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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DULOXETINE HYDROCHLORIDE AND METHYLCOBALAMIN IN THEIR PHARMACEUTICAL DOSAGE FORM BY ABSORPTION CORRECTION METHOD

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ABSTRACT

A simple, economical, precise and accurate method is described for the simultaneous determination of Duloxetine hydrochloride (DULO) and methylcobalamin (MCA) in combined capsule dosage form. The method was based on absorption correction method which involves direct estimation of MCA at 351 nm, as at this wavelength DULO has zero absorbance and shows no interference. For estimation of DULO, corrected absorbance was calculated at 289 nm due to the interference of MCA at this wavelength using distilled water as a solvent. The two drugs follow Beer-Lambert's law over the concentration range of 10-50µg/mL in both the drugs. The concentrations of the drugs were determined by using absorbance correction method at both the wavelengths. The proposed methods can be successfully applied in routine work for the determination of DULO and MCA in combined dosage form. The method was validated statistically.

KEYWORDS

Duloxetine hydrochloride (DULO), Methylcobalamin (MCA), Absorption correction method, Distilled water and Validation.

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INTRODUCTION

Duloxetine hydrochloride (Figure No.1)[1, 2] is a selective serotonin and norepinephrine reuptake inhibitor (SSNRI) is used for the treatment of major depressive disorder and anxiety. Its chemical designation is (+)-(S)-N-methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride. Methyl cobalamin (Figure No.2)[3, 4] (MeB12) is a form of

vitamin B12 used in the treatment of trigeminal neuralgia, megaloplastic anemia, diabetic neuropathy and facial paralysis in Bell's palsy syndrome. It is chemicallyCoα-[α-(5,6-dimethylbenz-1H-imid azolyl)]-Coβmethylcobamide.

The combined dosage forms of these drugs are used for the treatment of neuropathic pain associated with peripheral neuropathy especially diabetic poly neuropathy. It restores the balance of neuro transmitters in the brain like serotonin and norepinephrine. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of DULO and MCA in their combined dosage forms. Literature review revealed that analysis of DULO and MCA is mainly carried out on single or with other drugs combination by UV-Spectrophotometry, HPLC and HPTLC with derivatization. There is only one UV-spectro photometric (simultaneous equation)[5] methods have been reported for simultaneous estimation of DULO and MCA in their combined dosage form. The present work describes simple, specific, rapid, accurate and precise chromatographic method based on Absorption correction method[6] for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIAL AND METHOD

Instruments

A Shimadzu UV 1800 double beam UV/Visible spectrophotometer with spectral width of 2nm, wavelength accuracy of 0.5nm and a pair of 10mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Scale-tec^{TM} analytical balance and ultrasonic bath (Hintron, CD-4820) was used in the study.

Materials

Pharmaceutically pure sample of DULO and MCA were obtained as gift sample from Gujarat pharma Pvt. Ltd., Ahmedabad, Gujarat, India and Max Life Science Pvt. Ltd., Ahmedabad, Gujarat, India, respectively. All chemicals were of analytical grade. A combination of Duloxetine hydrochloride (20 mg) and Methylcobalamin (1.5 mg) in capsule formulation was procured from local market (Dulane M 20, Sun Pharma, Sikkim, India).

Preparation of standard stock solution

A 10mg of standard DULO and MCA were weighed and transferred to 100ml separate volumetric flasks and dissolved in distil water. The flasks were shaken and volumes were made upto mark with distil water to give a solution containing 100µg/ml each of DULO and MCA.

Application of absorption correction method

The working standard solutions of DULO and MCA were prepared by taking suitable aliquots of drug solution from the standard stock solutions and the volume was made up to 10 mL with distil water to get concentrations of 10-50 µg/mL for both drugs. From the over lay spectra of two drugs two wavelengths from spectra, 289nm (DULO) and 351(MCA) nm were selected. The absorptivity values of DULO and MCA were determined at selected wavelengths. At 351 nm, estimation of MCA was done where DULO has no interference. The absorbance of working standard solution of MCA at 289 nm was calculated using equation (1) and then from the total absorbance of sample mixture at wavelength 289 nm, the contribution due to MCA was subtracted. The calculated absorbance was called as corrected absorbance for DULO. The concentrations of DULO (Cy) at 289 nm using the corrected absorbance was determine using absorptivity value.

The concentration of two drugs in the mixture can be calculate using following equations

$$C_X = A_1/a_{X1} \quad \text{..... (1)}$$

$$C_Y = A_2-(a_{X2}. C_X)/a_{Y2} \quad \text{..... (2)}$$

Where,

A_1 and A_2 are absorbance of sample solution at 351 nm and 289 nm respectively.

a_{X1} and a_{X2} are absorptivity value of MCA at 351 nm and 289 nm,

a_{Y2} is absorptivity value of DULO at 289 nm.

Preparation of sample solution

For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely

powdered. Appropriate quantity equivalent to 1.5 mg MCA and 20 mg DULO was accurately weighed and as per standard addition method[4], 18.5 mg of pure MCA was added because of very law absorbance of MCA in mixture to achieve 1:1 ratio of MCA and DULO. The powder was transferred to 100 ml volumetric flask and shaken vigorously with distil water for 15 min and filtered. Necessary dilutions are made with distil water to give final concentration20µg/ml of MCA and DULO respectively. The absorbance's values were read at 289 and 351nm and concentration was obtained by solving the absorption correction equations. Results of analysis of tablet formulation are shown in Table No.1.

METHOD VALIDATION

Linearity

Linearity was studied by analyzing five standard solutions (n=3) covering the range of 10-50µg/mL for both drugs. Standard solutions containing 100 µg/mL of each drug in solvent were prepared in triplicate. Aliquots of these solutions were diluted to five different concentrations covering the above mention range. Calibration curves (Figure No.3 and 4) with concentration verses absorbance was plotted and the obtained data were subjected to regression analysis using the least squares method.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for MCA and DULO, 40µg/ml and 30 µg/ml respectively without changing the parameter of the proposed spectrophotometry method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of MCA (20, 30, and 40µg/ml) and for DULO (10, 20 and 30µg/ml). The result was reported in terms of relative standard deviation (% RSD).

Limit of detection and limit of quantitaton

The limit of detection (LOD) and limit of quantitaton (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (2) and (3), respectively.

LOD = 3.3 δ/S ……. (3)

LOQ =10 δ/S …….. (4)

Where,

δ: standard of y-intercept and S: slope of calibration curve.

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of MCA and DULO by the standard addition method. Known amounts of standard solutions of MCA and DULO were added at 50, 100 and 150 % level to prequantified sample solutions of MCA and DULO (20µg/ml for MCA and 20µg/ml for DULO) (Figure No.5). The amounts of MCA and DULO were estimated by applying obtained values to the respective regression line equations.

RESULTS AND DISCUSSION

In absorption correction method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 289 nm (λ-max of DULO) and 351 nm (λ-max of MCA) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of DULO (289 nm) and MCA (351 nm) showing zero absorbance of DULO at 351 nm in distil water is shown in (Figure No.6). The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined (Table No. 2). The method was successfully used to determine the amounts of MCA and DULO present in the Tablet mixture. The results obtained were in good agreement with the

corresponding labeled amount (Table No.3). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method is given in (Table No.4). By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in combined dosage form.

Table No.1: Regression analysis data

S.No	Parameters	MCA at λ- max 351	DULO at λ- max 289
1	Concentration range (µg/mL)	10-50	10-50
2	Slope	0.0163	0.0175
3	Intercept	0.0216	0.0351
4	Correlation coefficient	0.9991	0.9981

Table No.2: Recovery data of MCA and DULO by Spectrophotometric Method

S.No	Drug	Level	Amount of Sample Taken (µG/ML)	Amount of Standard Spiked (%)	% Recovery ± SD (N=6)	% RSD
1	MCA	I	20	50 %	100.08±0.32	0.32 %
		II	20	100 %	102.25±1.23	1.21 %
		III	20	150 %	100.86 ±1.14	0.14 %
2	DULO	I	20	50 %	99.30 ±1.46	1.47 %
		II	20	100 %	100.90 ±0.77	0.77 %
		III	20	150 %	99.60 ±0.89	0.90 %

Table No.3: Analysis of formulation using the UV (Absorption Correction method)

S.No	Mixture	Label claim	% Label Claim ± SD (N=6)	% RSD
1	MCA (1.5 mg)+ DULO(20 mg)	MCA – 1.5 mg	99.33 ± 1.64 %	1.62%
		DULO – 20 mg	101.00 ± 1.38 %	1.39%

Table No.4: Validation parameters of evaluated method

S.No	Parameters	MCA at λ- max 351	DULO at λ- max 289
1	Accuracy ±SD[a] (% Recovery, n=6)	100.08 ± 0.32 % - 102.25±1.23 %	99.30 ±1.46 - 100.90 ± 0.77%
2	LOD[b] (µg/mL)	0.2366	0.5164
3	LOQ[c](µg/mL)	0.7171	1.5651
4	Repeatability (%RSD[d],n = 6)	0.1716	0.4784
5	Precision (% RSD) Intraday (n = 3) Interday (n = 3)	 0.71 – 1.03 % 0.62 – 1.33 %	 0.44 – 1.05 % 0.20 – 1.04 %

[a]SD=Standard deviation, [b]LOD = Limit of detection, [c]LOQ = Limit of quantification, [d]RSD = Relative standard deviation.

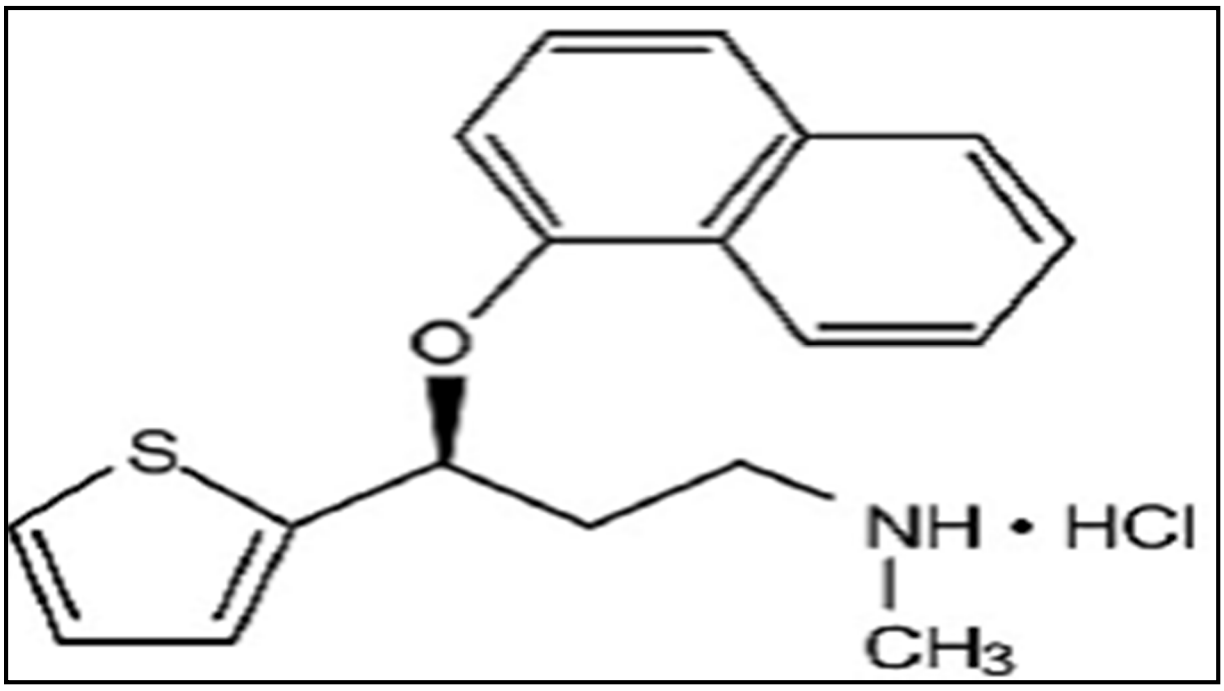

Figure No.1: Structure of Duloxetine hydrochloride

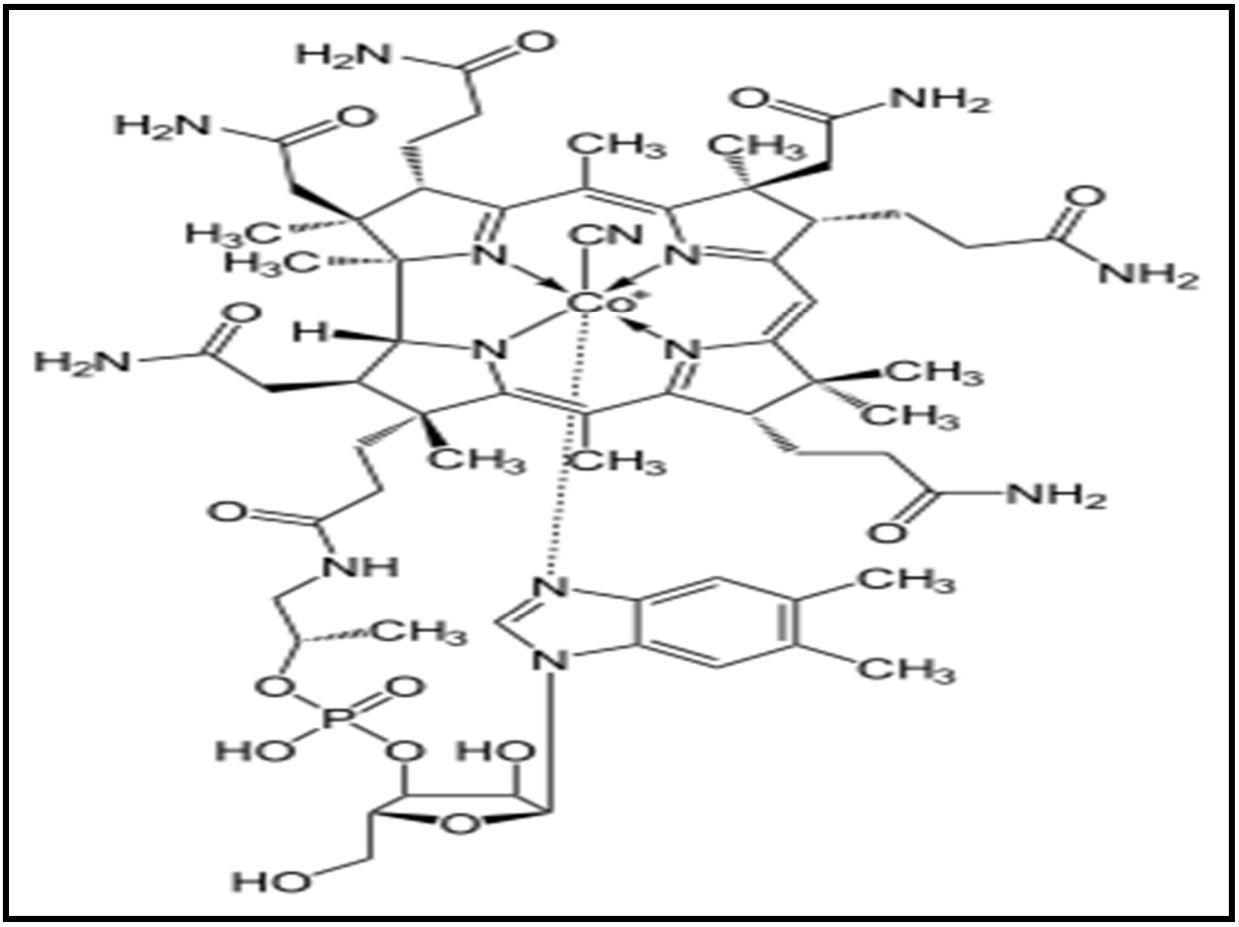

Figure No.2: Structure of Methylcobalamin

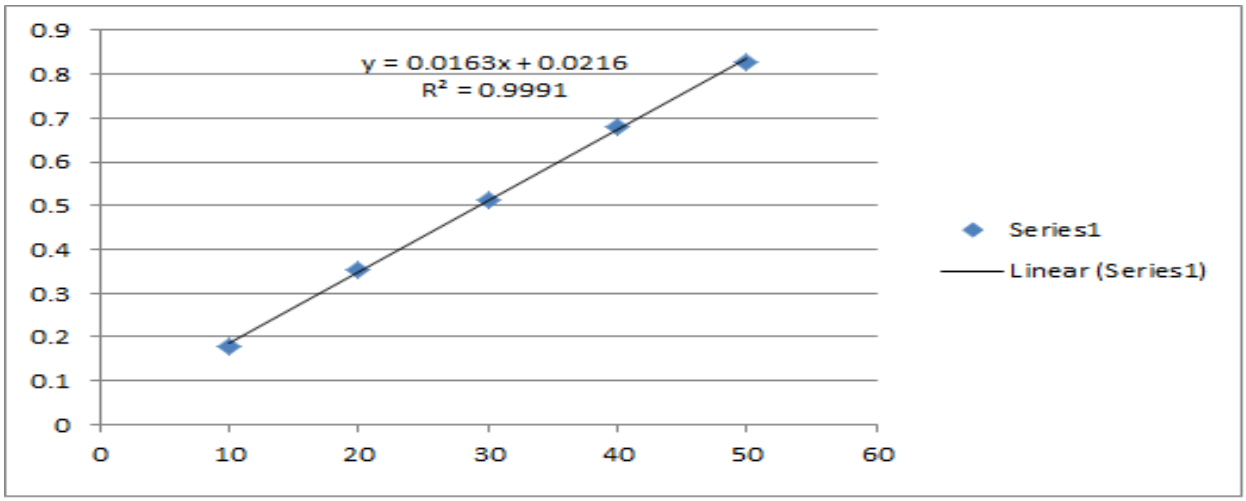

Figure No.3: Calibration curve of MCA in distil water at λ- max 351

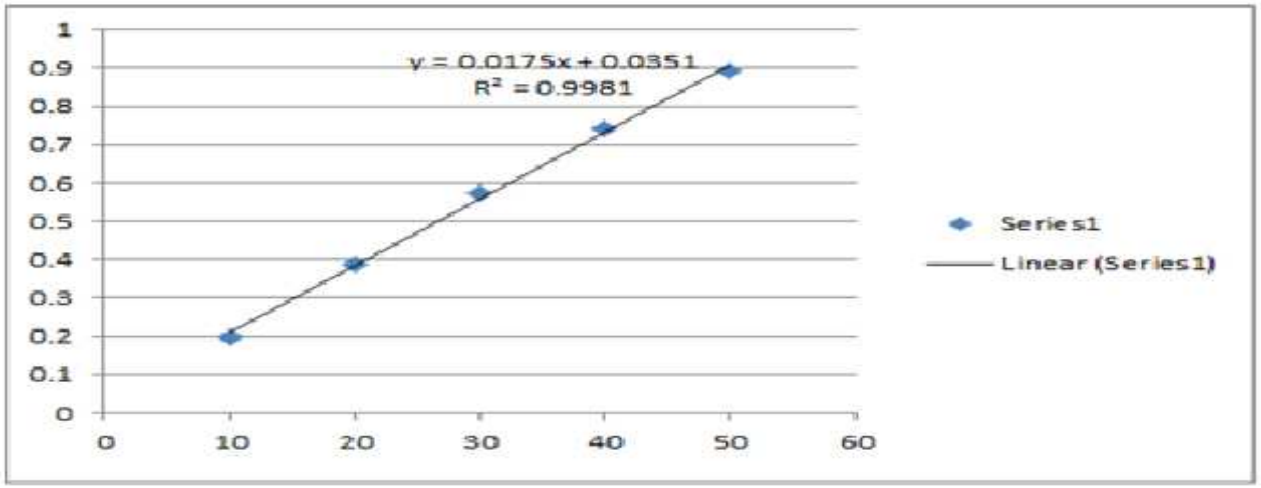

Figure No.4: Calibration curve of DULO in Distil water at λ- max 289

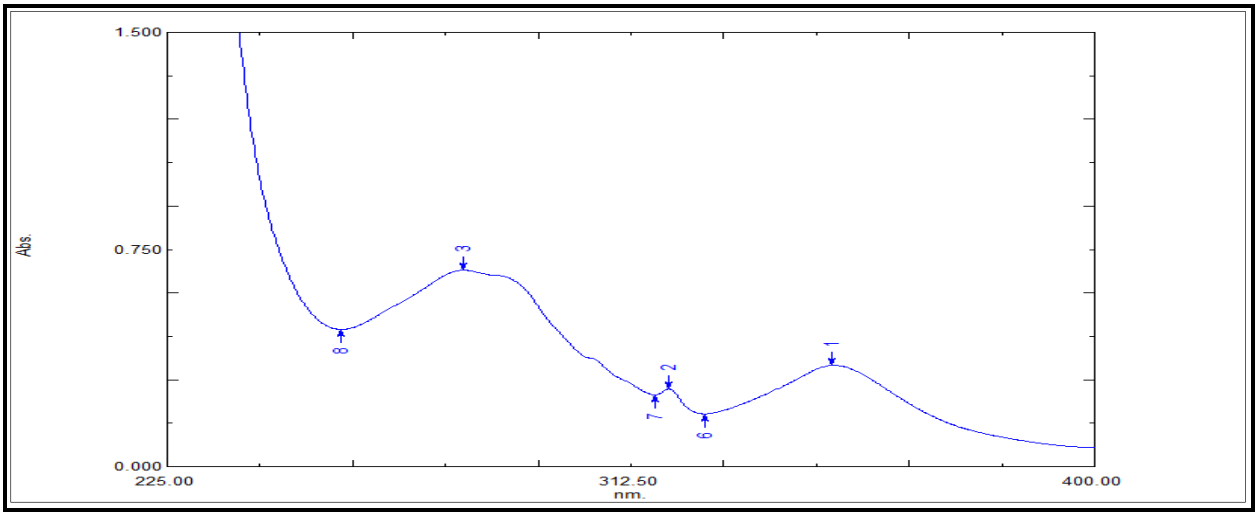

Figure No.5: Overlain spectrum of mixture (MCA-20 µg + DULO-20 µg)

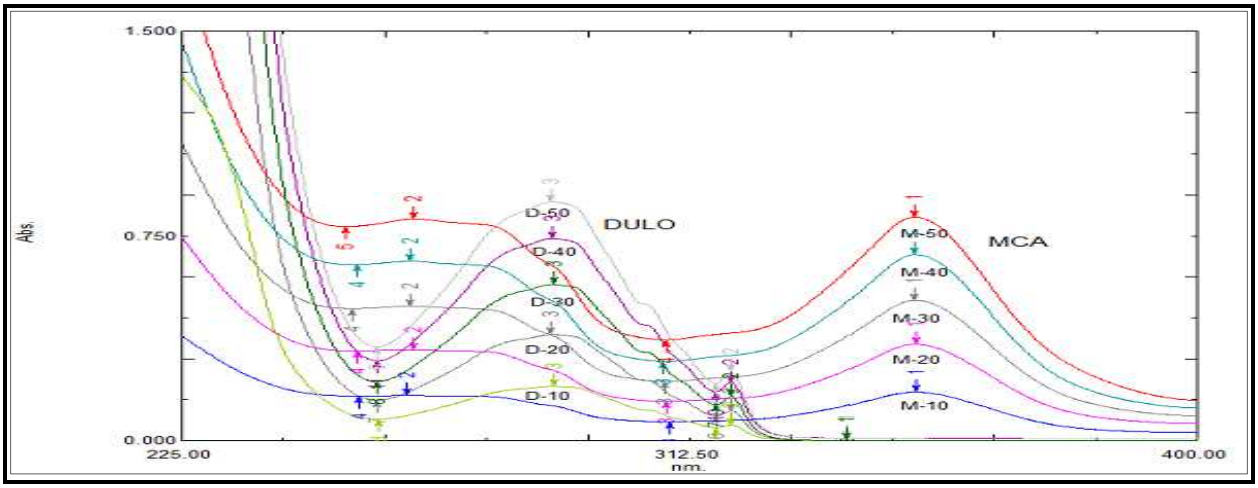

Figure No.6: Linearity of MCA and DULO in distilled water

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of MCA and DULO in tablet mixture. The method utilizes easily available and cheap solvent for analysis of MCA and DULO hence the method was also economic for estimation of MCA and DULO from Tablet mixture. The common excipients and other additives are usually present in the Tablet mixture do not interfere in the analysis of MCA and DULO in distil water, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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