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Novel Ultra-Fast Liquid Chromatography Method for Simultaneous Quantification of Metformin Hydrochloride and Remogliflozin Etabonate

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An advanced formulation having remogliflozin etabonate (REM) and metformin hydrochloride (MET) for the treatment of diabetes was recently approved. However, there hasn't been any information reported on a UFLC technique for simultaneously quantifying both active substances. Therefore, aim of the study was the development of an ultra-fast isocratic liquid chromatography method for quantification of MET and REM in API and dosage form. The reversed phase-UFLC method was developed with a very short run time of only 3 min with no interference due to blank. The compounds were separated using Shimadzu Sim-pack XR ODS (75 mm × 3 mm, 2.2 µ) column with mobile phase consisting of buffer (0.1% phosphoric acid):acetonitrile (30:70). The developed method was validated as per ICH-Q2(R1). The linearity was established in the range of 0.01-125 ppm and 0.01-25 ppm for MET and REM, respectively. The average assay was found 99.4% for MET and 99.1% for REM. The accuracy was observed 98.29-99.49% for MET and 98.39-99.73% for REM. The proposed method is very fast and simple however precise and accurate. This method is very useful for regular quality control laboratory to quantify MET and REM in API and dosages forms.

Keywords: UFLC, Chromatography, Remogliflozin etabonate, Metformin.

INTRODUCTION

Type-2 diabetes is a metabolic disorder categorized by high blood sugar levels that are either the result of insufficient insulin synthesis or the inability of muscle cells to use glucose as fuel. Today, type-2 diabetes affects a lot of people. 25% of individuals under 65 years and 10% of the overall population also develop this variant of hyperglycemia. Diabetic patients who had COVID-19 infections seemed to have an increased death rate [1-4]. It is crucial to maintain the blood sugar levels normal to prevent complications and organ damage. To maintain normal blood glucose and low Hb1AC levels, a range of oral anti-diabetic drugs, including inhibitors of dipeptidyl peptidase-4 (DPP-4) and sodium glucose cotransporter-2 (SGLT-2) are being evaluated [5-8].

Remogliflozin Etabonate is the newest accumulation to the recently approved SGLT2 inhibitor class of medications to control diabetes with type 2 in the Indian area. One of most recent addition to the recently permitted SGLT2 inhibitor class of medications for the management of type-2 diabetes is REM (Fig. 1). Remogliflozin is the prodrug of remogliflozin etabonate, which improves glycemic control by increasing urine glucose excretion. After administration and absorption, when the inactive prodrug is changed into the active form remogliflozin, sodium-glucose co-transporter subtype 2 is the only target of the drug's selective activity (SGLT2) [9-11].

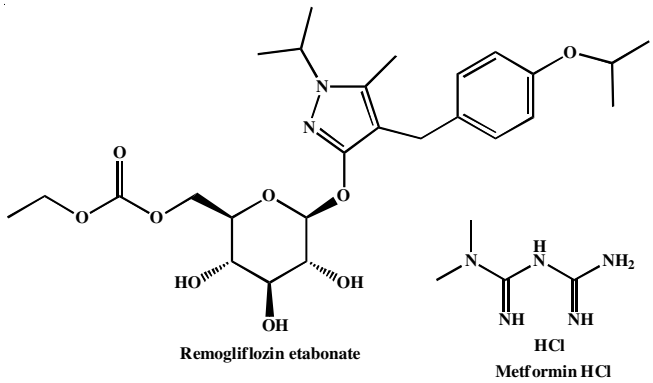

Fig. 1. Structures of remogliflozin etabonate (REM) and metformin·HCl (MET)

Metformin hydrochloride enhances glycemic control by increasing insulin sensitivity and reducing glucose absorption through the intestinal tract. MET can provide antineoplastic benefits by inhibiting the mammalian target of rapamycin (m TOR), which is up-regulated in many cancer tissues, *via* AMPK-mediated or AMPK-independent processes. Additionally, this agent suppresses tumor cell migration and invasion by blocking matrix metalloproteinase-9 expression, which is mediated by suppressing transcription activator protein-1 (AP-1) activation. The combination of MET and REM improved glycemic control and provided additional benefits [12,13].

Every laboratory requires an analysis technique to evaluate the formulation's content precise and accurately. So, the objective of the research was to establish a validated the method for the assessment of combination of remogliflozin etabonate (REM) and metformin hydrochloride (MET) and using various quickest techniques and with accurate results. Hence, the purpose of the study was development of method for the content determination of REM along with MET in API and dosage form by fast UFLC and validation as per ICH guidelines.

EXPERIMENTAL

Marketed formulation (REMO-M) was purchased from local medical store and pure remogliflozin etabonate (REM) and metformin hydrochloride (MET) were received from Glenmark Life Science Pvt. Ltd. as free sample. Acetonitrile (AR grade) from S.D. Fine Chemicals purchased from local supplier. Proper mixing and sonication of stock solutions were accomplished using an ultra-bath sonicator (3.5 L capacity, PCI analytics). Analytical Balance (Mettler Toledo, XS205) was used for weighing and water purification systems ELIX 10 (Millipore, USA) were used for purified water. All the equipment and apparatus were calibrated and verified.

Chromatographic conditions: The chromatographic analysis was conducted on Shimadzu Prominence UFLC system having quaternary pump, auto sampler, auto injector, columns oven, UV detector with Lab solution software. Shimadzu Sim-pack XR ODS (75 mm × 3 mm, 2.2 µ) column was used for separation. Column oven temperature was kept 40 ºC; resolution was accomplished by mixture of buffer (0.1% orthophosphoric acid):acetonitrile (30:70). Speed of the pump was 1.0 ml min, amount injected 20 µL and UV detector having 225 nm wavelength.

Mobile phase preparation: To prepare the buffer, 1 mL orthophosphoric acid mixed with 1000 mL of purified water. 300 mL of this buffer was mixed with 700 mL of acetonitrile, mixed well, and sonicated for 5 min for degassing.

Standard preparation: Precisely weighed 100 mg of MET and 20 mg of REM and to 100 mL volumetric flask separately. Diluent (60 mL) added to both the flasks and sonicated for 2 min to dissolve the substances. Solutions were shaken occasionally and volume made by diluent and got stock solution 1 and 2 separately (1000 µg/mL MET and 200 µg/mL REM). Each stock solutions (5 mL) was diluted to100 mL by diluent to get 50 µg/mL MET and 10 µg/mL REM.

Sample preparation: The typical weight of tablet found by weighing 20 tablets. Then these tablets crushed to fine powder and mixed well to obtain homogeneous powder. Accurate weighed equivalent weight corresponding 100 mg of MET and 20 mg REM to 100 mL volumetric flask containing 50 mL of methanol and dissolved by sonication for 15 min. After sonication diluted to 100 mL with diluent and then filtered the above solution through 0.45 µ filter followed by the dilution of 5 mL to 100 mL using diluent. A sample solution for API mixture was prepared by similar preparation as per standard preparation. Accurately weighed 100 mg MET and 20 mg REM was dissolved to 60 mL diluent in a 100 mL volumetric flask, sonicated for 2 min and then make up to the volume with diluent. Finally, 5 mL form above solutions was further diluted to100 mL with diluent. The final test solution obtained containing 50 ppm of MET and 10 ppm of REM.

RESULTS AND DISCUSSION

Method development: Standard solutions containing each drug concentration of REM and MET 10 µg/mL were prepared in diluent. The spectra of this solution were obtained by scanning between 200 to 400 nm in UV and their spectra were obtained. Wavelength was selected as 225 nm based on zero-order spectra of REM and MET.

Different chromatographic conditions were tried for separation of the compounds to get good resolution between peaks, good peak shape with tailing factor less than 2.0 and theoretical plates more than 2000 and shorter run time less than 5 min. The trails included different pH of buffer, different mobile phase ratio, flow rate. Shimadzu Sim-pack XR ODS (75 mm × 3 mm, 2.2 µ) column was tried for separation. Phosphate buffer was prepared and pH of buffer was kept from 1.8 to 7.0 with orthophosphoric acid and mobile phase compositions were explored in different ratio of buffer with acetonitrile. Injection volume was tried as 20 µL to get higher area response. The oven temperature for column was kept 40 ºC to get sharp peak shape (Fig. 2).

After initial trials final optimized method finalized and validated for the combination of REM and MET. For buffer preparation 0.1% orthophosphoric acid was prepared to simplify the buffer preparation. Phosphate buffer and acetonitrile were combined having ratio 70:30 to prepare the mobile phase. This phase was filtered from Millipore filter paper (0.45 µ). Shimadzu Sim-pack XR ODS (75 mm × 3 mm, 2.2 µ) column was selected to get fast separation of the peaks. Column oven temperature was kept at 40 ºC. The flow rate was kept at 1.0 mL/min and 225 nm wavelength was set for detector channel. The volume of injection adjusted to 20 µL and the auto sampler temperature was set at 10 ºC for better solution stability.

Linearity: The linearity study was conducted by analyzing a variety of analyte concentrations range including 0.01-100 ppm for MET and 0.01-200 ppm. Different concentration 0.01 ppm (LOD) to 125 ppm (250%) for MET and 0.01 ppm (LOD) to 25 ppm (250%) for REM. The linearity plot for concentration against area of peaks generated and the outcome was calculated. The data inferred from linearity shown in Table-1. The linearity plot of the REM and MET drugs are shown in Fig. 3, which displayed an excellent linear relationship among peak region and concentration. The correlation coefficient observed 0.99988 for MET and 0.99989 for REM.

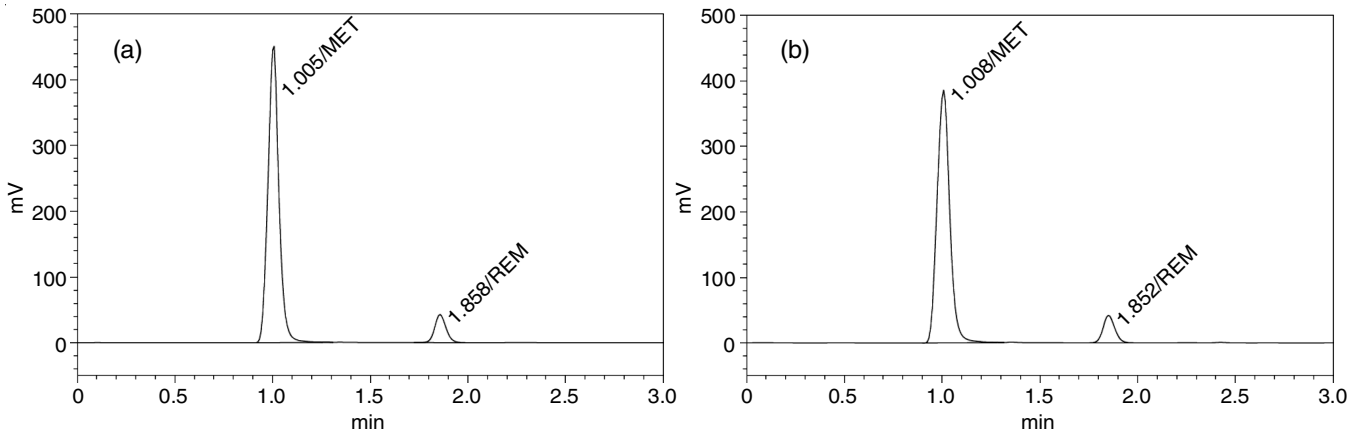

Fig. 2. Chromatogram of metformin hydrochloride (MET) and remogliflozin etabonate (REM) in (a) standard preparation and (b) sample preparation

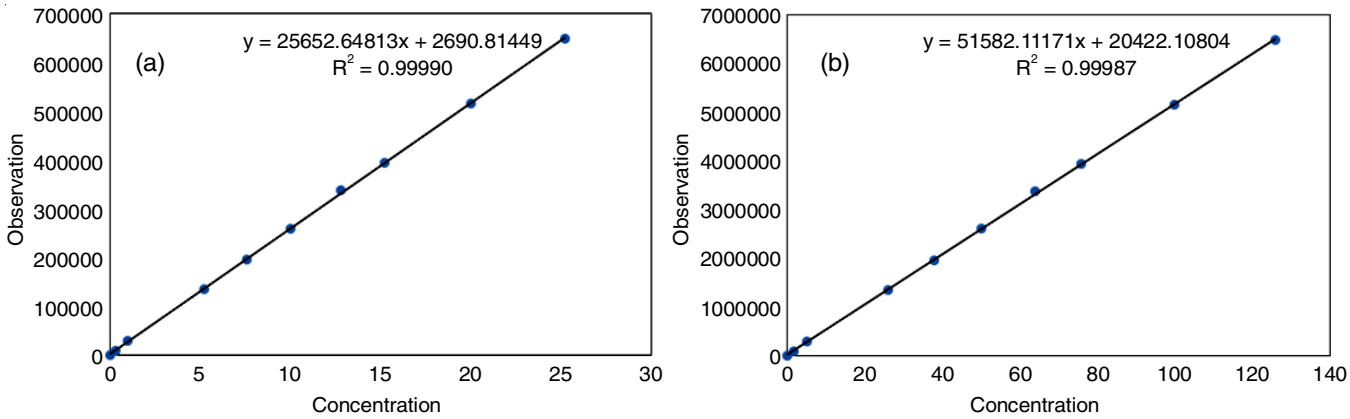

Fig. 3. Linearity of (a) remogliflozin etabonate (REM) and (b) metformin hydrochloride (MET)

TABLE-1
RESULTING PARAMETER FROM LINEARITY

Parameter	MET	REM
Linear range (ppm)	0.01-125	0.01-25
Slope	34181.27749	17083.03065
Intercept	4182.58351	1902.52369
C.C.	0.99994	0.99994
R2	0.99988	0.99989
Residual sum of squares	2335619362	21939541.4

Detection and quantitation limits: Two approaches were used to determine LOD and LOQ of the method. In visual area method minimum 1000 area response was set as LOD and LOD × 3 was set as LOQ. In Calibration curve technique, Standard variation and slope were used to define the LOD and LOQ. The LOD were 3.3 times and LOQ was 10 times the intercept's standard deviations and slope of the linearity, respectively. LOD and LOQ determined using both approaches and observed a very low LOQ indicating the sensitiveness of the suggested analytical technique (Table-2).

TABLE-2
VALUES OF LOD AND LOQ

Compound	Visual method		Calibration method	
	LOD (ppm)	LOQ (ppm)	LOD (ppm)	LOQ (ppm)
MET	0.01	0.03	1.421	4.307
REM	0.01	0.03	0.302	0.917

Precision (repeatability): The method precision was examined by performing determination of concentration of REM and MET in sample. The samples were prepared six times and assay of each component was calculated. The precision was checked for assay results. The intermediate precision checked by analyzing the above samples on another day. Table-3 shows the precision as RSD of six replicate results. The RSD values of method precision and intermediate precision was lower than 2.0 in both methods, which represents the good precision of the method.

TABLE-3
RESULTING PARAMETER FROM PRECISION STUDY

Sample No.	Method precision		Intermediate precision	
	MET	REM	MET	REM
1	99.7	99.1	99.2	98.9
2	99.2	98.9	99.6	99.4
3	99.4	99.0	99.5	99.2
4	99.5	98.9	99.6	99.3
5	99.1	99.1	99.5	99.2
6	99.6	99.5	99.9	99.0
Mean	99.4	99.1	99.6	99.2
%RSD	0.23	0.22	0.23	0.19

Accuracy: The spiking method (standard addition method) was used to test the accuracy of the method for both REM and MET at three different concentrations (50 ,100 and 150%). The

TABLE-4
RESULTING PARAMETER FROM ACCURACY STUDY

Level (%)	Replicate No.	Amount added (ppm)		Amount recovered (ppm)		Recovery (%)		Mean recovery (%)		RSD (%)	
		MET	REM	MET	REM	MET	REM	MET	REM	MET	REM
50	1	25.992	5.230	25.492	5.142	98.34	98.32	98.29	98.39	0.04	0.07
	2	25.992	5.230	25.476	5.149	98.28	98.45				
	3	25.992	5.230	25.470	5.147	98.26	98.41				
100	1	49.85	10.059	49.627	10.038	99.55	99.79	99.49	99.73	0.13	0.10
	2	49.85	10.059	49.519	10.020	99.34	99.61				
	3	49.85	10.059	49.540	10.038	99.58	99.79				
150	1	75.771	15.289	74.983	15.140	98.96	99.03	99.09	99.15	0.14	0.12
	2	75.771	15.289	75.065	15.165	99.07	99.19				
	3	75.771	15.289	75.188	15.176	99.23	99.26				

TABLE-5
SYSTEM SUITABILITY PARAMETERS

S. No.	MET				REM				Resolution
	RT	Area	T. Factor	T. Plate	RT	Area	T. Factor	T. Plate	
1	1.01	1759269	1.09	7960	1.86	170096	1.11	25116	7.18
2	1.00	1758339	1.10	7894	1.85	171409	1.12	25583	7.22
3	1.00	1758712	1.10	7913	1.85	170131	1.12	24949	7.17
4	1.00	1758268	1.10	7899	1.86	170189	1.11	25810	7.24
5	1.00	1757589	1.10	7906	1.86	170220	1.11	24933	7.18
6	1.00	1757783	1.10	7915	1.86	170021	1.11	25076	7.19
MEAN	1.00	1758327			1.86	170344			
%RSD	0.41	0.03			0.28	0.31			

mixture was examined and the outcome data were compared with those from standard solutions, indicating procedures had accurate results. The concentration of analyte peak was calculated against standard solutions (Table-4).

Specificity: The specificity was assessed by overlaying the chromatograms of blank, placebo, standard and sample. A 20 µL of each solution were injected into UFLC system separately. No co-elution peak at retention time of active compounds were observed representing no interfering from placebo confirmed the specificity of the method.

System suitability: The system suitability parameters evaluated by injecting the standard in six replicates. The parameters like retention time, tailing factor, theoretical plates, RSD of area were checked and found within the limits (Table-5).

Forced degradation: The degradation study was conducted by physical and chemical degradation of the dry samples and their dilutions. Chemical degradation was done on sample solution by adding 0.1 N HCl, 0.1 N NaOH and 3% H_2O_2 solutions (Fig. 4). Thermal degradation was carried out by heating the samples at 60 ºC for 24 h. Photolytic degradation was done by exposure of the samples in UV light. Humidity degradation was done exploring ammonium hydroxide solution to the dry sample in a closed container and kept for 8 h. The degradation was calculated for all conditions and described in Table-6.

TABLE-6
FORCED DEGRADATION STUDY

Stress parameter	Assay (%)		Degradation (%)	
	MET	REM	MET	REM
As such	99.5	99.0	–	–
Acid degradation	99.4	45.2	0.1	53.8
Alkali degradation	99.1	43.8	0.4	55.2
Peroxide degradation	94.5	74.2	5.0	24.8
Thermal degradation	99.7	97.5	0.0	1.5
Photolytic degradation	99.3	97.8	0.2	1.2
Humidity degradation	96.9	94.7	2.6	4.3

Robustness: A robustness study was carried out for altering the instrumental parameter including wavelength for 2 nm, flow

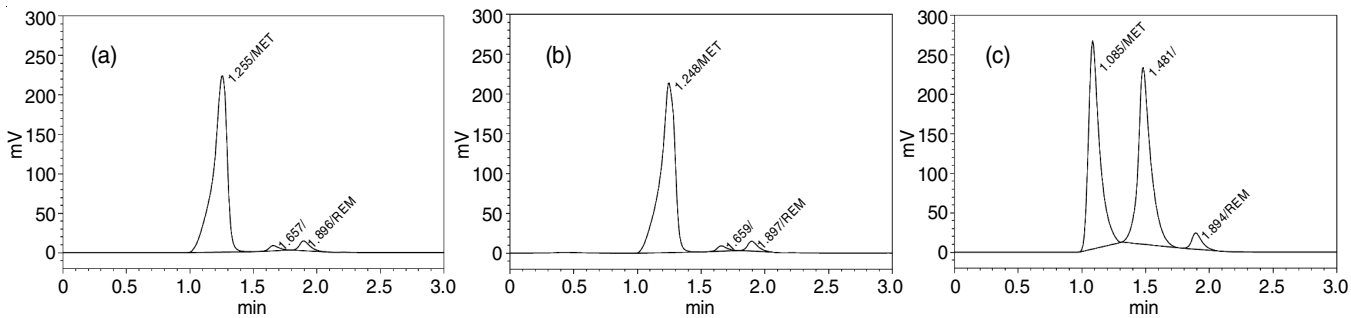

Fig. 4. Chromatogram of (a) acidic degradation, (b) alkali degradation and (c) peroxide degradation

TABLE-7
THE RESULTS OF ROBUSTNESS STUDY

Change in method parameter	MET			REM		
	Mean area standard	Mean area sample	Assay (%)	Mean area standard	Mean area sample	Assay (%)
As such	1722421	1723269	99.4	161981	162434	99.1
-WL (-2 nm)	1582936	1583685	99.4	153660	154359	99.3
+WL (+2 nm)	1908665	1908727	99.4	167756	168792	99.4
-Flow (-0.1 mL)	1898889	1901878	99.6	179623	179771	98.9
+Flow (+0.1 mL)	1567256	1570082	99.6	151021	150007	99.4
-Oven temp. (-5 °C)	1708146	1709387	99.5	162087	163387	99.6
+Oven temp. (+5 °C)	1710642	1715586	99.7	161624	163342	99.7
-Buffer ratio (-2%)	2018085	2021708	99.6	191249	190581	98.5
+Buffer ratio (+2%)	1505360	1505940	99.4	142881	142023	99.0

TABLE-8
COMPARISION OF PREVIOUSLY REPORTED CHROMATOGRAPHIC METHODS WITH PROPOSED METHOD

Instrument	RUN time	LOD (ppm)		LOQ (ppm)		Linear range (ppm)		Ref.
		MET	REM	MET	REM	MET	REM	
HPLC	3.5	1.49	0.48	4.53	1.56	5-200	2-150	[27]
UHPLC	6.0	1.93	1.47	6.44	4.92	20-100	20-100	[29]
UPLC	8.0	10.00	5.00	50.00	10.00	50-500	10-100	[30]
UFLC	3.0	0.01	0.01	0.03	0.03	0.01-125	0.01-25	Present study

rate by 0.1 mL, oven temperature by 5 ºC. Mobile phase ratio was altered for 2% absolute. The results are shown in Table-7.

Comparative studies: There are few methods reported for REM individually and in combination with utilization of UV, HPLC [14-17]. Several methods including LC-MS, HPTLC, UPLC, HPLC and UV methods have been reported for REM in combination with vildagliptin [18-22]. One HPLC method, has been reported for REM in combination with teneligliptin [23]. There are few methods comprising of HPTLC, UHPLC, UPLC, HPLC and UV methods have been reported for REM in combination with MET [24-30], but no method reported for REM with MET by UFLC. Every laboratory requires an analysis technique to evaluate the formulation's content precise and accurately. So, the objective of the research was to establish a validated the method for the assessment of the combination of metformin hydrochloride (MET) and remogliflozin etabonate (REM) and using various quickest techniques and with accurate results. Hence, the purpose of the study was development of method for the content determination of REM along with MET in API and dosage form by fast UFLC and validation as per ICH guidelines.

Applications: The simultaneous determination of MET and REM in the formulation was performed by using proposed UFLC method. The results given in Table-9 demonstrated the correctness of the drug quantification with excellent accord with API quantity mentioned on the label of medicine and verified that the presence of excipients had no influence on the assay of either drug. The excipient effects are eliminated by the proposed procedures, making it simple to utilize them in quality control laboratories.

TABLE- 9
RESULTS OF DOSAGE TABLET

Drug name	Content (mg)	UV method	
		Amount (mg)	Assay (%)
MET	500	500.20	100.0
REM	100	100.05	100.1

Conclusion

Novel reversed phase UFLC method for the quantitation of metformin hydrochloride (MET) and remogliflozin etabonate (REM) in dosage and API was established and validated as per ICH-Q2(R1) [31,32]. The technique is quick, accurate and easy. The assay values achieved using all this methodology are in satisfactory correlation. In case of pharmaceutical dosage forms or other matrices, the UFLC technique gives benefit for being the selective, fast and accurate. The APIs and pharmaceutical formulation were determined by UFLC method for the quantitation of REM and MET and the results were found successfully. The approach has the benefit of being extremely quick and it is straightforward to adapt it for usage in the simple laboratory.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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