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A Validated Stability indicating High Performance Thin Layer Chromatographic Method for Determination of Remogliflozin Etabonate in Tablet Dosage Form

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Abstract : The present work describes development and validation of a new simple, accurate and precise stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of Remogliflozin etabonate as bulk drug andin tablet dosage form.As stability testing is major step in the development of new drug as well as formulation, stress degradation studies were carried out according to ICH guidelines. Remogliflozin etabonate was found susceptible to all the analyzed stress conditions. HPTLC plates precoated with silica gel 60 F_{254} were used as the stationary phase and chromatographic separation was achieved by using Toluene: Methanol (8.5:1.5,v/v) as mobile phase. Densitometric detection was carried out at 224 nm. The retention factor was found to be 0.35 ± 0.03. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. The developed method was found to be linear in the concentration range of 50-250 ng band^{-1}. The LOD and LOQ for Remogliflozin etabonate was found to be 13.04 ng band^{-1}and 35.04 ng band^{-1}, respectively. The developed method has been effectively applied for the drug estimation in tablet dosage form.
Keywords : Remogliflozin etabonate, Stability indicating method, HPTLC, Forced degradation studies.

Introduction

Remogliflozin etabonate, chemically, Ethyl [(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-{[5-methyl-1-(propan-2-yl)-4-{[4-(propan-2-yloxy)phenyl]methyl}-1H-pyrazol-3-yl]oxy}oxan-2-yl]methyl carbonate is a potent benzyl pyrazole glucoside-based inhibitor of renal sodium-glucose co-transporter subtype 2 (SGLT2)and is used mainly for the treatment of diabetes [1-3].

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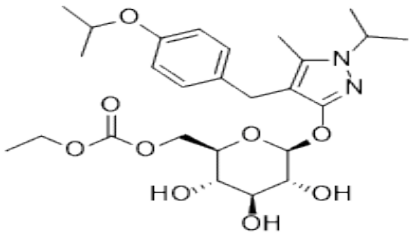


Figure 1: Chemical structure of Remogliflozin etabonate

An extensive literature survey revealed that different analytical methods have been reported for quantitative analysis of Remogliflozin etabonate. UV spectrophotometric methods for determination of Remogliflozin etabonate either as single or in combination with other drugs in bulk and its tablet formulation has been reported[4-6]. RP-HPLC methods for estimation of Remogliflozin etabonate either as single or in combination with other drugs in pure and pharmaceutical formulations are also reported[7-10]. Liquid chromatography coupled with mass spectrometric (LC-MS) methods for determination of Remogliflozin etabonate in human plasma are also reported[11, 12].

One of the HPTLC method reported by Shah DA *et al*.,[13] stating the stability indicating estimation of Remogliflozin etabonate, wherein the drug was exposed to acid and alkaline hydrolysis, oxidative stress condition, dry heat degradation and photolytic stress condition. Unfortunately, neutral hydrolytic degradation study which is also needs to be performed as recommended by ICH was not reported during this study. The study also reported 100% degradation of Remogliflozin etabonate when exposed alkali hydrolysis, which is no longer recommended. The less amount of literature provides the need for developing a new suitable stability indicating densitometric method for determination of Remogliflozin etabonate. Based on this fact, an attempt was made in this study to develop and validate a more sensitive stability-indicating HPTLC method for estimation of Remogliflozin etabonate in bulk and pharmaceutical formulation to overcome the shortcomings of reported stability indicating method with well resolved peak from its degradation products and acceptable degradation. The drug was degraded by all the stress conditions including neutral hydrolytic degradation to check the stability in accordance with International Conference on Harmonization Guidelines[14, 15].

Experimental

Chemicals and reagents

Pharmaceutical grade working standard Remogliflozin etabonate was obtained as a gift sample from Actis Generics Pvt Ltd., Hydrabad, India.The pharmaceutical tablet dosage form Remo 100 labelled to contain 100 mg(Glenmark Pharmaceuticals Ltd.) was procured from local pharmacy. All chemicals and reagents used for analysis were of analytical grade. Chemicals used viz. Methanol, Toluene were purchased from Loba Chemie Pvt Ltd., India.

Instrumentation and Chromatographic Conditions

Chromatographic resolution of the drug was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm × 10 cm with 250 µm layer thickness) from E. MERCK, Darmstadt, Germany, using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with a 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland). A constant application rate of 0.1 µL sec^{-1} was employed.

Linear ascending development was carried out in 10 ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using toluene: methanol (8.5: 1.5, v/v) as mobile phase. The saturation of mobile phase was done for 20 min in the chamber at room temperature. The length of chromatogram run was 82 mm. Densitometric scanning was performed on a CAMAG TLC scanner III at 224 nm for all developments operated by winCATS software version 1.4.2.Deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm was used as radiation source.

Preparation of working standard stocksolution

Accurately weighed 10 mg of Remogliflozin etabonate transferred to 10 mL volumetric flask and dissolved in methanol to acquiresolution of concentration 1000 ng μL^{-1} which was diluted further using methanol to get working standard solution of 50 ng μL^{-1}.

Analysis of tablet dosage form

Commercial brand of tablets Remo 100 containing 100 mg of drug was used to estimate the amount of Remogliflozin etabonate in existing tablet formulation. For this, 20 tablets were weighed accurately and powdered. Powder quantity equivalent to 10 mg of was weighed and transferred to the 10 mL volumetric flask and 5 mL methanol was added and sonicated for 10 min. The solution was filtered using Whatman filter paper No. 41, and the volume was made up to the mark with methanol. The resulting solution was diluted further with methanol to get final concentration 50 ng μL^{-1}. Two micro-liter volume of this solution was applied to a TLC plate to provide final concentration of 100 ng $band^{-1}$. After chromatographic development the peak areas of the bands were measured at 242 nm and the amount of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies

Stressdegradation studies were carried out to confirm the stability by exposing the bulk drug to different physical stress conditions recommended by ICH. The study was carried out at concentration of 1000 ng μL^{-1}. The acid and base hydrolytic studies were performed by treating stock drug solution separately with 0.1 N HCl and 0.1 N NaOH at room temperature for 30 min and 15 min, respectively. The acid and alkali stressed samples were neutralized with NaOH and HCl, respectively to provide the final concentration of 250 ng $band^{-1}$. The drug was treated with distilled water at room temperature for 1 hfor neutral hydrolysis. Standard drug solution was treated with 3 % H_2O_2 at room temperature for 30 min to perform the oxidative degradation and was diluted with methanol to obtain 250 ng $band^{-1}$ solution. Thermal stress degradation was performed by keeping drug in oven at 60ºC for period of 18 h.The solid drug powder was exposed UV light up to 200-watt hour $square\ meter^{-1}$ to check photolytic degradation. Thermal and photolytic samples were diluted with methanol to get concentration of250 ng $band^{-1}$.

Results and Discussion

Optimization of chromatographic conditions

The TLC procedure was optimized with a view to develop a stability indicating method for Remogliflozin etabonate which would be proficient to give the satisfactory resolution.Varied solvent systems comprisingdifferent ratios of benzene, chloroform, toluene, methanol, ethyl acetate were examined (data not shown) to achieve better separation and to resolve spot of Remogliflozin etabonatefrom its impurities and other excipients present in formulation. Finally, the mobile phase comprising of toluene: methanol (8.5: 1.5, v/v)was chosen as optimumwhich gave acceptable resolution of drug with symmetrical peak shape. Densitometric detection was carried out at 224 nm. The retention factor (Rf) was found to be 0.35±0.03. Representative densitogram of standard solution of Remogliflozin etabonate is represented in Figure 2.

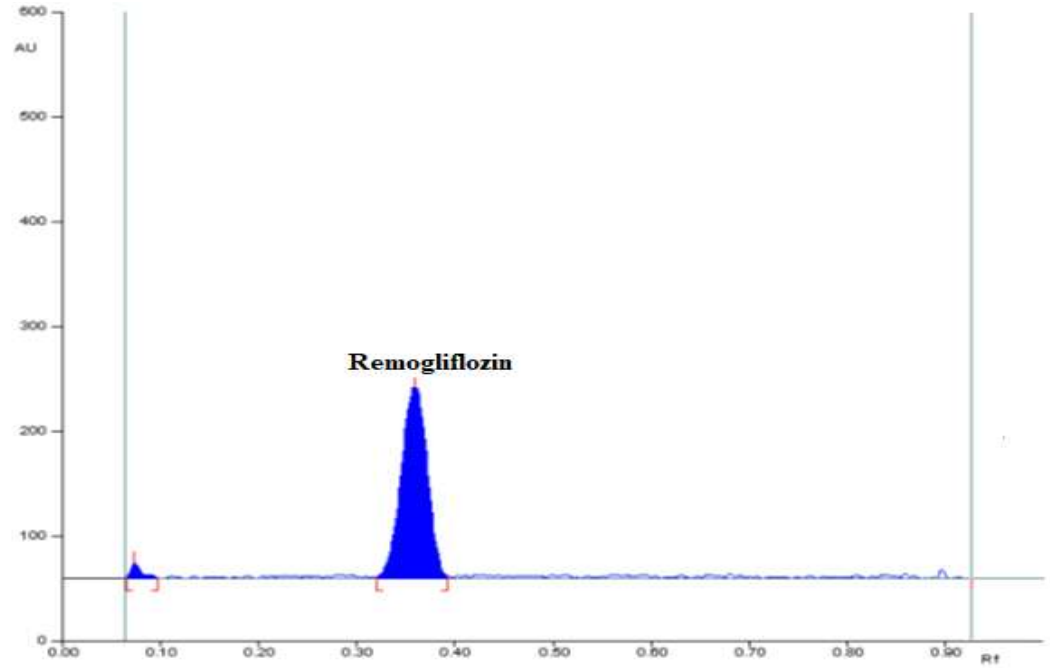


Figure 2:Representative densitogram of standard solution of Remogliflozin etabonate (150 ng band^{-1}, Rf= 0.35±0.03)

The acid and alkali degraded samples of Remogliflozinshowed products of degradation D1 and D2 at Rf 0.54 and 0.22, respectively and degradation products were well resolved from the drug peak (Figure 3 and 4).Remogliflozintreated with distilled water at room temperature for 1 h exhibited product of degradation D3 at Rf 0.49 which was well separated from drug peak (Figure 5). The peroxide treated sample also found susceptibile to oxidative degradation and showed the peak for degradation product D4 at Rf 0.63 (Figure 6).Remogliflozin after exposure to 60ºC for 5 h showed significant degradation with appearance of degradation peak D5 at Rf 0.72 (Figure 7). Remogliflozin after exposure to UV light exhibited the degradation without any degradation peak but there was decrease in the area of drug as compared to initial area. The stress degradation results demonstrated susceptibility of drug to all the analyzed stress conditions. The findings of degradation studies along with % degradation and % of drug recovered are summarized in Table 1.

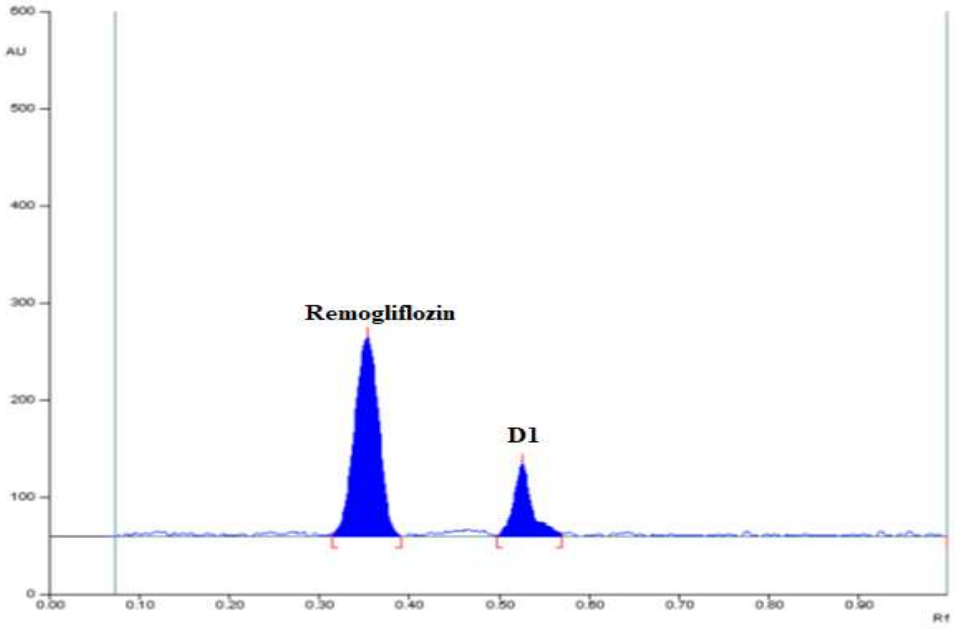


Figure 3: Densitogram obtained after treatment with 0.1 N HCl

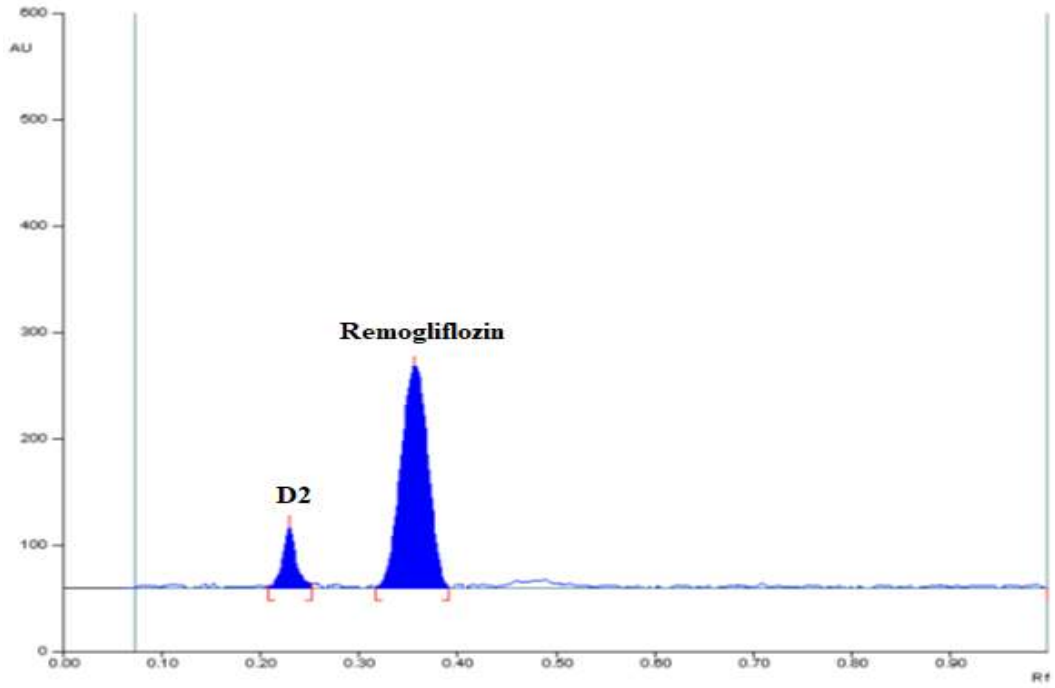
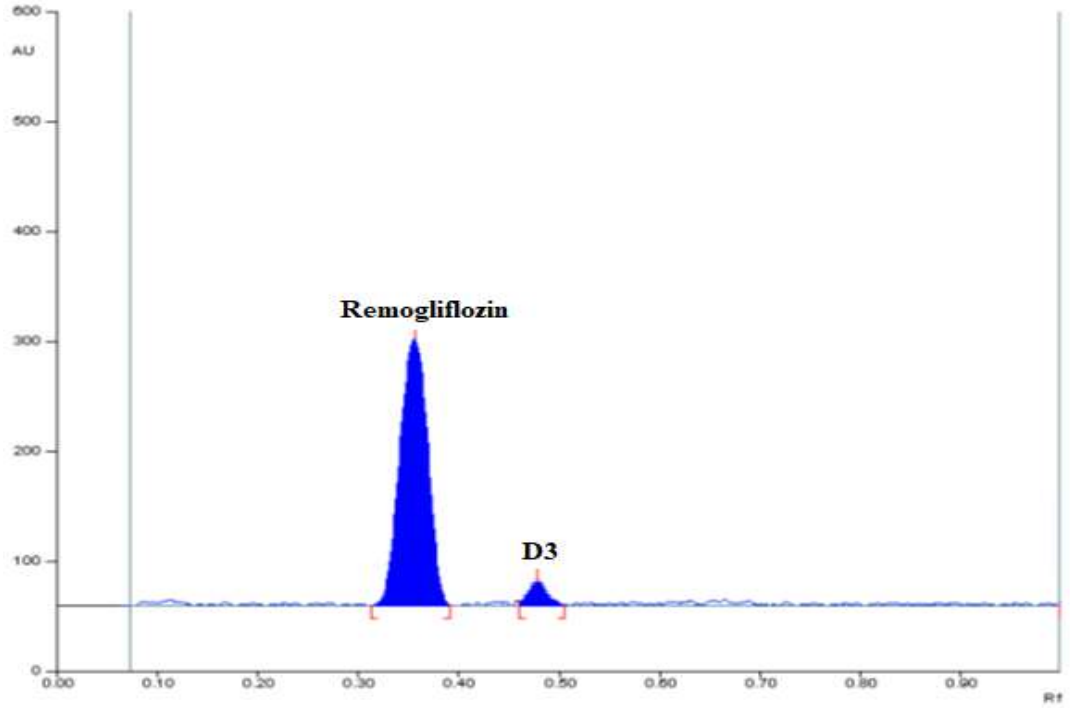


Figure 4: Densitogram obtained after treatment with 0.1 N NaOH



Figur 5: Densitogram after neutral hydrolysis with distilled water

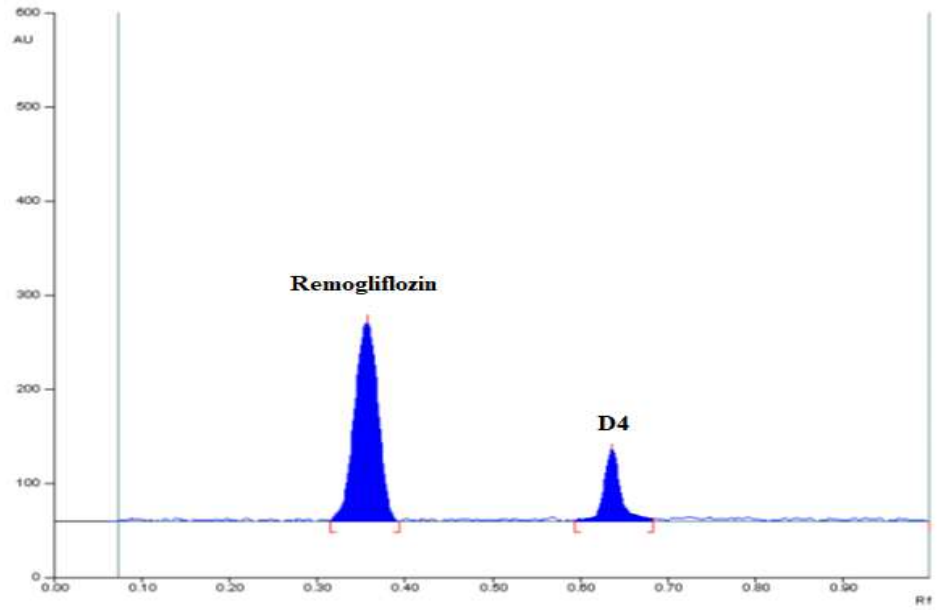


Figure 6:Densitogram after treatment with 3% H_2O_2

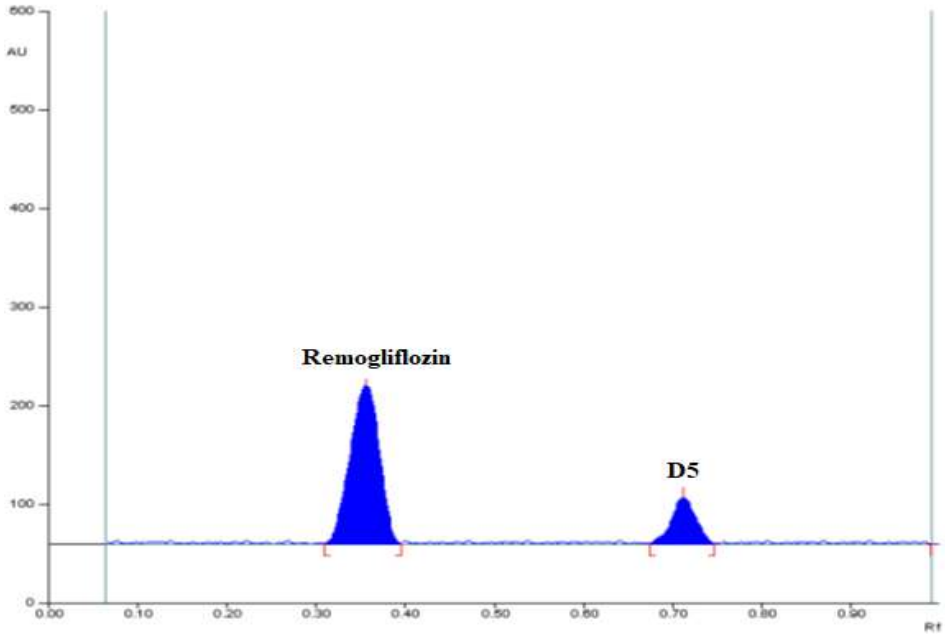


Figure 7: Densitogram obtained after exposure to 60ºC for 5 h

Table 1: Stress degradation studies

Stress conditions	**%Recovery**	**% Degradation**
Acid hydrolysis(0.1 N HCl, Kept at RT for 30 min)	82.04	18.39
Base hydrolysis (0.1 N NaOH, Kept at RT for 15 min)	81.60	18.40
Neutral hydrolysis (Distilled water Kept at RT for 1 h)	86.40	13.60
Oxidative degradation (3 % H_2O_2, Kept at RT for 30 min)	85.54	14.45
Thermal degradation (70º C for 18 h)	78.39	21.61
Photolytic degradation (UV light, 200 watt h) square meter^{-1})	81.51	18.49

Method Validation

The developed method was validated in terms oflinearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH Q2 (R1) guidelines.

Linearity

The linearity of proposed method was checked by spotting volumes 1, 2, 3, 4 and 5µL of standard solution of Remogliflozin(50 ng μL^{-1}) onto the TLC plates, developed and scanned under optimized chromatographic conditions.The method was found to be linear in the concentration range 50-250 ng $band^{-1}$ with high correlation coefficient. The linear regression equation was found to be y = 2.7474x + 1512.7with correlation coefficient (R^2) value of 0.995.The calibration curve was obtained by plot of concentration vs peak area of drug.A 3D densitogram of obtained in the concentration range 50-250 ng $band^{-1}$is shown in Figure 8.

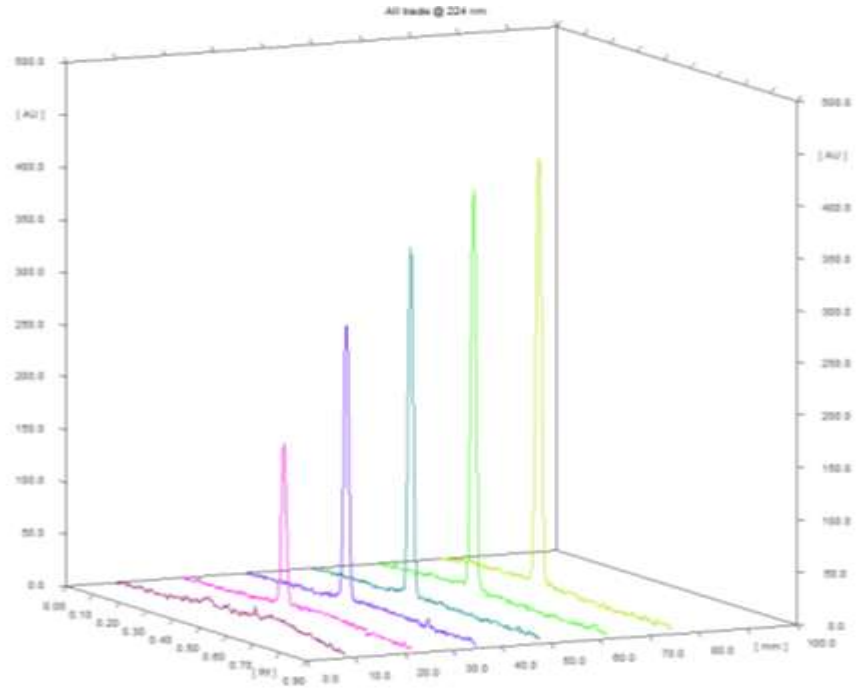


Figure8: 3D densitogram in concentration range 50-250 ng $band^{-1}$

Precision

The precision of the method was demonstrated by intraday and interday variation studies in which three replicates of three concentrations within the linearity range were analyzed on the same day and on three consecutive days, respectively and percentage R.S.D. was calculated. The % R.S.D. values obtained for intraday and interday variations were found to be < 2 which indicated that method is precise.The results obtained for intraday and inter-day precision studiesare shown in Table2 and 3, respectively.

Table 2: Intraday precision studies

Spotted concentration (ng $band^{-1}$)	Average Area	S. D.	%R.S.D.*
1000	4270.9	26.29	0.62
1500	5655.05	40.03	0.71
2000	7011.67	38.78	0.55

* Average of three determinations

Table 3: Inter-day precision studies

Spotted concentration (ng band^{-1})	Average Area	S. D.	%R.S.D.*
1000	4261.4	65.83	1.54
1500	5662.67	63.53	1.12
2000	6955.4	106.51	1.53

* Average of three determinations

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be13.04 ng band^{-1}and 35.04, respectively.

Assay of tablet formulation

From the prepared sample solution, sample concentration 1000 ng band^{-1}was applied, developed and scanned under optimized chromatographic conditions.Procedure was repeated six times. There was no interference from the excipients commonly present in the tablet. The drug content (mean ± S.D.) was found to be 99.91± 0.97.

Accuracy

Accuracy of developed method was checked by performing recovery studies by standard addition method. It involved addition standard drug solution to pre-analyzedsample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 1000ngband^{-1}from tablet solution.The results of the recovery studies indicated accurateness of developed method forestimation of drug in tablet formulation (Table 4).

Table 4: Recovery studies

Drug	Concentration taken (ng band^{-1})	Concentration added (ng band^{-1})	Concentration found (ng band^{-1})	% Recovery±R.S.D.*
Remogliflozin	1000	800	1803.77	100.21 ±1.18
	1000	1000	1995.69	99.78 ±0.65
	1000	1200	2201.34	100.06 ±0.89

*Average of three determinations

Robustness

By introducing deliberate variation in the method parameters,the effects on the results were examined to check the robustness of the method. The parameters varied were mobile phase composition (± 1 % methanol), wavelength (± 1 nm)and the effect on the area of drug was noted. The areas of peaks of interest remained unaffected by small changes of the operational parameters which indicated robustness of the method.

Conclusion

Stability-indicating HPTLC-densitometric method without interference from degradants has been developed and validated using all the stress conditions including neutral hydrolytic study for the estimation of Remogliflozin etabonate as bulk drug and in tablet dosage form. The drug was found to be susceptible all analyzed stress conditions including heat and light. Compared with HPTLC method reported [13], the established method is more sensitive as the range for the method developed starts from 50 ng band^{-1}whereas range starts from 500 ng band^{-1} for reported method. The developed method is simple, sensitive, precise, accurate, and reproducible.The developed method can be used for quantitative analysis of drugin pharmaceutical dosage form. As the method is stability indicating, it may be extended to study the degradation kinetics of drug.

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