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Research Article

Simultaneous Estimation of Metformin Hydrochloride and Alpha Lipoic Acid by HPTLC Method in Tablet Dosage Form

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ABSTRACT

High Performance Thin Layer Chromatographic Method has been developed to quantify Metformin Hydrochloride and Alpha Lipoic Acid. Separation of both the drugs was carried out by using silica gel 60F254 plates. The mobile phase comprised of Toluene, Ammonium Acetate (4%), Ethyl Acetate (5:4:1 v/v/v). The detection wavelength was found to be 227 nm. The Rf values of Metformin Hydrochloride and Alpha Lipoic Acid were found to be 0.28 and 0.65 respectively. The method was linear over concentration range 1500-7500 ng /band for Metformin Hydrochloride and 600-3000 ng / band for Alpha Lipoic Acid. The developed method was validated according to ICH guidelines. Linearity, regression value, recovery and %RSD of Intraday and interlay precision values were found within the limits and the method was found to be satisfactory. The developed HPTLC method was found to be simple, accurate and precise.

Keywords: Metformin Hydrochloride; Alpha Lipoic Acid; HPTLC; Validation

INTRODUCTION

Metformin Hydrochloride (MET) is chemically, 1-carbamimidamido-N-N-dimethylmethanimidamide Figure 1 [1]. It is an oral anti-diabetic drug from the biguanide class [2]. It is the first-line drug for the treatment of type-2 diabetes, particularly in overweight and obese people and those with normal kidney function and evidence suggest it may be the best choice for the people with heart failure. The major action of MET is increasing glucose transport across the cell membrane in skeletal muscle [3].

Alpha lipoic acid (ALA) is chemically 5-[(3R)-dithiolan-3-yl] pentanoic acid is an antioxidant drug [4]. Lipoic Acid is generally involved in oxidative decarboxylation's of keto acids and is presented as a growth factor for some organisms. Lipoic acid exists as two enantiomers, the R-enantiomer and the S-enantiomer. Normally only the R-enantiomer of an amino acid is biologically active, but for lipoic acid the S-enantiomer assists in the reduction of the R-enantiomer when a racemic mixture is given. Some recent studies have suggested that the S-enantiomer in fact has an inhibiting effect on the R-enantiomer, reducing its biological activity substantially and actually adding to oxidative stress rather than reducing it. Furthermore, the S-enantiomer has been found to reduce the expression of GLUT-4s in cells, responsible for glucose uptake, and hence reduce insulin sensitivity [5,6].

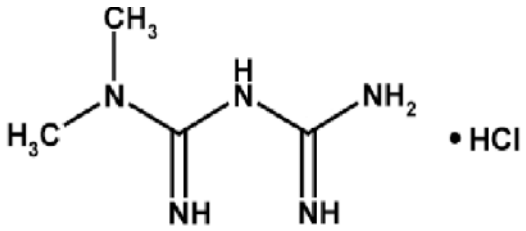

Figure 1: Metformin Hydrochloride.

Various methods had been developed for individual analysis of metformin HCl, including HPLC, HPTLC and spectrophotometry [7-9]. Alpha Lipoic Acid also had been subjected to different methods of analysis including HPLC and spectrophotometry [10,11]. Literature review also reveals that HPLC and spectrophotometry methods have been reported for the determination of mixed Metformin HCl and Alpha Lipoic Acid in tablet dosage form by HPLC and spectrophotometry [12,13]. Literature survey does not reveal any HPTLC method for the determination of mixed Metformin HCl and Alpha Lipoic Acid in tablet dosage forms. The present developed HPTLC method is simple, precise and accurate for determination of both drugs in tablet dosage forms as per the good validation requirements.

The principle of separation is adsorption [14]. One or more

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compounds are spotted on thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action (against gravitational force). The components move according to their affinities towards the adsorbent Figure 2. The component with more affinity towards the stationary phase travels faster. Thus, the components are separated to a thin layer chromatographic plate based on the affinity of the components towards the stationary phase [15].

METHODS AND MATERIALS

Apparatus and Instruments

A Camag HPTLC system comprising of Linnomet 5 (Camag) sampler, Camag twin through chamber, Camag TLC scanner, UV cabinet and stationary phase pre coated with silica gel 60F254 were used. TLC aluminium plates pre-coated with silica gel 60F254 (10 x 10 cm) were from Merck. Samples were applied on the TLC plates using the spray on technique under nitrogen gas flow, and developed in a Camag 10 x 10 cm twin trough chambers Figure 3.

Materials and Reagents

Metformin Hydrochloride and Alpha Lipoic Acid reference standards were obtained from Aarti Drugs and Sami Labs Limited respectively. Tablets containing these two compounds, (MET and ALA) were brought from retail pharmacy in Vapi (Gujarat, India).

Chromatographic Conditions

The chromatographic estimation was performed using following conditions:

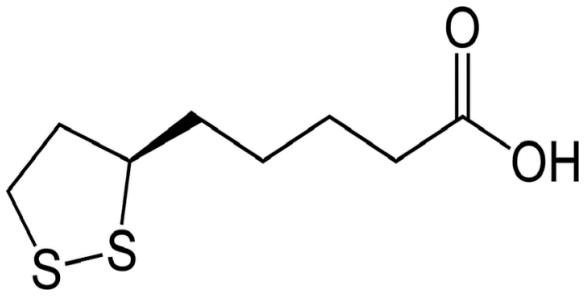

Figure 2: Alpha Lipoic Acid.

- Mobile phase: Toluene, Ammonium Acetate (4%), Ethyl Acetate (5:4:1 v/v/v)
- Chamber saturation time: 20 min
- Ambient temperature: 25-26°C
- Wavelength of detection: 227 nm
- Slit dimensions: 6 x 0.45 mm (micro)
- Syringe capacity: 100 µl

Method

Preparation of ammonium acetate solution: Ammonium Acetate solution (4%) was prepared by accurately weighing 4 gm of Ammonium Acetate and dissolving in methanol and diluted up to 100 ml with methanol in the volumetric flask Figure 4.

Preparation of mobile phase: A mixture of 5 ml Toluene, 4 ml Ammonium Acetate and 1 ml Ethyl Acetate were mixed properly and it was used as a mobile phase Table 1.

Preparation of working standard stock solution of MET and ALA: A mixed standard stock solution of MET (3000µg/ml) and ALA (1200 µg/ml) were prepared by accurately weighing MET (30 mg) and ALA (12 mg) and dissolving in methanol and diluted to 10 ml with methanol in the same volumetric flask.

Preparation of sample solution: Aliquots of 1 ml from stock solution of MET (3000 µg/ml) andALA (1200 µg/ml) were taken into common volumetric flask and diluted upto 10 ml with methanol to make final concentration MET (300 µg/ml) and ALA (120 µg/ml).

Table 1: Linearity data of MET at 227 nm.

Sr.no	Concentration(ng/band)	Peak area Mean ± SD (n=5)	% RSD
1	1500	9553 ± 30.2729	0.3168
2	3000	12662 ± 36.8381	0.2909
3	4500	15134 ± 77.3464	0.5110
4	6000	17627 ± 46.6904	0.2648
5	7500	19993 ± 121.00	0.6052

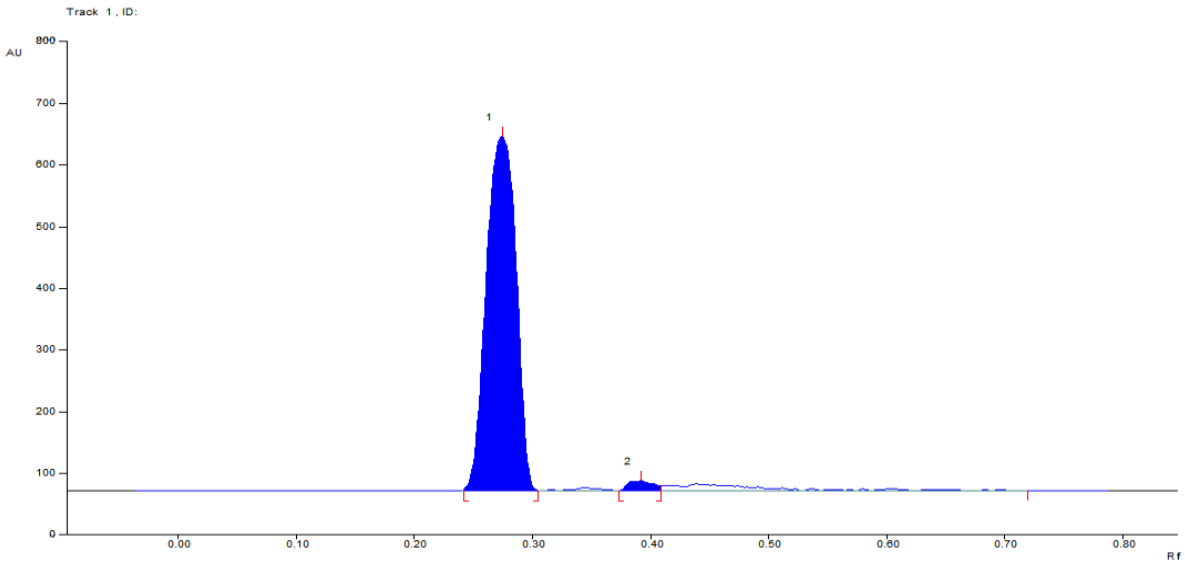

Figure 3: HPTLC Chromatogram of MET (Rf value 0.28).

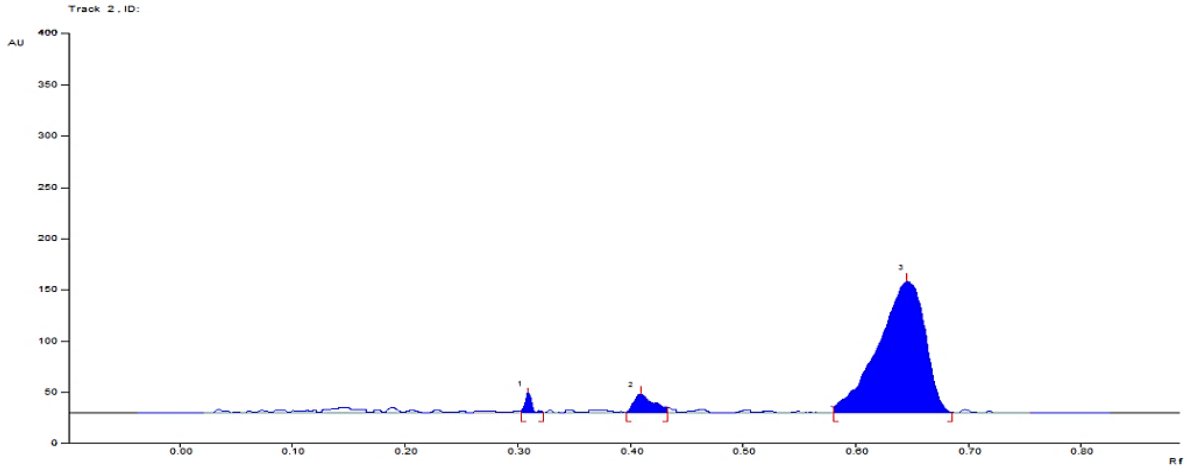

Figure 4: HPTLC Chromatogram of ALA (Rf value 0.65).

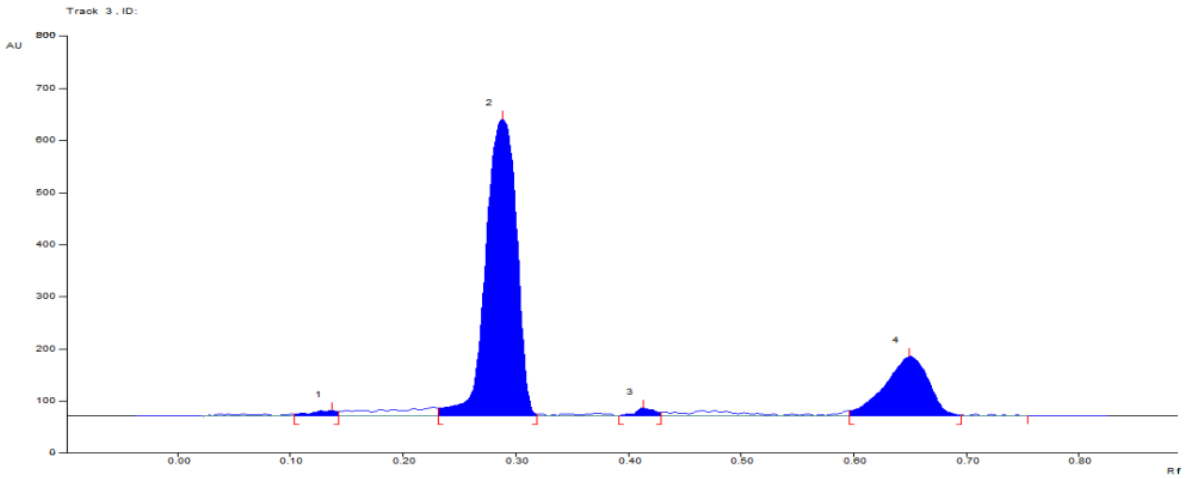

Figure 5: Chromatogram of MET and ALA at 227 nm (3000:1200 ng/Band).

Determination of analytical wavelength: The solution of MET and ALA were applied to silica gel 60F254 HPTLC plates by means of applicator under the chromatographic condition mentioned above. The plate was developed in a twin-trough chamber previously saturated for 20 min with the mobile phase. HPTLC plate was dried by hair drier. Scanning was performed in the reflectance-absorption mode using a UV detector in the range of 200-700 nm. Both components showed reasonably good response at 227 nm. So, they were detected at this analytical wavelength Table 2.

METHOD VALIDATION

Linearity

Calibration curves were plotted over the concentration range of 1500 – 7500 ng/band and 600-3000 ng/band for MET and ALA respectively. Accurately prepared mixed standard solutions ofMET and ALA (5, 10, 15, 20, and 25 µl) were applied to the plate. The calibration curves were constructed by plotting peak areas Vs concentrations [16].

Precision

Repeatability: Repeatability of the developed method was assessed by analysing samples from the same batch 6 times with standard solutions containing concentrations 4500 ng/band for MET and 1800 ng/band for ALA and % R.S.D. was calculated Figure 5.

Table 2: Linearity data of ALA at 227 nm.

Sr.no	Concentration (ng/ band)	Peak area Mean ± SD (n=5)	% RSD
1	600	1980 ± 13.0843	0.6608
2	1200	4466 ± 14.7737	0.3308
3	1800	6831 ± 19.5959	0.2868
4	2400	8736 ± 35.3553	0.4047
5	3000	11032 ± 33.7624	0.3060

Intraday precision: The intraday precision of the proposed method was determined by analyzing mixed standard solution of MET and ALA at 3 different concentrations (3000, 4500 and 6000 ng/bnad for MET; 1200, 1800 and 2400 ng/band for ALA) 3 times on the same day. The results are reported in terms of relative standard deviation (%RSD).

Interday precision: The interday precision of the proposed method was determined by analyzing mixed standard solution of MET and ALA at 3 different concentrations (3000, 4500 and 6000 ng/bnad for MET; 1200, 1800 and 2400 ng/band for ALA) 3 times on different days. The results are reported in terms of relative standard deviation (%RSD) Figure 6.

Accuracy (% Recovery): The accuracy of the method was determined by calculating recovery of MET and ALA by the standard addition method. Known amounts of mixed standard solutions of MET and ALA were added at 80, 100 and 120%

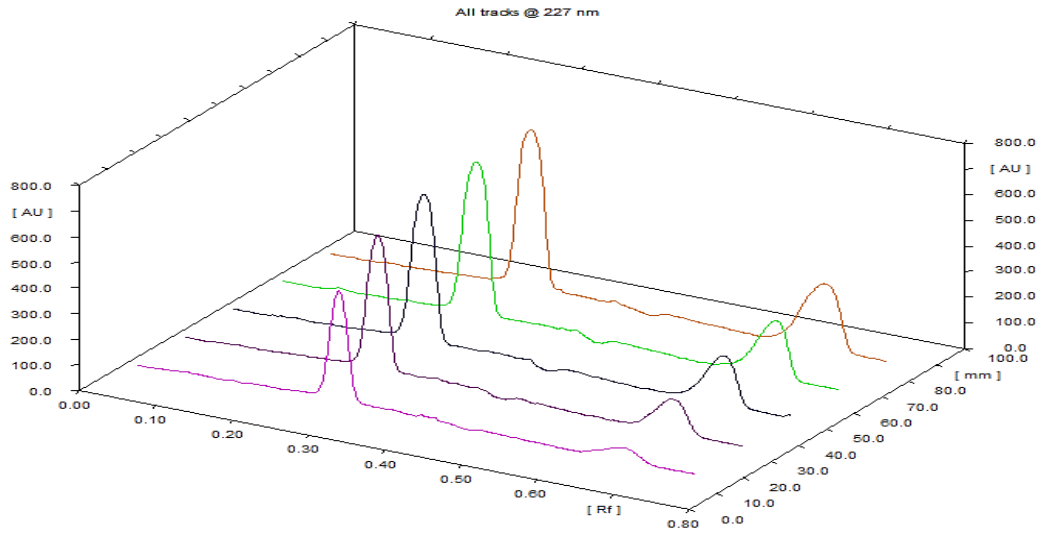

Figure 6: 3-D Chromatogram of MET and ALA at 227 nm.

levels to prequantified sample solutions of MET and ALA (3000 & 1200 ng/band respectively). The amounts of MET and ALA were estimated by using the regression equation of the calibration curve

Limit of detection and limit of quantification: LOD and LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times s/S$$

$$LOQ = 10 \times s/S$$

Where s = the standard deviation of the response

S = Slope of calibration curve

Specificity : The specificity of the method was ascertained by analysing standard drug and sample. The bands for MET and ALA in sample were confirmed by comparing the Rf values and UV spectra of the bands with those obtained from the standard. The peak purity of MET and ALA were assessed by comparing the spectra acquired at three different positions on the band, i.e. peak start (s), peak apex (m) and peak end (e).

Robustness: Robustness of the method was determined by subjecting the method to slight change in the method condition like,

Mobile Phase Ratio

Analysis of pharmaceutical formulation: The response of the sample solution was measured at 227 nm for the quantitation of MET and ALA in available marketed formulation. The amounts of the MET and ALA present in the sample solution were calculated by fitting the responses into the regression equation for MET and ALA in the proposed method.

RESULTS AND DISCUSSIONS

Several mobile phases were tried to accomplish good separation of MET and ALA. Using the mobile phase Toluene: Ammonium Acetate: Ethyl Acetate (5:4:1 v/v/v) and 10×10 cm HPTLC silica gel 60F254 aluminum plates, good separation was attained with retardation factor (Rf) values of 0.28 for MET and 0.658 for ALA. Wavelength 227 nm was used for the quantitation of the drugs. Resolution of the peaks with clear baseline separation was found.

METHOD VALIDATION

Linearity: Calibration data for 1500-7500 ng/band and 600-3000 ng/band of MET and ALA respectively are presented in Tables 1 and 2. A 3D chromatogram showing linearity of MET and ALA is shown in Figure 6. Calibration curves of peak area versus concentration were constructed Figures 7 & 8.

Precision

Repeatability: The data for repeatability for MET and ALA at 227 nm is shown in Table 3.

Intraday precision: The data for intraday precision for MET and ALA at 227nm is shown in Tables 4 & 5 respectively.

Interday precision: The data for Interday precision for MET and ALA at 227 nm is shown in Tables 6 & 7 respectively.

Accuracy: Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition.

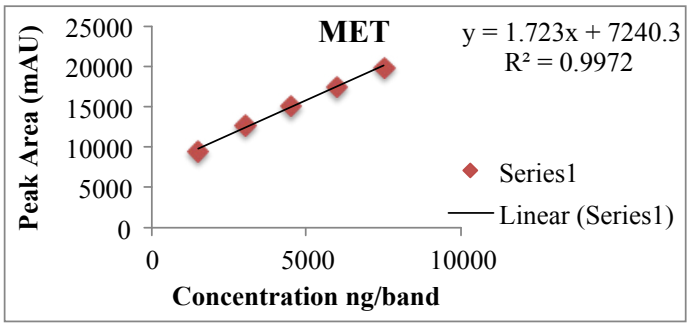

Figure 7: Calibration curve for MET at 227nm.

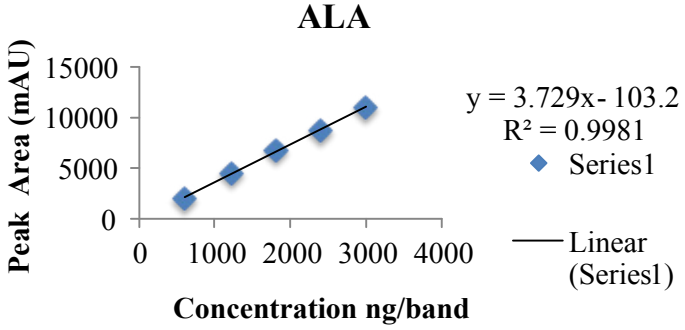

Figure 8: Calibration curve for ALA at 227nm.

Percentage recovery for MET was found to be in range of 99-100% and for ALA was found to be in range of 98-100%. The results are shown in Table 8.

Robustness: Small change in Mobile phase wasToluene: Ammonium Acetate: Ethyl Acetate. (5.8:4.2:0.1 v/v/v). Hence, the method was found to be robustness for the estimation of MET and ALA. The result shown in Table 9.

Specificity: Specificity is carried out by taking peak purity of standard and sample of each drug and standard and sample peak spectra were overlain to check specificity of each individual drug peak. The peak purity for Metformin and Alpha Lipoic acid was tested by correlation of spectra Tables 10 & 11.

Assay

Assay of Pharmaceutical formulation: Applicability of the proposed method was tested by analyzing the commercially available marketed formulation (METMIN-A) containing 500 mg of MET and 200 mg of ALA. The results are shown in Tables 12 & 13. The proposed validated method was successfully applied to the simultaneous determination of MET and ALA in Tablet dosage form. The assay results were comparable to labeled value of each drug in Solid dosage form. These results indicate that the developed method is simple, accurate and precise. It can be used in the routine quality control of dosage form in industries.

Table 3: Repeatability data of MET and ALA at 227 nm.

Drugs	Concentration (ng/ band)	Mean Peak Area ± S.D. (n=5)	% R.S.D.
MET	4500	15139 ± 90.5290	0.5979
ALA	1800	6827 ± 22.5610	0.3304

Table 4: Intraday precision data of MET at 227 nm.

Sr.no.	Conc. (ng/ band)	Mean Peak area. ± S.D. (n=3)	%R.S.D.
1.	3000	12646 ± 44.7101	0.3535
2.	4500	15127 ± 96.5349	0.6381
3.	6000	17646 ± 63.6631	0.3607

Table 5: Intraday precision data of ALA at 227 nm.

Sr.no.	Conc. (ng/band)	Mean Peak area. ± S.D. (n=3)	%R.S.D.
1.	1200	4451 ± 20.0748	0.4022
2.	1800	6859 ± 28.0535	0.7074
3.	2400	8758 ± 47.6235	0.5016

Table 6: Interday precision data of MET at 227 nm.

Sr.no.	Conc. (ng/ band)	Mean Peak area. ± S.D. (n=3)	%R.S.D.
1.	3000	12637 ± 50.292	0.4130
2.	4500	15111 ± 106.3344	0.7036
3.	6000	17667 ± 88.0681	0.4984

Table 7: Interday precision data of ALA at 227 nm.

Sr.no.	Conc. (ng/ band)	Mean Peak area. ± S.D. (n=3)	%R.S.D.
1.	1200	4480 ± 26.9629	0.6018
2.	1800	6862 ± 39.3954	0.5741
3.	2400	8776 ± 55.0181	0.6269

Table 8: Determination of Accuracy for MET and ALA.

Drug	Level	Amount of sample takenng/band	Amount. of standard spikedng/band	Total Amount	Mean Peak Area ± S.D. (n=3)	Amount of sample found ng/band	% Recovery
MET	0%	3000	0	3000	12465 ± 41.7080	3032.32	101.07
	80%	3000	2400	5400	16458 ± 37.7226	5349.79	99.07
	100%	3000	3000	6000	17656 ± 32.7871	6045.09	100.75
	120%	3000	3600	6600	17554 ± 40.5832	6566.27	99.48
ALA	0%	1200	0	1200	4382 ± 20.7766	1202.78	100.23
	80%	1200	960	2160	7942 ± 37.6876	2157.46	99.88
	100%	1200	1200	2400	8749 ± 42.1544	2373.88	98.91
	120%	1200	1440	2640	9656 ± 3695	2617.1	99.13

Table 9: Robustness data of MET and ALA.

Parameters	Mobile Phase(± 0.5)	Mean Peak Area ± S.D. (n=3)	% R.S.D	Rf ± S.D. (n=3)	% R.S.D
MET4500 ng/band	5.5:3.5:1 v/v/v	15010 ± 23.5159	0.1566	0.278 ± 0.0010	0.3597
	4.5:4.5:1 v/v/v	15267 ± 18.0831	0.1184	0.289 ± 0.0020	0.6944
ALA1800 ng/band	5.5:3.5:1 v/v/v	6775 ± 17.6918	0.2611	0.656 ± 0.0020	0.3170
	4.5:4.5:1 v/v/v	6865 ± 11.7898	0.1717	0.66 ± 0.0026	0.3954

Table 10: Specificity data for MET.

Sr. No.		r(s,m)	r(m,e)
1	Standard	0.999	0.999
2	Sample	0.999	0.999

Table 11: Specificity data for ALA.

Sr. No.		r(s,m)	r(m,e)
1	Standard	0.999	0.999
2	Sample	0.999	0.999

Table 12: Analysis of marketed formulation.

Formulation (Tablet)	Actual concentrationng/band		Area		% MET ±SD(n=3)	% ALA ±SD(n=3)
	MET	ALA	MET	ALA		
METMIN-A (MET 500 mg + ALA 200 mg)	4500	1800	4485.18	1778.54	99.52 ± 0.2986	98.79 ± 0.3085

Table 13: Optical Characteristic and Validation Parameter of MET and ALA.

Parameter	HPTLC METHOD	
	MET	ALA
Detection wavelength(nm)	227	227
Linearity (n=5)	1500-7500	600-3000
Regression equation	y = 1.723x + 7240.3	3.729x – 103.2
Slope (m)	1.723	3.729
Intercept (c)	7240.3	103.2
Regression Co-efficient (R^2)	0.9972	0.9981
Correlation Coefficient (r)	0.9985	0.9990
Repeatability (n=6) (% RSD)	0.5979	0.3304
Intraday precision (n=3)(% RSD)	0.3532 – 0.6381	0.4090 – 0.5437
Interday precision (n=3)(% RSD)	0.3977-0.7036	0.5744-0.6269
LOD (n=5)	23.53	12.94
LOQ (n=5)	71.30	39.23
% Recovery (n=3)	99.07 – 101.07	98.91 – 100.23
% Assay ± S.D. (n = 3)	99.52% ± 0.2986	98.79% ± 0.3085

CONCLUSION

The HPTLC method was developed and validated as per ICH guidelines. This validated method was new, reproducible, accurate and precise. This method gives good resolution between two compounds. The standard deviation and %RSD calculated for the proposed method are within limits, indicating high degree of precision of the method. The proposed HPTLC method can be useful for routine analysis of metformin hydrochloride and Alpha Lipoic Acid in Tablet dosage form.

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