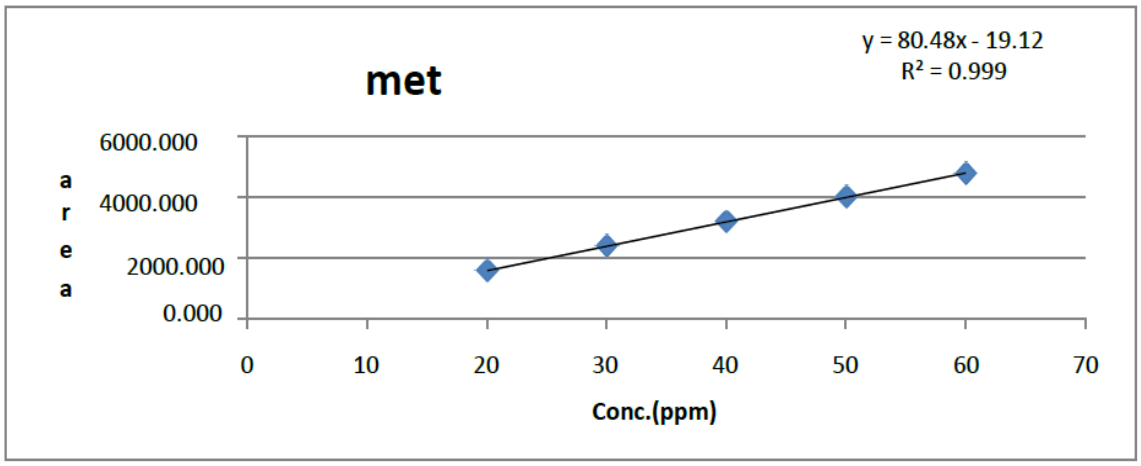
Sr. No	Concentration (µg/ml)	Area
1	5	1363.447
2	7.5	2044.602
3	10	2746.260
4	12.5	3488.236
5	15	4116.664

Table 6.19: 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



Calibration Curve of remogliflozin etabonate (5-15 µg/ml)



Calibration Curve of Metformin HCl (20-60 µg/ml)

Precision (intraday precision)

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

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)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
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

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)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
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.

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	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		10	10	10.01	100.09	
5	100%	10	10	10.11	101.08	100.49± 0.52
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	

Recovery data for Metformin hydrochloride

SR. NO.	Conc. Level(%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
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-interceptsof 3 calibration curves. Slope = Mean slope of the 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.

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

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 rate of 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

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
% R.S.D	0.770	0.501	1.906	1.260	0.700	0.994

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
3	5361.944	4825.483	5294.13	4797.421	5026.97	5135.115
% R.S.D	0.648	0.865	0.906	0.876	1.281	0.352

FORCED DEGRADATION STUDIES

Acid Hydrolysis

Acid degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and put for …..min at RT. Then the volume was adjusted with diluent to get 50µg/ml for Metformin hydrochloride and 50µg/ml for Remogliflozin.

Alkaline Hydrolysis

Base degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for hrs at RT. Then the volume was adjusted with diluent to get 50µg/ml for Metformin hydrochloride and 50µg/ml for Remogliflozin.

Oxidative Hydrolysis

Oxidation degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. Two ml of 3% H2O2 solutions was added and mixed well and put for 2, 4, 6 hrs at RT. Then the volume was adjusted with diluent to get 50µg/ml for Metformin

hydrochloride and 50µg/ml for Remogliflozin**.**

Thermal Degradation

A 50 mg of Metformin hydrochloride and 50 mg of Remogliflozin were taken in same Volumetric flask and was put in oven for 2, 5 and 8 hrs at 800C temperature, than after Volumetric flask was removed and cool at room temperature, than this combined powder was transferred to 100ml volumetric flask and volume was made up with mobile phase, 1ml of this solution was transferred in 10ml volumetric and volume was made up with mobile phase to make 50µg/ml for Metformin hydrochloride and 50µg/ml for Remogliflozin.

Photolytic degradation

A 50 mg of Metformin hydrochloride and 50 mg of Remogliflozin were taken in same Volumetric flask and was put in oven for 2, 5 and 8 hrs at 800C temperature, than after Volumetric flask was removed and cool at room temperature, than this combined powder was transferred to 100ml volumetric flask and volume was made up with mobile phase, 1ml of this solution was transferred in 10ml volumetric and volume was made up with mobile phase to make 50µg/ml for Metformin hydrochloride and 50µg/ml for Remogliflozin.

RESULT AND DISCUSSION

METHOD VALIDATION

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.

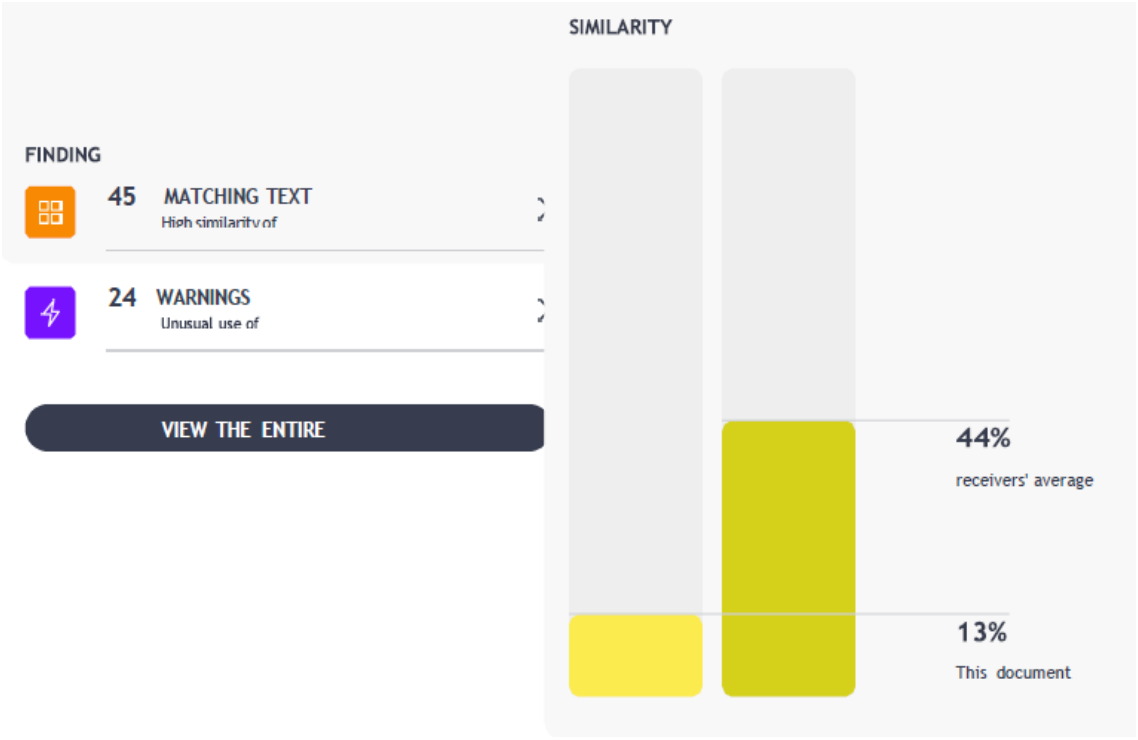
Applicability of the proposed method was tested by analyzing the commercially available formulation.

Table 6.30: Analysis of marketed formulation.

Formulation	Label claim		Assay (% of label claim) Mean ± S. D.	
	remogliflozin etabonate	Metformin hcl	%remogliflozin etabonate	%metformin hcl
Synthetic Mixture	50 mg	50 mg	99.344 ± 0.422	100.025 ± 0.142

*Average of three determinations

ANALYSIS OVERVIEW



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 and all degradants are well separated from drug. The method was successfully validated for all the validation parameters as per ICH guidelines. The method is stability-indicating and can be used to assess the stability of Remoglifilozin Etabonateand metformin hcl in the API and tablet dosage form. The method can be conveniently used for assay of Remoglifilozin Etabonateand metformin hcl in API and tablet dosage form.

AKNOWLEDGEMENT

The author is highly graceful to Ms. Bhumi Patel and Mr. Jaymin patel for his guidance in research work.

REFERENCES

1. Waller DG, Sampson AP, Renwick AG, Hiller K. Medical pharmacology and therapeutics; 4th edn, 457.
2. Tripathi, KD. Essential of medical pharmacology; 7th edn; Jaypee brothers medical publisher PVT. LTD. New Delhi, 2013; 258.
3. Shethi PD,"HPLC-Quantitative Analysis of Pharmaceutical Formulations" CBS Publishers & Distributers, New Delhi, 1996; 3-46.
4. Chatwal GR, "Instrumental Method of Chemical Analysis", Part-1, Himalaya Publishing House, 5th Edition, 2002; 2.624-2.631.
5. Robinson JW, Skelly Frame EM and Frame GM, "Undergraduate Instrumental Analysis", 6th Edition, Marcel Dekker, 2005; 806.
6. Ahuja S and Scypinski S, "Handbook of Modern Pharmaceutical Analysis", Academic Press, 2001; 3: 356-367.
7. FDA, "Guidance for Industry; Analytical Procedures and Methods Validation (Draft guidance), Food & Drug Administration," Rockville, US Department of Health and Human Services, 2000.
8. ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva, 1996.
9. Brummer H, "How To Approach A Forced Degradation Study", Life sci. tech bul., 2011.
10. Urvi S, Nilesh P, Ashok P, Amit V, "Stability Indicating RP-HPL Method Developmen and Validation of remogliflozin etaboante and metformin hcl and its Degradant in its Capsule Dosage Form" J. Pharm. Sci. and Res., 2017; 9(9): 1549-155.
11. Harne UV, Birajdar L, Damle MC, "Development and validation of stability indicating HPLC method for determination ofremogliflozin etaboante" World J. Pharm. Res., 2017; 6(11): 430-441.
12. Harne UV, Birajdar L, Damle MC, "Development and validation of stability indicating HPLC method for determination of metformin hcl" World J. Pharm. Res., 2017; 6(11): 430-432.
13. Saxena V, Zaheer Z, Farooqui M, "Stability Indicating RP-HPLC Method for the Determination of remogliflozin etaboante and metformin hcl in Pharmaceutical formulation" Ind. J. of Chem. Tech. Res., 2006; 13: 242-246.
14. Vasanthraju SG, Mishra A, Karthik A, Mausmade PP, Udupa N, "Stability Indicating RP-HPLC Method For Determination of metformin hcl in Solid Dosage Form" J. Pharm. Res., 2009; 8(4): 184-186.

15. Shanmuga SD, Vamshikrishna D, Ragapallavi C, "A simple method development and validation for the simultaneous estimation of remogliflozin etaboante and metformin hcl by RP-HPLC method" Ind. Ame. J. Pharm. Res., 2013; 3(10): 8169-8177.
16. Sidhhartha B, Babu IS, Gupta CR, Parthiban C, "Analytical method development and validation for simultaneous estimation of remogliflozin etaboante and metformin hcl in bulk & pharmaceutical dosage form by RP-HPLC method" Asi. J. Pharm. and Cloi. Res., 2014; 7(2): 156-160.