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Simultaneous Estimation of Metformin Hydrochloride and Repaglinide in Pharmaceutical Formulation by HPTLC-Densitometry Method

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Abstract

A simple, precise, rapid, selective, and economic reversed phase high-performance liquid chromatography (HPTLC) method has been established for simultaneous analysis of Metformin Hydrochloride and Repaglinide. HPTLC method was developed using on precoated silica gel G60 F254 plates as stationary phase, using methanol:ammonium sulphate (0.25%) (pH-5.7) (2.5:7.5, v/v) as mobile phase. The plates were scanned at approximately 243 and 236 nm for HPLC and HPTLC both respectively. In HPTLC method both the drugs were resolved using proposed mobile phase and R_f value was found to be 0.34 for MET and R_f 0.60 for REPA. The method was found to linear in the range 500-2500 ng/band for MET, and 100-500 ng/band based for REPA respectively. This HPTLC procedure is economic, sensitive, and less time consuming than other chromatographic procedures. It is a user-friendly and importance tool for analysis of combined dosage form.

Introduction

Metformin Hydrochloride (MET; (1-carbamimidamido-N,Ndimethylmethanimidamide; Figure 1a is a widely used for treating non-insulin-dependent diabetes mellitus (NIDDM) [1]. Repaglinide (REPA; (S)-(+)-2-ethoxy-4-[2-(3-methyl-1-[2-(piperidin- 1-yl) phenyl] butylamino)-2 oxoethyl]benzoic acid Figure 1b. It is used for the treatment of non-insulin dependent diabetes mellitus in conjunction with diet and exercise [2].

Literature survey revealed that various analytical methods like HPLC [3-17], UV [18-23] and HPTLC [24,25] have been reported for the determination of MET and REPA either individually or combination with some other drugs. The review of literature prompted us to develop an accurate, selective and precise simultaneous method for the estimation of MET and REPA in combined dosage forms.

Experimental

Chemicals and materials

Metformin Hydrochloride was procured from Torrent Pharmaceuticals Ltd., Ahemdabad and Repaglinide was obtained from Torrent Pharmaceuticals Ltd, Ahemdabad. Methanol (HPLC Grade), Toluene (AR Grade), Ammonium Sulphate, Isopropylalcohol (AR Grade), Ammonia and Ethyl-Acetate were used as solvents to prepare the mobile phase. Tablet formulation EUREPA MF (Torrent Pharmaceuticals Ltd, Ahemdabad) was procured from local market.

Figure 1: Structure of Metformin Hydrochloride (a) and Repaglinide (b).

Chromatographic conditions

A Pre-coated silica gel G60–F254 aluminum sheet (100×100 mm, thickness layer 0.2 mm) pre washed with methanol was used as stationary phase. The linear ascending development was carried out in a CAMAG twin-trough glass chamber (20×20 cm) equilibrated with the mobile phase methanol:ammonium sulphate (0.25%) (pH 5.7) (2.5:7.5 v/v) for 30 min at room temperature. The length of the chromatogram run was 70 mm. Quantitative evaluation of the plate was performed in absorbance mode at 236 nm. The slit dimensions were 5 mm length and 0.45 mm width, with a scanning rate of 20 mm/s with a computerized CAMAG TLC scanner -3 integrated with win CATS 4 software.

Preparation of ammonium sulphate solution (0.25%): Weigh accurtly 0.25 g of ammonium sulphate in 100 ml volumetric flask and add 25 ml of distilled water, shake well and make up the volume up to mark with distilled water.

Sample preparation

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 250 mg of MET (1 mg of REPA) was accurately weighed and transferred to a 10 ml volumetric flask. A few ml of methanol was added to the above flask and flask was sonicated for 5 min. The solution was filtered using Whatman filter paper No.1 in another 10 ml volumetric flask and volume was diluted to the mark with the methanol. 1 ml was taken from above in 10 ml volumetric flask and dilute upto mark with Methanol 1 ml aliquot from the above solution was taken, add 1 ml 500 ng/band Standard solution of REPA
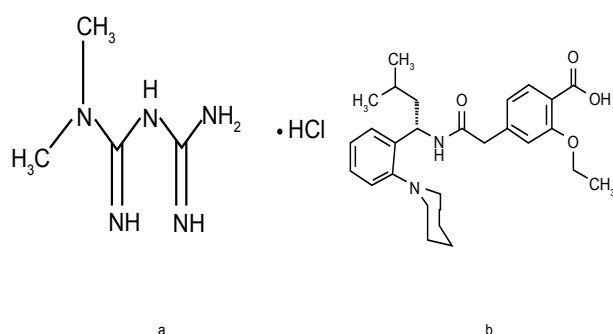
(STANDARD ADDITION) diluted to 10 ml with methanol to obtain final concentration of 250 ng/band of MET (and 51 ng/band of REPA). Appropriate aliquot of these solutions were applied to HPTLC plates and analyzed for MET and REPA content using the proposed method as described earlier.

Preparation of standard solution: REPA (10 mg) was accurately weighed and transferred to 10 ml volumetric flasks and dissolved in few ml of methanol. The volume was made up to the mark with methanol to yield a solution containing 1000 μg/ml REPA. MET (10 mg) was accurately weighed and transferred to 10 ml volumetric flask and dissolved in few ml of methanol. The volume was made up to the mark with methanol to yield a solution containing 1000 μg/ml MET. This stock solutions are used for constructing the calibration curve.

Method validation

The developed method was validated for linearity and range, specificity, accuracy, precision, Limit of detection, Limit of quantization, robustness and solution stability as per ICH guidelines.

Linearity and range: Linearity of the method was evaluated by constructing calibration curves at five concentration levels over a range of 500-2500 ng/band and 100-500 ng/band of MET and REPA respectively. The calibration curves were developed by plotting peak area versus concentration with the help of the winCATS software.

Specificity: The specificity of the method was ascertained by analyzing MET and REPA in presence of excipients like cellulose microcrystalline, Magnesium stearate, Povidone, Propyleneglycol, Sorbitol, Talc, Titaniumdioxide, Polyethylene glycols commonly used for Tablet formulations. The bands of MET and REPA were confirmed by comparing R_f values and respective spectra of sample with those of standards. The peak purity of MET and REPA was assured by comparing the spectra at three different levels, that is, peak start, peak apex and peak end positions.

Accuracy (% Recovery): The accuracy of the method was determined by calculating recoveries of MET and REPA by method of standard additions. Known amount of MET (0%, 50%, 100%, 150%) and REPA (0%, 50%, 100%, 150%) were added to a pre quantified sample and the amount of MET and REPA were estimated by measuring the peak area and by fitting these values to the straight-line equation of calibration curve.

Method precision (Repeatability): Repeatability of measurement of peak area was determined by analyzing MET and REPA sample (1500 and 300 ng/band) six times without changing the position of plate.

Intermediate precision (Reproducibility): Precision was evaluated in terms of intraday and interday precisions. Intraday precision was determined by analyzing sample solutions of MET (1000, 1500, 2000 ng/band) and REPA (200, 300, 300 ng/band) at three levels covering low, medium, and high concentrations of the calibration curve three times on the same day (n=3). Interday precision was determined by analyzing sample solutions of MET (1000, 1500, 2000 ng/band) and REPA (200, 300, 400 ng/band) at three levels covering low, medium, and high concentrations over a period of 3 days (n=3). The peak areas obtained were used to calculate mean and %RSD values.

Limits of detection (LOD) and limits of quantitation (LOQ): The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with

suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines. LOD=3.3×σ/S; LOQ=10×σ/S; Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Robustness: Small changes in the chamber saturation time, solvent migration distance was introduced and the effects on the results were examined. Robustness of the method was determined in triplicate at a concentration level of 1500 ng/band and 300 ng/band of MET and REPA respectively. The mean and %RSD of peak areas were calculated.

Application of validated method to pharmaceutical formulation

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 250 mg of MET (1 mg of REPA) was accurately weighed and transferred to a 10 ml volumetric flask. A few ml of methanol was added to the above flask and flask was sonicated for 5 min. The solution was filtered using Whatman filter paper No.1 in another 10 ml volumetric flask and volume was diluted to the mark with the methanol. 1 ml from above was taken in 10 ml volumetric flask and dilute upto mark with methanol 1 ml aliquot from the above solution was taken, add 1 ml 500 ng/band Standard solution of REPA(STANDARD ADDITION) diluted to 10 ml with methanol to obtain final concentration of 250 ng/band of MET (and 51 ng/band of REPA). Appropriate aliquot of these solutions were applied to HPTLC plates and analyzed for MET and REPA content using the proposed method as described earlier.

Results and Discussion

Method development and optimization of chromatographic conditions

A mobile phase consisting of methanol:ammonium sulphate (0.25%) (pH 5.7) (2.5:7.5, v/v) gave good separation of MET and REPA from its matrix. It was also observed that chamber saturation time and solvent migration distance were crucial in the chromatographic separation as chamber saturation time of less than 20 min and solvent migration distances greater than 70 mm resulted in diffusion of the analyte band. Therefore, methanol:ammonium sulphate (0.25%) (pH 5.7) (2.5:7.5, v/v) mobile phase with linear ascending development was carried out in a twin-trough glass chamber equilibrated with the mobile phase vapors for 30 min at room temperature. 10 milliliters of the mobile phase was used for each development and was allowed to migrate a distance of 70 mm. After development, the HPTLC plates were dried completely. These chromatographic conditions produced a well-defined, compact band of MET and REPA with optimum migration at R_f 0.34 ± 0.002 and 0.60 ± 0.01 respectively. Developed plate was subjected to densitometric measurements in scanning mode in the UV region of 200-400 nm and the overlain spectrum was recorded using Camag TLC Scanner 3. The overlain spectra showed that both the drugs absorbs appreciably at 236 nm so, it was selected for the densitometric analysis (Figure 2).

Validation of the method

Linearity: The method was found to be linear in a concentration range of 500-2500 ng/band and 100-500 ng/band of MET and REPA respectively, (n=5) with a co-relation co-efficient of 0.999 and 0.995 of MET and REPA respectively (Figure 3). The regression data showed good linear relationship over the concentration range studied, demonstrating the suitability of the method for analysis (table 5). Figure 5 displays a three-dimensional overlay of HPTLC densitograms of the
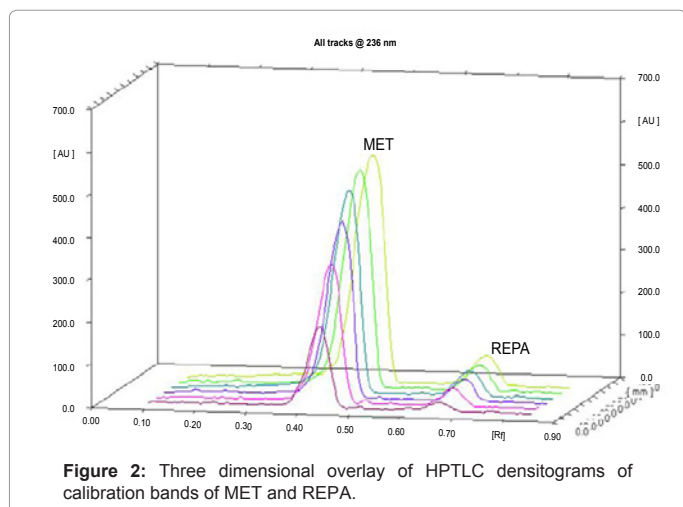
Figure 2: Three dimensional overlay of HPTLC densitograms of calibration bands of MET and REPA.
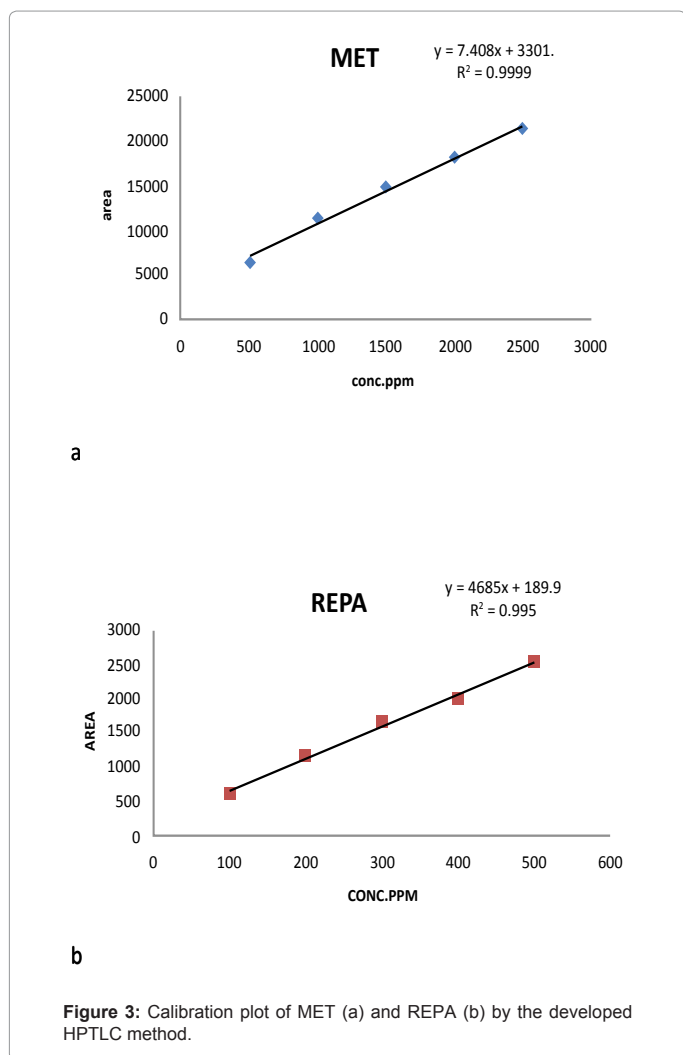

a

b

Figure 3: Calibration plot of MET (a) and REPA (b) by the developed HPTLC method.

calibration bands of MET and REPA at 236 nm.

Specificity: Specificity is the ability of an analytical method to determine the analyte unequivocally in the presence of sample matrix. Specificity of the method for MET and REPA was proved from the

spectral scan, and peak purity correlation (r) results for MET and REPA in tablet formulations indicate that there is no co-eluting peak with MET and REPA, so there is no interference from any excipients present in tablet formulation (Figures 4 and 5) (Table 1).

Accuracy: Accuracy of an analytical method is the closeness of test results to the true value. It was determined by the application of analytical procedure to recovery studies, where a known amount of standard is spiked into pre analyzed samples solutions. Percentage recovery was found to be 99.23-101.76% and 97.85-100.78% for MET and REPA respectively. Recovery values demonstrated the accuracy of the method in the desired range (Table 2).

Precision: The %RSD value for inter day and intraday precision were found 0.14-0.50 and 0.16-0.33 respectively for MET. The %RSD value for inter day and intraday precision were found 0.63-1.76 and 0.89-1.96 respectively for REPA. In all instances, %RSD values were less than 2%, confirming the precision of the method. Repeatability of the scanning device was studied by applying and analyzing MET and REPA sample (1500 and 300 ng/band) three times. RSD was less than 2%, which was well below the instrumental specifications (Table 3).
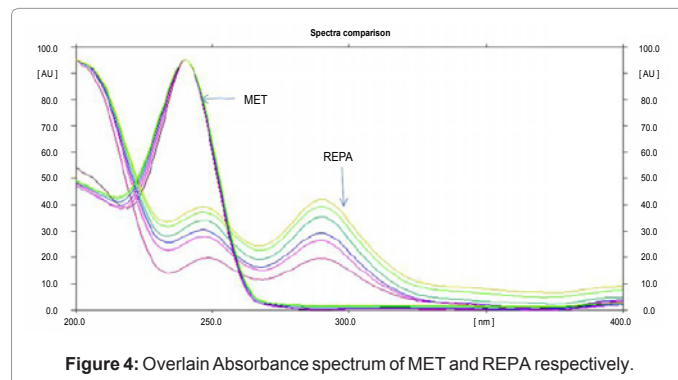

Figure 4: Overlain Absorbance spectrum of MET and REPA respectively.
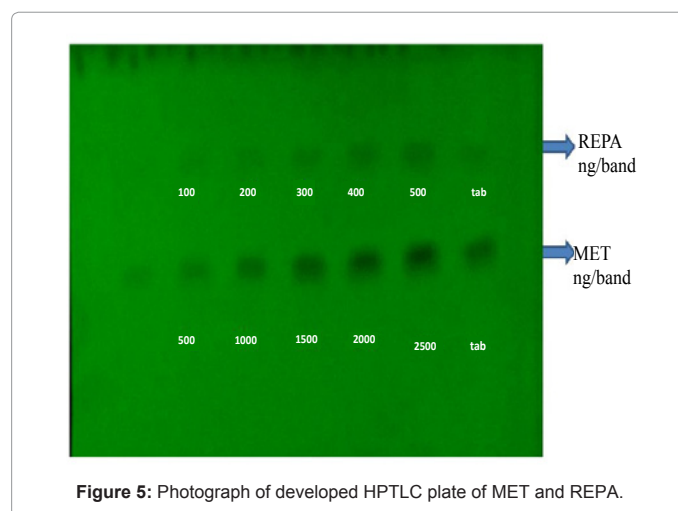
Figure 5: Photograph of developed HPTLC plate of MET and REPA.

Sample	Correlation of center and slope spectra	
	r (s, m)	r (m, e)
MET	0.998	0.996
MET in tablet formulation	0.996	0.998
REPA	0.999	0.997
REPA in tablet formulation	0.997	0.996

Table 1: Peak purity correlation results of MET and REPA in formulation at peak start, middle and end using PDA detector.

Amount of sample Taken (ng/band)		Amount of standard drug added (ng/band)		Standard addition (ng/band)	Amount of drug recovered (ng/band)		% recovery ± % RSD (n=3)	
MET	REPA	MET	REPA	REPA	MET	REPA	MET	REPA
500	2	0	0	100	744.27	101.12	101.76 ± 1.56	99.14 ± 0.91
500	2	250	1	100	744.27	101.3	99.23 ± 0.98	98.35 ± 1.17
500	2	500	2	100	1005.21	101.76	100.49 ± 1.29	97.85 ± 0.97
500	2	1000	3	100	1475.4	105.81	98.35 ± 1.37	100.78 ± 0.67

Table 2: Results from accuracy study.

Parameters	MET	REPA
Range (ng/band)	500-2500	100-500
Tailing factor	1.46	1.31
Detection limit (ng/band)	98	17
Quantitation limit (ng/band)	296	51
Accuracy (%)	98.35-101.76%	97.85-100.78%
Precision (% RSD)		
Intra-day (n=3)	0.16-0.33	0.89-1.96
Inter-day (n=3)	0.14-0.50	0.63-1.76
Instrument precision (%RSD)	0.16	0.89
Specificity	Specific	Specific

Table 3: Summary of validation parameters of developed HPTLC method.

Parameter	Deliberate changes	%Rsd of peak area	
		MET	REPA
Chamber saturation time(± 20%)	24 min	2.12	2.39
	36 min	1.06	0.69
Development distance from spot application (± 10%)	7.7 cm	2.69	1.94
	6.3 cm	1.56	1.89

Table 4: Results from the robustness study of method.

Formulation	Label claim (mg)		% of label claim (n=5) ± % RSD (n=5)	
	MET	REPA	MET	REPA
EUREPA MF	500	2	99.19 ± 1.02	98.77 ± 0.63

n=number of determinations

Table 5: Results from analysis of MET and REPA in the combined tablet dosage form.

Limits of detection (LOD) and Limits of quantification (LOQ): Under the experimental conditions used, the lowest amount of drug that could be detected (LOD) for MET and REPA were found to be 98 and 17 ng/band respectively. The limit of quantification (LOQ) for MET and REPA were found to be 296 and 51 ng/band respectively LOD and LOQ were calculated using following equation as per ICH guidelines. LOD=3.3×σ/S; LOQ=10×σ/S; Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve (Table 3).

Robustness: The robustness study was carried out by introducing small but deliberate change in experimental condition.

Robustness: Acceptable %RSD values obtained after making small deliberate changes in the developed HPLC method indicates that the method is robust for the intended purpose (Table 4).

Method application

Marketed formulation was analyzed using proposed method which gave percentage recovery for MET and REPA were 99.19 ± 0.64 and 98.77 ± 0.78 respectively of EUREPA MF (Table 5). A single band at R_f 0.34 ± 0.002 and 0.60 ± 0.01 was observed in the chromatogram for MET and REPA, and no interference from the excipients present in the marketed tablet formulation was observed.

Conclusions

This developed and validated method for simultaneous analysis of MET and REPA in pharmaceutical preparations is very rapid, accurate, and precise. The method was successfully applied for determination of MET and REPA in its pharmaceutical tablet formulations. Moreover it has advantages of short run time and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence this method can be conveniently used for routine quality control analysis of MET and REPA in its pharmaceutical formulations.

Acknowledgment

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