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HPTLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TWO ANTIDIABETIC DRUGS IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Metformin HCl (MET) and Remogliflozin Etabonate (REM) is a new drug combination used for treating diabetes mellitus. It is necessary to estimate the drugs in the combined dosage form. For the simultaneous quantification of metformin HCl and remogliflozin etabonate, high-performance thin layer chromatography (HPTLC) was devised in the current investigation. For separation Merck pre-coated silica gel aluminium plate 60 F_{254} was used as stationary phase and Acetone: methanol: chloroform: Formic acid (3: 1: 5: 1) as mobile phase. A camag TLC scanner was used to densitometrically scan plates at 224 nm to quantify the medication. The method has been tested for linearity, precision, accuracy, and robustness by ICH criteria. The response was found linear over the concentration range of 100-600

ng/band with a regression value of 0.999 for both the drugs. Metformin's LOD and LOQ were reported to be 5.34 and 17.81 ng/band, but remogliflozin's LOD and LOQ were reported to be 11.03 and 33.45 ng/band. This method can be used for its intended purpose because they are highly sensitive, accurate and precise.

Keywords: Metformin HCl, Remogliflozin etabonate, Validation, Densitometric, High-Performance Thin Layer chromatography

INTRODUCTION

Diabetes mellitus is the leading cause of death worldwide **[1-3]**. Various medications are used to lessen the effects of diabetes on consequences such as renal failure, retinopathy, neuropathy, and cardiovascular issues **[4]**. The chemical name for remogliflozin etabonate is 5-Methyl-4-[4-(1-methylethoxy)benzyl]-1-(1-methylethyl)-1H-pyrazol-3-yl 6-O-(ethoxycarbonyl)-β-D-glucopyranoside. It belongs to the class of SGLT 2 inhibitors. The reabsorption of glucose by the kidney is carried out by these transporters. By preventing the transporters, blood glucose is excreted in the urine. **Figure 1 [5-7]** depicts the structural formula. Oral hypoglycemic medication is metformin. Through the various mode of action metformin act on body glucose levels such as decreasing the rate of hepatic gluconeogenesis and insulin sensitivity resulting in an increase in the utilization of glucose in peripheral cells **[8-10]**. Chemically metformin is known as 1,1-dimethyl biguanide hydrochloride **[11]**.

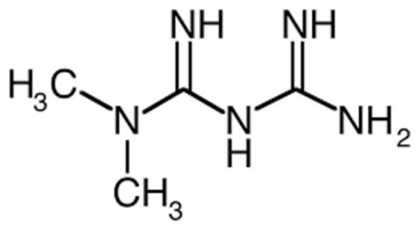

Figure 1: Chemical structure of Metformin

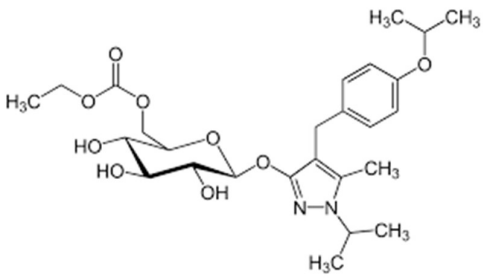

Figure 2: Chemical structure of Remogliflozin Etabonate

According to a review of the literature, dapagliflozin, empagliflozin, and canagliflozin have some publications published while remogliflozin etabonate has none **[12-17]**. Analytical methods such as spectrophotometric **[18]**, HPLC **[19]**, and LC-MS **[20]** were reported for quantification of metformin alone in pharmaceutical dosage form and biological samples. By using HPLC metformin is estimated in combination with empagliflozin **[21]** and canagliflozin **[22]**.

In the present work, efforts are made to develop a simple, precise and accurate HPTLC method for estimation of remogliflozin etabonate in bulk and pharmaceutical dosage forms. The method is validated according to ICH guidelines **[23-24]**.

MATERIAL AND METHOD

Chemicals and Reagents

Remogliflozin etabonate and metformin HCl were purchased from Sigma Aldrich, India. Acetone, chloroform, formic acid and methanol of HPLC grade were used. In this study, the tablet was used in pharmaceutical dosage form and it was acquired at a neighbourhood drug store in Vadodara, India (Trade name is Remozen M containing 100 mg remogliflozin and 500 mg metformin manufactured by Glenmark Pharmaceuticals).

Camag HPTLC (Camag, Muttenz, Switzerland) was used. It consists of spotting tool camag linomat v with nitrogen gas flow and it was semi-automatic. Camag TLC scanner with winCATS software was used. Camag glass twin trough development chamber and camag UV chamber was employed. Silica gel 60 F254 TLC Aluminum with pre-coat sheets 20×20cm was used. TLC scanner with winCATS software is used for densitometric analysis.

OPTIMIZATION OF HPTLC METHOD

Stock Solution

1) Metformin HCl

About 50 mg of MET is dissolved in 50 ml of distilled water to obtain 1000 µg/ml. From this, different aliquots are withdrawn to prepare a 10 µg/ml working solution.

2) Remogliflozin Etabonate

About 50 mg of REM is dissolved in 50 ml of distilled water and make-up was done with ethanol up to 100 ml to obtain 1000 µg/ml. From these different aliquots are withdrawn to prepare a 10 µg/ml working solution.

Sample Solution

Stationary Phase

Pre-coated Silica gel 60 F254 TLC Aluminium sheets 20×20cm were used.

Selection of mobile phase

Acetone: Methanol: Chloroform: Formic acid (3: 1: 5: 1) was used as the Mobile Phase in a Twin-Trough Chamber. The saturation time for the developing chamber was 20min.

Selection of analytical method

Both the drugs were scanned from the 200-800 nm range. The spectrum of MET and REM was obtained at 224 nm.

Optimization of chromatographic conditions for HPTLC

In initial experimental conditions development of the plate was performed Acetone: Methanol: Chloroform: formic acid (3: 1: 5: 1) since the mobile phase into CAMAG chamber before saturated with 10

ml of solvent for 20 min. A continuous supply of nitrogen gas aided in sample application under the pressure of more than 2000 Pa. Densitometric scanning was carried out in a vacuum on top of the situation, with the help of the Win CATS planar chromatography software. The most important thing about the radiation detection method is a deuterium lamp and a band of characters to be scanned at 224 nm.

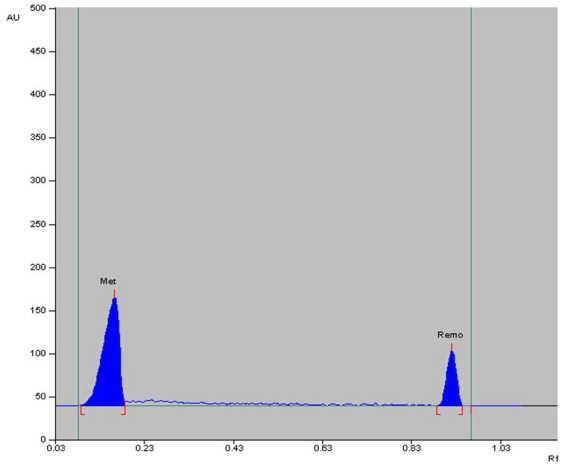

Figure 3: Optimised HPTLC chromatogram of Metformin and Remogliflozin

VALIDATION PARAMETERS OF THE DEVELOPED METHOD

Linearity

The linearity of MET and REM is evaluated by plotting calibration curves under the range of 100 -600 ng/band with 6 concentrations. Calibration curves were developed by plotting peak area against concentration (n =6).

Limit of Detection and Limit of Quantification

LOD & LOQ were calculated as per the ICH guideline equation which is LOD = 3.3 ×σ/S and LOQ = 10 × σ/S. Here, σ place the standard deviation and y-intercepts of regression lines & S place the slope of the calibration curve.

Precision

The precision of the given method was done by using the above-mentioned linearity range. Both interday and intraday precision was studied using the same chromatographic condition described above to estimate the comparable answers on the same day on three different days over the course of one week. Results are expressed as a percentage of RSD.

Accuracy (Recovery studies)

Recovery studies are studied by employing three different percentages of standard additions in the formulation (80, 100, and 120%). The same chromatographic conditions as before are used to perform three determinations at each level.

Robustness

By purposefully altering the mobile phase's composition, the resilience was

investigated, and results were compared to those obtained using standard chromatographic bands. The method's precision is tested on three occasions at a 300 ng/band API concentration. The values of the mean and %RSD for the Rf were calculated.

RESULTS AND DISCUSSION

Optimisation of Method

Optimisation of HPTLC parameters was done by using several mobile phases in different combinations such as acetone and methanol, formic acid and ethanol, water and chloroform and satisfactory results were obtained by using acetone; methanol; toluene; formic acid (4; 3; 2; 1) with 224 nm UV detector. With an Rf, a sharp and symmetric peak was resolved. As per Q2B guidelines validation results were found satisfactory.

Range and Linearity

The linearity of MET and REM is evaluated by plotting calibration curves under the range of 100 -600 ng/band with 6 concentrations. Calibration curves were developed by plotting peak area against concentration (n =6). Linearity data show linear relationships between peak area (AUC) and concentrations of MET and REM over a concentration range of 100-600 ng/band, standard showing a good regression coefficient of 0.999 for both the drugs. The correlation coefficient and Regression equation are shown in **Table 3**.

Precision

The precision of the given method was done by using the above-mentioned linearity range. Both interday and intraday precision was studied using the same chromatographic condition described above to estimate the comparable answers on the same day on three different days over the course of one week. Results are expressed as a percentage of RSD. Interday and intraday values of metformin over the three concentrations were found to be 0.77-1.79 %RSD and 1.1-1.42%RSD and for remogliflozin interday and intraday values were found to be 1.28-1.54%RSD and 1.52-1.67%RSD shown in **Table 4 and Table 5**.

Repeatability

It expresses the precision under the same operating conditions over a short interval of time. Repeatability also termed intra-assay precision was shown in **Table 6**.

Accuracy (Recovery studies)

Recovery studies shown in **Table 7** were performed by employing three different percentages of standard additions in the formulation (80, 100, and 120%). The same chromatographic conditions as before are used to perform three determinations at each level. %Recovery of MET and REM are found to be within the 98-102% limit. %RSD not more than 2.

Robustness

By purposefully altering the mobile phase's composition, the resilience was investigated, and results were compared to those obtained using standard chromatographic bands. The method's precision is tested on three occasions at a 300 ng/band API concentration as shown in **Table 8**. The values of the mean and %RSD for the Rf were calculated, by changing the mobile phase composition and development distance no change in R_f value was found.

Limit of Detection and Limit of Quantification

LOD & LOQ were calculated as per the ICH guideline equation which is LOD = 3.3 ×σ/S and LOQ = 10 × σ/S. Here, σ place the standard deviation and y-intercepts of regression lines & S place the slope of the calibration curve. LOD and LOQ of metformin were found to be 5.34 and 17.81 ng/ml while for remogliflozin LOD and LOQ were found to be 11.03 and 33.45 ng/ml as shown in **Table 9**.

Table 1: Optimised chromatographic conditions for HPTLC analysis of MET and REM

Parameters	Optimised conditions
Stationary phase	Pre-coated Silica gel 60 F254 TLC Aluminium sheets 20×20cm.
Mobile phase	Acetone: Methanol: Chloroform: Formic acid (3: 1: 5: 1).
Scanner wavelength	224 nm
Development chamber	Twin Trough Glass Chamber
Software	Wincats–version1.4.3
Development distance	7.0 cm
Tank saturation time	20 min

Table 2: Linearity results for Metformin and Remogliflozin

Concentration	Area	
	Metformin	Remogliflozin
100	2545	420.36
200	4823.2	1016.23
300	7023.6	1520.23
400	9023.2	2172.3
500	11423	2675.3
600	13245	3214.5

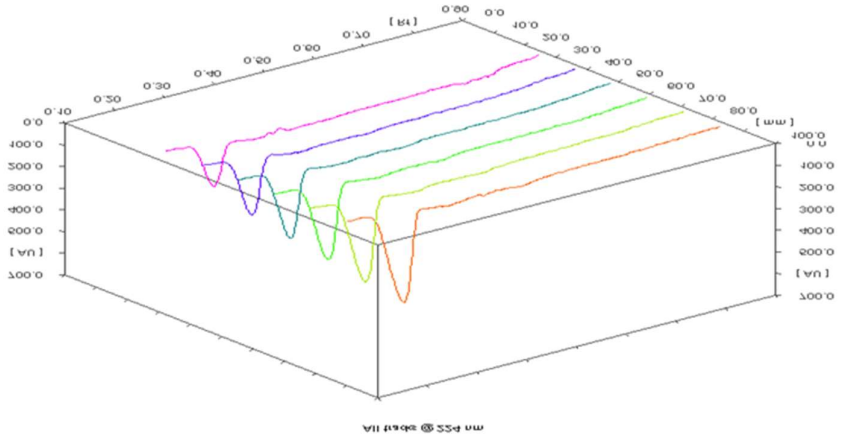

Figure 4: 3D chromatogram for linearity of Metformin

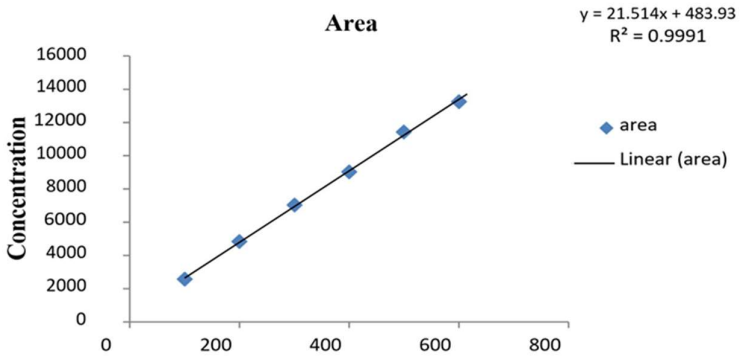

Figure 5: Linearity curve of Metformin

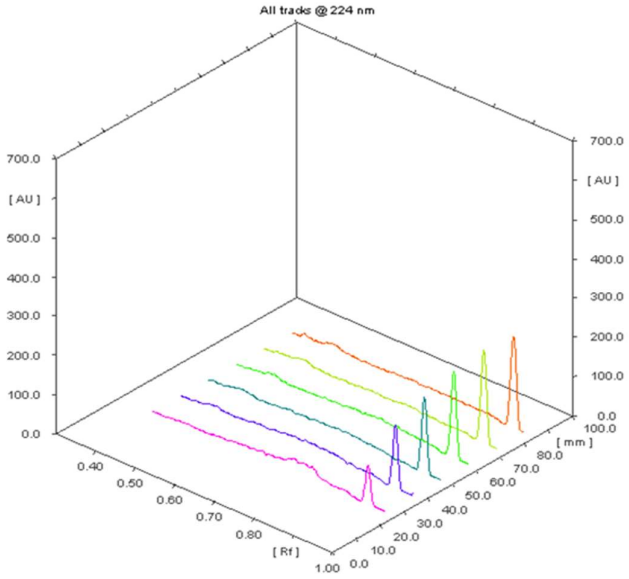

Figure 6: 3D chromatogram for linearity of Remogliflozin

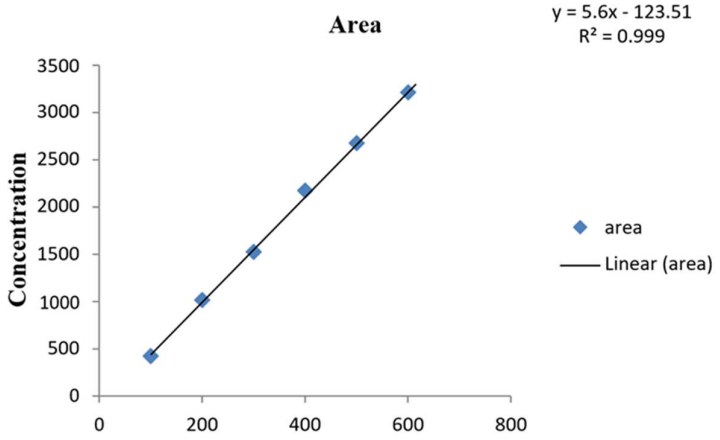

Figure 7: Linearity curve of Remogliflozin

Table 3: Statistical data of calibration curves of Metformin and Remogliflozin

Parameters	Results	
	Metformin	Remogliflozin
Linearity Range	100 – 600	100-600
Regression Equation (R^2)	Y = 21.514x + 483.93	Y = 5.6x - 123.51
Correlation Coefficient	0.999	0.999
Slope	21.51	5.6
Intercept	483.90	123.51

Table 4: Interday study of Metformin and Remogliflozin

Drug level (ng/band)	Interday			
	Metformin		Remogliflozin	
	Average peak area	%RSD	Average peak area	% RSD
200	4856	1.79	1027.6	1.28
400	9144.3	1.26	2200.3	1.44
600	13347.3	0.77	3271	1.54

Table 5: Intraday study of Metformin and Remogliflozin

Drug level (ng/band)	Intraday			
	Metformin		Remogliflozin	
	Average peak area	%RSD	Average peak area	% RSD
200	4945.3	1.42	1093.3	1.6
400	9250.4	1.10	2182.6	1.52
600	13436	1.12	3358.3	1.62

Table 6: Repeatability of Metformin and Remogliflozin

Drug	Concentration (ng/band)	Mean area (Scanner)	% RSD (Scanner)	Mean area (Injector)	% RSD (Injector)
Metformin	300	7134.5	1.46	7144.8	1.40
Remogliflozin	300	1544.83	1.94	1550.83	1.88

Table 7: Recovery studies of MET and REM by HPTLC

Drug	API concentration (ng/band)	Standard drug spiked (ng/band)	Drug recovery (ng/band)	Mean ± S.D	% Recovery	% RSD
Metformin	300	450	447.12	2568.3 ± 21.54	99.33	0.83
	300	600	605.05	4936.6 ± 40.41	100.08	0.81
	300	750	747.45	7074 ± 33.06	99.65	0.46
Remogliflozin	300	150	447.11	480 ± 39.12	99.35	0.081
	300	300	605.80	1596.3 ± 67.09	100.91	0.042
	300	450	747.15	2603 ± 51.17	99.61	0.019

Table 8: Robustness of Method for MET and REM

Parameters	Initial condition	Change in condition	Effect found
Mobile phase composition	Acetone: Chloroform: Methanol: Formic acid (3:1:5:1)	Acetone: Chloroform: Methanol: Formic acid (3:1:5:0.5)	No change in R_f value
Development distance	7 cm	6 cm	No change in R_f value
Saturation time	20 min	20 min	No change in R_f value

Table 9: LOD and LOQ for MET and REM

Parameters	Metformin	Remogliflozin
LOD	5.34 ng/band	11.03 ng/band
LOQ	17.81 ng/band	33.45 ng/band

CONCLUSION

According to studies and based on the results, it can be said that the created HPTLC method is straightforward, picky, sensitive, and precise. MET and REM can be estimated using this newly developed and verified approach in both bulk drugs and formulations. No interference of excipients is observed during analysis. Time and cost of analysis are minimised by analysing several samples at a time and using the minimum amount of mobile phase. Expensive chemicals and solvents are not required for analysis and they can be utilised as an alternative to HPLC.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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