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Development and Validation of RP-HPLC Method for Simultaneous Estimation of A- Lipoic Acid and Metformin in their Tablet Dosage Form

Patel PK[1]*, Patel GH[2], Modha NB[3] and Upadhyay UM[4]

[1,2,3,4] Sigma Institute of Pharmacy, Bakrol, Vadodara, Gujarat, India

Abstract

The combination of Alpha- Lipoic Acid and Metformin HCl is used in treatment of Diabetes. The present work involves the development and validation of RP-HPLC method for the estimation of α- Lipoic Acid (ALA) and Metformin HCl (MET) in tablet dosage form. A specific, precise and selective RP-HPLC method was developed and validated for the estimation of α- Lipoic Acid and Metformin HCl in Tablet dosage form using Zodiac C18 (150mm x 4.6mm i.d., 5µm). Phosphate buffer: Acetonitrile (60:40) was selected as mobile phase. Flow rate was selected as 1.0 ml/min and detection was carried out at 220 nm. The retention time of α- Lipoic Acid and Metformin HCl was 8.983 min and 2.003 min was obtained respectively. The linearity range was found to be 2-12µg/ml and 150-500 µg/ml for α- Lipoic Acid and Metformin HCl respectively. The Correlation Coefficient r^2= 0.9984 and r^2= 0.9989 was obtained for α- Lipoic Acid and Metformin HCl respectively. The mean recovery was found to be 99.20±1.14 and 99.28± 0.70 for α- Lipoic Acid and Metformin HCl respectively. The developed method was economic, precise, specific and validated as per ICH Guidelines.

Keywords

α- Lipoic Acid, Metformin HCl, RP-HPLC Method,Validation

Greentree Group

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INTRODUCTION

Alpha Lipoic acid is Chemically 5-(1,2-dithiolan-3-yl) pentanoic acid (Fig. 1). It is a universal antioxidant present naturally in all prokaryotic and eukaryotic cells[1-2].It is used in combination with Metformin HCl to manage type-2 diabetes.

Fig 1 Chemical Structure of ALA

Metformin HCl is chemically 1-carbamimidamido-N,N-dimethylmethanimidamide (Fig.2). It is an anti-hyperglycemic drug used to manage type-2 diabetes[3-5].It is a unique and widely used anti hyper glycemic drug throughout the world.

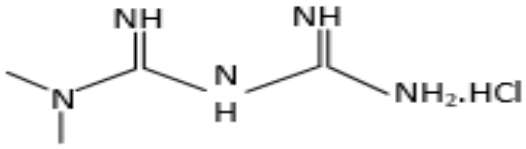

Fig 2 Chemical Structure of MET

The combination of Alpha- Lipoic Acid and Metformin HCl is used in treatment of Diabetes. Literature survey shows that there was no method has been found for the estimation of Alpha Lipoic Acid and Metformin in combination till date. Thepresent work involves the development of simple, precise and accurate UV Spectrophotometry and RP-HPLC method for simultaneous estimation of α- Lipoic Acid (ALA) and Metformin HCl (MET) in their combined dosage form. Developed analytical method was then validated by using various validation parameters as per ICH guidelines and assay of marketed drug was performed for comparison study.

EXPERIMENTAL WORK[6-10]

Materials and Reagents

Pure drug sample of Alpha Lipoic Acid was gifted by Jenburkt pharmaceutical ltd., Bhavnagar and pure drug sample of Metformin HCl was gifted by Captab Laboratory, Vadodara.

Table 1 Material's and Reagents

Materials and Reagents	Grade	Manufacturer
Alpha Lipoic Acid	-	Jenburkt Pharmaceuticals LTD, Bhavnagar
Metformin HCL	-	Kaptab Pharmaceuticals, Vadodara
Acetonitrile	HPLC	Merch laboratories, New Delhi
Methanol	HPLC	Merch laboratories, New Delhi
Ortho phosphoric acid	HPLC	Merch laboratories, New Delhi

Selection of diluent and mobile phase:

Alpha Lipoic Acid and Metformin were freely soluble in Methanol, Acetonitrile, Phosphate Buffer and Water. The mobile phase for reverse phase chromatography was selected in such a way to achieve the proper peak shape with sufficient height, theoretical plates, resolution and purity. Phosphate Buffer and Acetonitrile were selected as a mobile phase due to sharp peak with sufficient height and good resolution in the ratio of 60:40 % v/v.

Preparation of standard solutions

MET stock solution:

Accurately weight 50 mg MET was taken in 100 ml volumetric flask and volume made up with solvent which make 500µg/ml.Appropriate aliquots of standard stock solution of MET (0.5, 0.75, 1.0, 1.25 and 1.5ml) were taken in different 10ml volumetric flask and volume were made upto the mark to obtained the final concentrations of MET as 25, 37.5, 50, 62.5, and 75 µg/ml respectively.

ALA stock solution:

Accurately weight 20 mg ALA was taken in 100 ml volumetric flask and volume made up with solvent. Appropriate aliquots of standard stock solution of ALA (0.5, 0.75, 1.0, 1.25 and 1.5ml) were taken in different 10ml volumetric flask and volume were made up to the mark to obtained the final concentrations of MET as 10, 15, 20, 25, and 30 µg/ml respectively.

Mixed standard solution:

From both the stock solutions 1ml from each transferred to 10 ml volumetric flask and made up to mark with solvent for preparation of mixture.

Selection of detection wavelength

A wavelength which gives good response for the drugs is to be selected. For that standard solution of ALA (200 mcg/ml) and MET (500 mcg/ml) were scanned over the range of 200 to 400nm. Two drugs detection were carried out at different wavelength maxima. Both the drugs shows good absorbance and higher isobestic point at 240nm and hence it was selected for detection wavelength (Fig. 3).

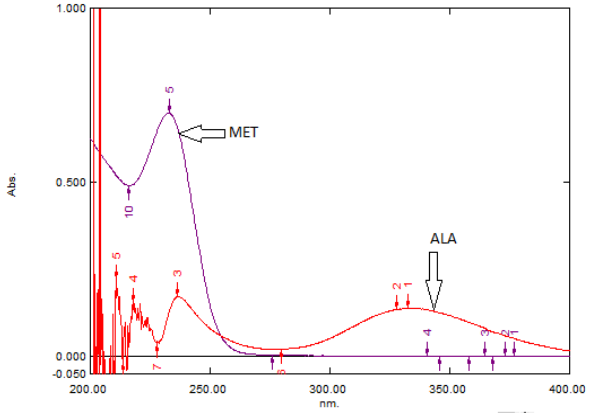

Figure 3 Overlaid spectra of MET (50 µg/ml) and (ALA 20 µg/ml) in Methanol

VALIDATION OF RP-HPLC METHOD[11-12]

Linearity and Range

Chromatogram of concentration ranges from 2-12 µg/ml of MET and 150-500µg/ml of ALA taken for calibration curve. Peak area (Voltage)vs concentration was plotted. The correlation co-efficient and regression equation were determined.

Precision

Repeatability: For repeatability, six replicates of standard mixture solution having MET (50µg/ml) and ALA (20µg/ml) were prepared. Peak area analyzed were recorded and %RSD was calculated. The experiment was repeated three times in a day for intraday and on three different days for Interday precision.

Accuracy

Accuracy was determined by performing recovery studies by spiking different concentrations of pure drug in pre analyzed sample solution of 25µg/ml of MET and 10µg/ml of ALA. To pre-analyzed sample solution, a known amount of standard stock solutions were added which was at different level 80, 100 and 120 %. The solutions were analyzed by proposed method. Mean % recovery was calculated.

LOD and LOQ

Calibration curves were repeated 5 times and standard deviation of intercept was calculated. Then LOD and LOQ were measured as per equations given here.

LOD= 3.3 (SD/Slope of calibration curve)

LOQ= 10 (SD/Slope of calibration curve)

SD = Standard deviation of intercept

Robustness

Robustness was performed using minute positive and negative change in various parameters like flow rate, pH and Mobile phase composition. Average of three reading was considered for calculation of % RSD.

Applicability of Method: Analysis of marketed formulation

Pharmaceutical formulation contains MET500 mg and ALA200 mg as tablet equivalentto 50mg MET powder of crushed 20 tablets (equivalent to 50µ of MET and 20µ of ALA) was taken in 100 ml volumetric flask and was diluted up to mark with mobile phase to get MET (50 µg/ml) and ALA (20 µg/ml).

RESULT AND DISCUSSION OF RP-HPLC METHOD

Linearity and Range:

Chromatogram of Concentrationranging from 2-12 µg/ml of MET and 150-500µg/ml of ALA taken for calibration curve. Peak

area (voltage)vs concentration was plotted. The correlation co-efficient and regression equation were determined.

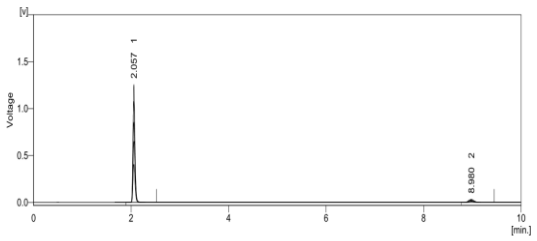

Figure 4 Overlay Chromatogram of MET and ALA (2D)

Table 2 Linearity data of MET

Concentration (µg/ml)	Peak Area (Voltage)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Avg Peak Area (V, n=5)
25	1148.96	1132.62	1189.22	1248.23	1146.29	1173.064
37.5	1844.97	1826.88	1898.56	1912.92	1821.36	1860.938
50	2414.06	2389.55	2369.59	2426.98	2367.29	2393.494
62.5	3035.47	2922.21	2981.85	3036.25	2982.69	2991.694
75	3544.45	3489.81	3569.72	3609.22	3545.37	3551.714

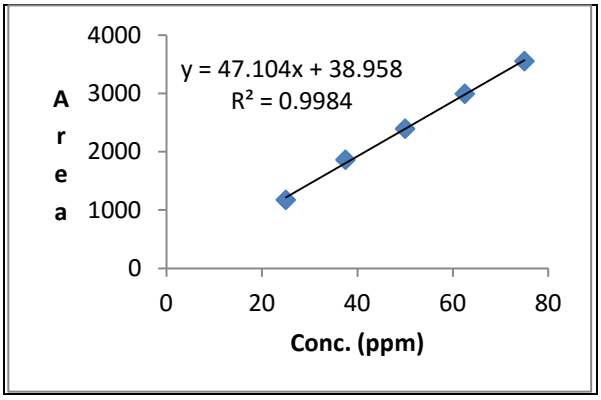

Figure 5 Calibration curve of MET

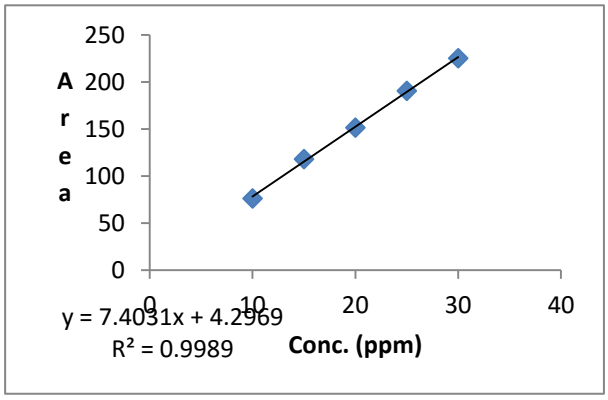

Figure 6 Calibration curve of ALA

Table 3 Linearity data of ALA

Concentration (µg/ml)	Peak Area(Voltage)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Avg Peak Area (V, n=5)
10	74.689	81.587	70.59	73.27	81.57	76.3412
15	119.988	125.295	116.58	103.58	125.29	118.1466
20	157.219	159.646	154.72	126.58	159.46	151.525
25	197.278	201.445	193.256	159.47	201.45	190.5798
30	230.86	239.489	223.552	192.22	239.89	225.2022

Table 4 Regression Analysis data for proposed method

Parameters	MET	ALA
Beer's law Limit (µg/ml)	2-12	150-500
Regression equation (y = mx + c)	y = 47.104x + 38.958	y = 7.4031x + 4.2969
Correlation co-efficient (r^2)	0.9984	0.9989
Slope (m)	47.104	7.4031
Intercept (c)	38.958	4.2969

Repeatability of MET & ALA

Table 5 Repeatability of MET & ALA

Drug	Sr. No.	Peak Area	Concentration (µg/ml)	RSD (%)
MET **(50µg/ml)**	1	2404.952	50	0.576
	2	2419.389	50	
	3	2395.241	50	
	4	2409.617	50	
	5	2436.128	50	
	6	2414.244	50	
ALA **(20µg/ml)**	1	156.638	20	0.519
	2	157.588	20	
	3	156.007	20	
	4	156.957	20	
	5	155.408	20	
	6	157.266	20	

Discussion: Linearity range, Correlation Coefficient and regression equations were found to be satisfactory for both drugs.

Precision

For repeatability, six replicates of standard mixture solution having MET (50µg/ml) and ALA (20µg/ml) were prepared analyzed peak area were recorded and %RSD calculated. The experiment was repeated three times in a day for intra-day and on three different days for inter-day precision.

Table 6 Intra-day Precision of MET

Drug	Concentration (µg/ml)	Peak Area (voltage)	Concentration Found (µg/ml)	Mean (µg/ml)	SD	RSD (%)
MET	25	1211.169	24.6	25.0	7.594	0.631
		1199.076	25.1			
		1197.164	25.2			
	50	2392.503	50.3	50.7	9.175	0.384
		2390.528	50.9			
		2375.716	51.0			
	75	3612.729	76.0	76.3	27.034	0.751
		3609.072	76.3			
		3564.182	76.5			

Table 7 Intra-day Precision of ALA

Drug	Concentration (µg/ml)	Peak Area (Voltage)	Concentration Found (µg/ml)	Mean (µg/ml)	SD	RSD (%)
ALA	10	78.738	10.55	10.27	0.476	0.609
		77.956	10.25			
		77.875	10.61			
	20	155.813	20.03	20.92	0.581	0.375
		154.924	19.56			
		154.718	20.15			
	30	235.306	30.40	30.41	1.292	0.551
		235.075	30.24			
		232.96	30.59			

Table 8 Inter-day Precision of MET

Drug	Concentration (µg/ml)	Peak Area (Voltage)	Concentration Found (µg/ml)	Mean (µg/ml)	SD	RSD (%)
MET	**25**	**1212.389**	**24.6**	**24.9**	**13.686**	**1.142**
		1196.644	**25.1**			
		1185.124	**25.1**			
	50	**2397.276**	**50.2**	**50.6**	**22.366**	**0.940**
		2381.652	**50.9**			
		2353.163	**50.8**			
	75	**3619.889**	**75.5**	**76.0**	**26.579**	**0.739**
		3590.825	**76.4**			
		3566.809	**76.2**			

Table 9 Inter-day Precision of ALA

Drug	Concentration (µg/ml)	Peak Area (Voltage)	Concentration Found (µg/ml)	Mean (µg/ml)	SD	RSD (%)
MET	**10**	**78.817**	**10.56**	**10.49**	**0.726**	**0.930**
		77.799	**10.34**			
		77.411	**10.56**			
	20	**156.128**	**20.05**	**20.90**	**1.607**	**1.038**
		155.126	**20.60**			
		152.982	**20.15**			
	30	**235.769**	**30.44**	**30.37**	**2.243**	**0.960**
		233.871	**30.22**			
		231.299	**30.44**			

Discussion: The %RSD for repeatability study was found to be 0.93 and 0.97 respectively for MET and ALA. The % RSD for intraday precision and interday precision were found to be less than 2% for both drugs. The limit for %RSD is NMT 2%.The values confirm that the method is precise.

Accuracy: Recovery study of MET

Concentration of Preanalysed sample taken was 25µg/ml and Concentration of Preanalysed sample was found to be 25.1µg/ml

Table 10 Recovery study of MET

Spike (%)	Amount of Std. spiked (µg/ml)	Total Conc. (µg/ml)	Total Amount found (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	Mean Recovery ± SD. (%, n=3)	RSD (%)
80	**25**	**20**	**45**	**20.11**	**100.58**	**100.94 + 0.64**	**0.6385**
			45	**20.33**	**101.69**		
			45	**20.11**	**100.56**		
100	**25**	**25**	**50**	**25.12**	**100.50**	**99.20 + 1.14**	**1.1525**
			50	**24.69**	**98.79**		
			50	**24.58**	**98.33**		
120	25	30	55	30.13	100.45	100.85 ± 0.59	0.5939
			55	30.46	101.54		
			55	30.17	100.57		

Recovery study of ALA

Concentration of Preanalysed sample taken was 10µg/ml and Concentration of Preanalysed sample was found to be 10.47µg/ml

Table 11 Recovery study of ALA

Spike (%)	Amount of Std. spiked (µg/ml)	Total Conc. (µg/ml)	Total Amount found (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	Mean Recovery ± SD. (%, n=3)	RSD (%)
80	**10**	**8**	**18**	**8.05**	**100.71**	**100.37 + 0.64**	**0.64**
			18	**8.06**	**100.77**		
			18	**7.97**	**99.62**		
100	**10**	**10**	**20**	**9.97**	**99.73**	**99.28 + 0.70**	**0.70**
			20	**9.96**	**99.64**		
			20	**9.84**	**98.47**		
120	**10**	**12**	**22**	**12.06**	**100.57**	**100.73 + 1.01**	**1.0**
			22	11.97	99.81		
			22	12.21	101.82		

Discussion: The results of recovery study were found within acceptance criteria. %RSD values are less than 2%. Result shows method is accurate.

LOD and LOQ:

LOD and LOQ values calculated using five calibration curves were given in Table for both drugs.

Table 12 LOD and LOQ values of MET and ALA

Parameter	MET	ALA
SD of Y-intercept	48.12	5.77
Mean of slope	98.134	6.16
LOD (µg/ml)	1.168	3.09

LOQ (µg/ml)	4.90	9.36

Robustness

Robustness was performed by changing ±0.2 in flow rate, ±0.2pH and ±2mobile phase composition. Each case repeated three times for both drugs.

Table 13 Robustness Evaluation of Method: MET (50µg/ml)

Parameter	Variation	Level of Variation	Average Peak Area (n = 3)	RSD (%)	System Suitability Parameters	
					Retention Time (min)	Tailing Factor
Flow rate (ml/min)	0.8	-0.2	2493.094	0.20	2.141	1.39
	1	0	2386.249	0.38	2.043	1.36
	1.2	+0.2	2295.659	0.26	1.976	1.27
pH of water	6.7	-0.2	2559.941	0.17	2.06	1.40
	6.9	0	2377.364	0.94	2.047	1.27
	7.1	+0.2	2214.808	0.34	2.054	1.34
Organic Content of Mobile Phase{ Phosphate Buffer:ACN }	60:40	-2	2336.604	0.66	2.202	1.31
	62:38	0	2379.109	0.43	2.060	1.364
	58:42	+2	2437.092	0.20	1.913	1.33

Table 14 Robustness Evaluation of Method: ALA (20µg/ml)

Parameter	Variation	Level of Variation	Average Peak Area (n = 3)	RSD (%)	System Suitability Parameters	
					Retention Time (min)	Tailing Factor
Flow rate (ml/min)	0.8	-0.2	161.24	1.37	9.341	1.28
	1	0	155.15	0.37	8.953	1.29
	1.2	+0.2	149.34	0.26	8.615	1.32
pH of water	6.7	-0.2	152.45	0.29	8.987	1.29
	6.9	0	154.74	1.03	8.980	1.34
	7.1	+0.2	157.74	0.50	8.957	1.27
Organic Content of Mobile Phase{Phosphate Buffer:ACN}	60:40	-2	166.30	0.41	9.603	1.30
	62:38	0	155.33	0.70	8.970	1.36
	58:42	+2	144.25	1.13	8.342	1.29

Applicability of the method

Applicability of proposed method was tested by analyzing marketed formulation. Acceptance limit for assay of eye drop formulation is within limit with low SD justified the assay of method.

Table 15 Analysis of Marketed formulation

Drug	Label	Conc.	Conc.	Conc.	Assay ± SD

	claim (%)	taken for Assay (µg/ml)	found for Assay (µg/ml)	found from Mixture as per labe1 claim (%)	(%, n=3)
MET	500	500	495.57	99.11	99.11 + 0.30
ALA	200	200	200.68	100.34	100.34 +0.82

Discussion: Both Drugs are found to be in acceptance limit so proposed method can be used for simultaneous estimation of MET and ALA.

CONCLUSION

The developed RP-HPLC Method for simultaneous estimation of MET and ALA is simple, precise, accurate and reproducible. The developed method was validated as per ICH guidelines Q2 (R1). Hence this method can be used for the simultaneous estimation of MET and ALA in routine analysis of their tablet dosage form.

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