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Novel Analytical Method Development and Validation for Simultaneous Estimation of Metformin Alpha Lipoic Acid in Bulk and Pharmaceutical Dosage Form by UV Spectrometric Method

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ABSTRACT: A rapid, simple, selective and precise UV- Visible Spectrophotometric simultaneous method (Vierodt's Method) has been developed for the determination of metformin hydrochloride (MFH) and alphalipoic acid (ALA) in bulk forms and solid dosage formulations. The spectrophotometric detection was as per carried out at an absorption maximum of 232 nm and 334nm for MFH and ALA respectively using phosphate buffer of pH 8 as solvent. The method was validated for specificity, linearity, accuracy, precision, robustness and ruggedness. The detector response for the MFH was linear over the selected concentration range 2 to 12µg/ml with a correlation coefficient of 0.993 and 100-500µg/ml with a correlation coefficient of 0.998. The accuracy was carried out as per recovery study and found between 99.1 % to 100.45% and 99.8% to 101.60% for MFH and ALA respectively. The results demonstrated that the excipients in the tablets did not interfere with the method and can be conveniently employed for routine quality control analysis of MFA and ALA in bulk and formulation.

Keywords: UV Spectroscopy; Method Development; Validation; Alpha lipoic acid; Metformin and Simultaneous estimation.

INTRODUCTION: Analysis is an important component in the formulation development of any drug molecule.[1-5] A suitable and validated method has to be available for the analysis of drug in the bulk, in drug delivery systems, from release dissolution studies and in biological samples. If a suitable method, for specific need, is not available then it becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug samples.[6-9] The efficient analytical method development and its validation are critical elements in the development of pharmaceuticals. An analytical method is selected on the basis of criteria such as accuracy, precision, sensitivity, selectivity, robustness, ruggedness, and the amount of available sample, the amount of analyte in the sample, time, cost, and availability of equipment.[10-16] Alpha lipoic acid (ALA) was first isolated by Reed and coworkers as an acetate replacing factor. It is slightly soluble in Water, and soluble in organic solvents. ALA is a chiral molecule. ALA is known by a variety of names, including thioctic acid; 1,2-diethylene-3 pentanoic acid; 1,2-diethylene-3 valeric acid; and 6,8-thioctic acid. ALA found to be synthesized by animals and humans.[17] ALA (thioctic acid) is a potent anti-oxidant that has been widely used in food supplement preparations. ALA has been used to alleviate peripheral pain in severe diabetic patients and its application in food preparations is getting popular. ALA is usually present in the mitochondrial matrix in the cells of organisms where cells metabolisms and energy production take place. Alpha lipoic acid normally exists in the reduced form in living organisms.[18] Various beneficial effects of ALA, e.g. skin whitening effect, inhibition of adipocytes production and growth promoting effect on ALA ingredient for weight loss, cosmetics and anti-oxidative preparations muscle cells. ALA is an antioxidant, an anti-diabetic drug which helps mainly to convert glucose (blood sugar) into energy. In patients with type II Diabetes Mellitus, both acute and chronic administration of ALA improves insulin resistance, reduces plasma fructosamine levels.[19] Metformin (MFH) is 1, 1-Dimethylbiguanide. It is official in IP, USP & BP. MFH is a biguanide hypoglycemic agent used in the

treatment of non-insulin-dependent diabetes mellitus not responding to dietary modification.[20] MFH is a white to off-white crystalline compound with a molecular formula of $C_4H_{11}N_5.HCl$ and a molecular weight of 165.63.[21] MFH is freely soluble in water and is practically insoluble in acetone, ether, and chloroform.[22] MFH used in the management of type 2 diabetes. MFH improves glycemic control by improving insulin sensitivity and decreasing intestinal absorption of glucose. Metformin is an oral diabetes medicine that helps to control blood sugar levels.[23]

Thus, present study was undertaken to develop and validate a simple sensitive, accurate, precise and reproducible imultaneous UV- method for determination of MFH and ALA.

MATERIAL AND METHODS:

Instruments: The analysis was performed by using the analytical balance (Mettler), pH meter (Cyber scan), UV spectrophotometer (UV-Lambda 25, Perkin Elmer equipped with variable wavelength detector and data integration software).

Reagents and solutions: Alpha lipoic acid and metformin, potassium dihydrogen phosphate, sodium hydroxide analytical grade were used in entire research work.

Preparation of solvent system:

Potassium dihydrogen phosphate (KH_2PO_4): 6.8 gm of dipotassium hydrogen phosphate was weighed accurately and transferred into a 1000 ml volumetric flask containing 900 ml of water and mixed well till clear solution obtained. pH of solution was adjusted up to 6.8 by using Sodium hydroxide. Finally volume make up to 1000ml with water

Standard stock solution of alpha lipoic acid and metformin: 100 mg of ALA or MFH weighted accurately and transferred into a 100 ml volumetric flask containing 60 ml water. Solution sonicated to dissolve ALA or MFH and cooled at room temperature then volume make up with water and mix well (Stock solution ALA-1 and MFH-1). Pipette out 2 ml of standard stock solution, mixed well and diluted up to volume with 6.8 phosphate buffer solution. The resulting solution contains 0.2 mg/ml of ALA and MFH.

Sample Stock Solution: Average weight of the tablets was determined and fine powder made with the help of mortar and pestle. Transferred accurately equivalent to one tablet weight into a 500 ml volumetric flask containing 250 ml water and sonicated till clear solution, finally cooled at room temperature. Final volume made with water and mixed well. Prepared solution then centrifuge at 3500 rpm for 5 minutes and used as standard test solution (Stock solution ALA-2 or MFH-2). 2 ml of clear supernatant sample stock solution were transferred into a 100 ml volumetric flask and dilute to volume with 6.8 phosphate buffer. The resulting solution contains 0.2 mg/ml of ALA or MFH.

Spectral study: The final stock solution scanned in UV spectrophotometer over the range 200-400nm (Figure 1).

RESULTS AND DISCUSSION: The methods discuss in the present work provide a convenient, precise and accurate way for simultaneous estimation of ALA and MFH in bulk and pharmaceutical dosage form. An absorption maximum of MFH and ALA were selected at 232nm and 334nm respectively for the analysis. Regression analysis shows linearity over the concentration range of 2-12µg/ml for MFH with correlation coefficient 0.993 and 100-500µg/ml with correlation coefficient 0.998 for ALA (Figures 2 and 3).

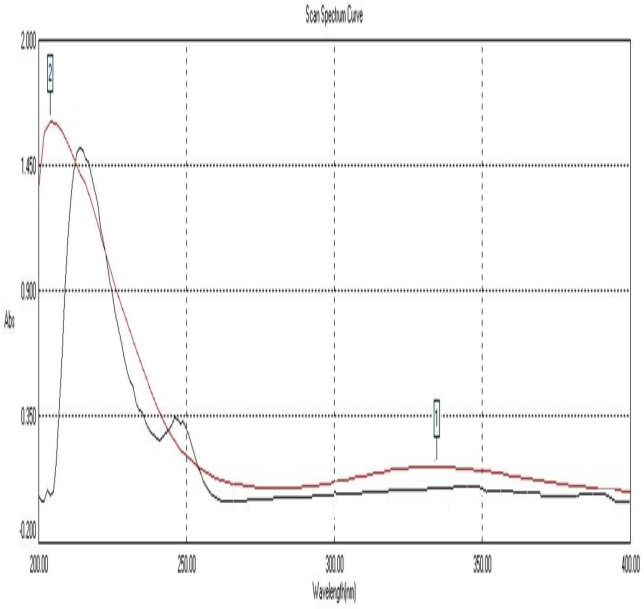

Figure 1: UV Spectra of MFH and ALA.

The % RSD for repeatability (n=6) precision was found to be less than 2% indicating the precision of method. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as percentage recovery. Percentage recovery for MFH and ALA was found within the range between 99.1 % to 100.45% and 99.8% to 101.60% respectively. The % RSD value for MFH and ALA

was found to be less than 2%. In this study simultaneous estimation of MFH and ALA was carried out by UV Spectroscopy method and all the validation parameters found satisfactorily. The result of developed method and validation was given in table 1.

Table 1: Result of method development and validation.

Sr. No.	Parameters	Observations	
		MFH	ALA
01.	SPECIFICITY (Interference of peaks)	No interference observed	No interference observed
02.	PRECISION 1. Precision of system (%RSD) 2. Precision of Method (%RSD)	0.01% 1.21%	0.02% 1.12%
03.	INTERMEDIATE PRECISION 1. Precision of system (%RSD) 2. Precision of Method (%RSD)	0.05%. 1.32%	0.02%. 1.12%
04.	LINEARITY (Correlation coefficient)	0.993	0.998
05.	ACCURACY (% Recovery)	99.1%-100.45%	99.8%-101.60%
06.	RUGGEDNESS (%RSD)	1.22%	2.1%
07.	ROBUSTNESS	Complies all deliberated changes.	Complies all deliberated changes.

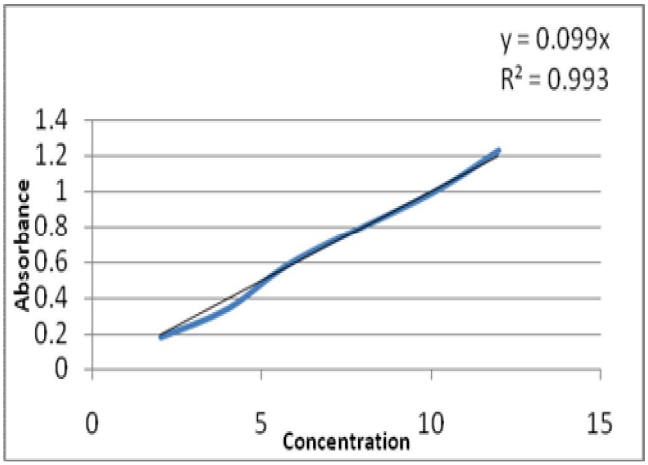

Figure 2: Calibration curve of MFH.

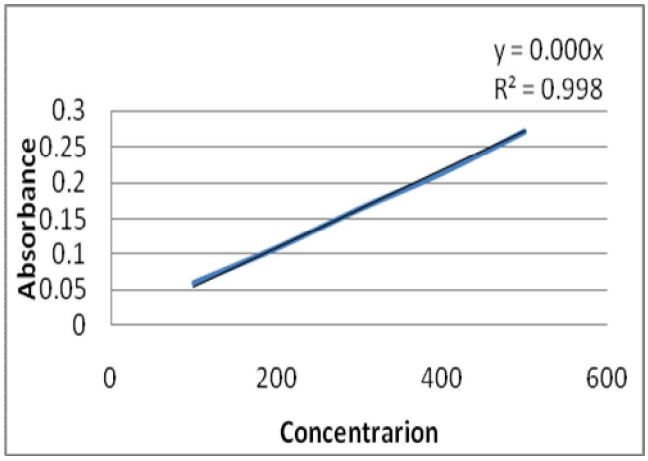

Figure 3: Calibration curve of ALA.

CONCLUSION: The analytical method for simultaneous estimation of MFH and ALA has been developed and validated according to validation protocol of ICH guidelines. All parameters mentioned in the protocol were tested and they fulfilled the requirement of ICH analytical method validation for the drug. The results obtained are well within the set limit; indicates that the described analytical method is suitable for simultaneous estimation of MFH and ALA in bulk as well as tablet formulation.

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