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Simultaneous Estimation of Duloxetine and Methylcobalamin in combined dosage form by Ultra-violet Spectrophotometry

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Abstract: A new, simple, precise and cost effective UV-spectrophotometric method has been developed for simultaneous estimation of Duloxetine and Methylcobalamin in combined dosage form. The formulation contains 20 mg of DUL and 1.5 mg MTH. The method shows maximum absorbance at 289 nm and 351 nm for DUL and MTH respectively. The proposed method is based upon simultaneous equation method where distilled water is used as solvent. The method employs formation and solving of simultaneous equation using 289 nm and 351 nm as the two wavelengths for forming equations. The calibration curves were found to be linear and in adherence to Beer's law over the concentration range of 10- 60 µg/ml for DUL and 1.5 – 9.0 µg/ml for MTH, the correlation coefficient were found to be 0.999 for both the drugs. The percentage recovery of DUL and MTH were found to be 99.79% & 99.87% respectively. The LOD values for DUL & MTH were found to be 0.05 µg/ml & 0.145 µg/ml respectively. LOQ values were found to be 0.166 µg/ml & 0.484 µg/ml for DUL & MTH respectively.
Keywords: Duloxetine, Methylcobalamin, UV, Simultaneous equation method.

Introduction

Duloxetine (Fig No. 1) is ((3S)-N-Methyl-3-naphthalen-1-yloxy-3-thiophen-2-ylpropan-1-amine). Duloxetine is a potent inhibitor of neuronal serotonin and norepinephrine reuptake and a less potent inhibitor of dopamine reuptake belongs to antidepressant category. It produces its antidepressant and pain inhibitory action by potentiation of serotonergic and noradrenergic activity in the CNS[1-2].

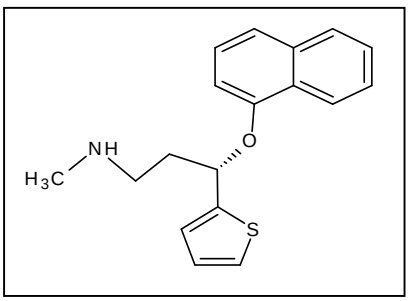

Fig No. 1 Structure for Duloxetine

Methylcobalamin (Fig.No.2) is MeCbl;Co(alpha)-[(alpha)-(5,6-Dimethyl benzimidazolyl)]-Co (beta)-methylcobamide. It is a cobalmine and it is a form of Vitamin B12. Vitamin B12 is used in the body in two forms such as Methylcobalamin and 5-deoxyadenosyl cobalamin. The methionine synthase is an enzyme that involved in conversion of the amino acid homocysteine into methionine and this enzyme requires Methylcobalamin as a cofactor. Methylcobalamin is also used in the treatment of peripheral neuropathy, diabetic neuropathy, and as a preliminary treatment for amyotrophic lateral sclerosis [3-4].

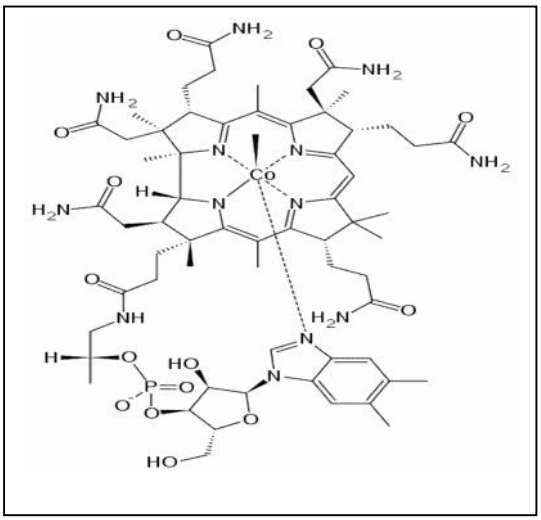

Fig No. 2 Structure for Methylcobalamin

Duloxetine and Methylcobalamin is a recent combination in the market used for treatment of antidepressant. This paper is in continuation with our work,[12-16] where we studied spectrophotometric method for single or multicomponent drugs. There are methods to estimate the drugs individually for DUL[5-7] and MTH[8,9] or in combination with other drugs, but not a single method is reported for its simultaneous estimation. So here an attempt has been made to develop simple, accurate, sensitive, rapid and economic method for simultaneous estimation of Duloxetine and Methylcobalamin in combined dosage form using UV – Visible spectroscopy. The proposed method was validated according to ICH guidelines. [10, 11]

Material and Method

Chemicals

Pharmaceutical grade Duloxetine and Methylcobalamin were obtained as gift sample from Lupin Pharmaceutical Ltd, Aurangabad, India. Sample used was *Dulane M20* capsule manufactured by Sun Pharma, Sikkim, containing 1.5 mg of Methylcobalamin and 20 mg of Duloxetine. Distilled water was used as solvent.

Instrumentation

A Shimadzu model UV-1800 double beam UV-Visible spectrophotometer attached with computer operated software UV probe 2.0 with spectral width of 2 nm, with a pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Analytical weighing balance (AA-2200), digital pH meter (Systronic) and ultrasonic bath (HMG India: CD-4820) were used during the study.

Procedure

Preparation of standard stock solution

An accurately weighed quantity of about 20 mg of pure drug of DUL was dissolved in water and diluted to 100 ml. Further dilutions carried out to get final concentration of 200 µg/ml.

An accurately weighed quantity of about 10 mg of pure drug of MTH was dissolved in water and diluted to 100 ml. Further dilutions carried out to get final concentration of 100 µg/ml.

Selection of analytical wavelengths

Appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. DUL and MTH showed absorbance maxima at 289 nm (Fig. No.3) and at 351 nm (Fig. No. 4) respectively. Fig. No.5 represents the overlain spectra of both the drugs.

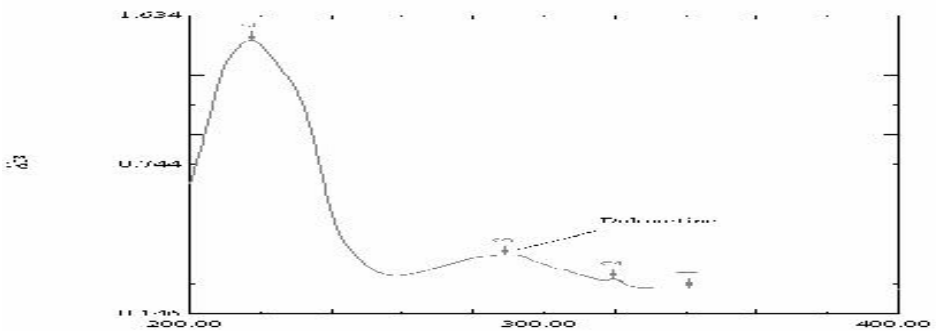

Fig. No. 3: UV spectrum of DUL

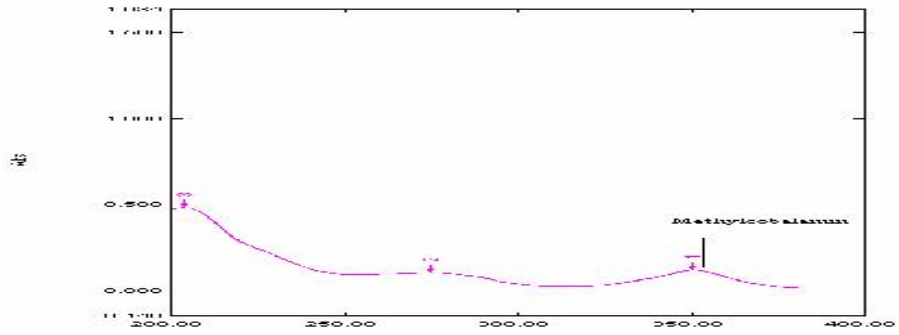

Fig. No.4: UV spectrum of MTH

Determination of absorptivity coefficients at analytical wavelengths

The absorptivity coefficients for the two drugs were determined at both the selected wavelengths. The values obtained as the mean of six independent determinations were used for forming the simultaneous equations.

The simultaneous equations formed were-

$A1 = 48.46 \times C1 + 11 \times C2$ ---------- (1) at 289nm
(For Duloxetine)

$A2 = 10.33 \times C1 + 51.08 \times C2$ ---------- (2) at 351 nm
(For Methylcobalamin)

Where A1 and A2 are the absorbance of sample solution at 289 nm and 351 nm respectively and C1 and C2 are the concentrations of Duloxetine and Methylcobalamin respectively (in grams per liter) in the sample solution. By solving the two simultaneous equations, the concentration of Duloxetine (C1) and Methylcobalamin (C2) in sample solutions can be obtained.

Procedure for analysis of mixture

The method was checked by analyzing a solution containing known concentration of both drugs. The mixed standards in the Beer- Lambert's range for each drug in the ratio of 1:13.33 containing 20, 40 and 60 µg/ml of DUL and 1.5, 3.0 and 4.5 µg/ml of MTH respectively were prepared by diluting appropriate volumes of standard stock solutions. The scanning of mixed standard solutions was carried out in the range of 400 nm to 200 nm in spectrum mode (Table No. 1). The absorbance of mixed standard solutions was measured at 289 nm and 351 nm. The concentrations of DUL and MTH present in mixed standards were calculated using the equation 1 and 2. (Table No. 2) Good results were obtained and hence the method was applied to the marketed capsule formulation.

Table No. 1: Absorbance of mixed standards containing DUL and MTH

Sr. No.	Mixed Standards		Abs. at 289 nm	Abs. at 351nm
	Conc. of DUL (µg/ml)	Conc. of MTH (µg/ml)		
1.	20	1.5	0.585	0.068
2.	40	3.0	1.283	0.148
3.	60	4.5	1.932	0.221

Table No. 2: Results of mixture containing DUL and MTH

Sr. No.	Amount Present * (µg/ml)		Amount Found* (µg/ml)		% Amount Found*	
	DUL	MTH	DUL	MTH	DUL	MTH
1.	20	1.5	19.89	1.49	99.48	99.55
2	40	3.0	19.90	1.48	99.53	99.32
3	60	4.5	20.03	1.50	100.15	100.45

*Each value is a mean of six observations

Result and Discussion

Validation of method

The proposed method was validated for accuracy, precision, recovery, linearity and robustness. The method validation was performed as per ICH guidelines.

Linearity and ranges:

From the standard stock solution of DUL, appropriate aliquots were pipetted out into 10 ml volumetric flasks and dilutions were made with water to obtain working standard solutions of concentrations 10- 60 µg/ml. Absorbance for these solutions were measured at 289 nm (Table No. 3) and a calibration curve of absorbance against concentration was plotted (Fig. No. 6).

Similarly, a series of standard solutions of concentration 1.5 – 9.0 µg/ml were prepared for MTH and their absorbance were measured at 351 nm (Table No. 3). A standard calibration curve of absorbance against concentration was plotted (Fig. No. 7). Both drugs followed the Beer's law in the range of 10-60 µg/ml and 1.5-9.0 µg/ml for DUL and MTH respectively. Table No.4 summaries the optical characteristics of both the drugs.

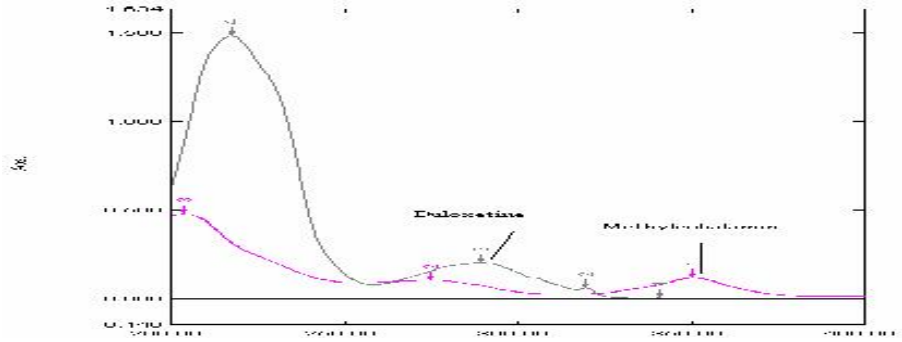

Fig. No.5: Overlain spectrum of the DUL and MTH

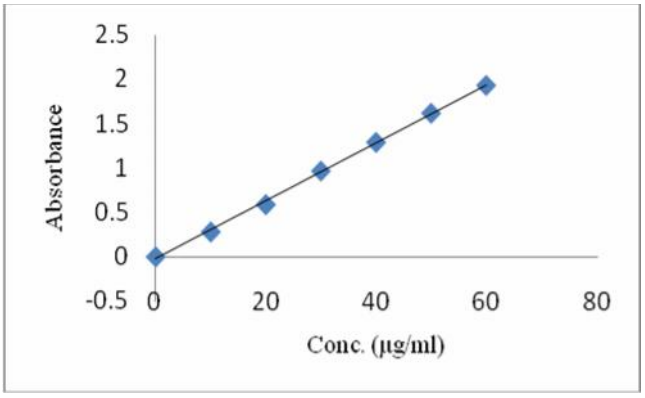

Fig. No. 6: Calibration curve of DUL

Table No.3: Standard calibration table for DUL and MTH

Sr. No.	For Duloxetine		For Methylcobalamin	
	Conc. («g/ml)	Abs.* at 289 nm	Conc. («g/ml)	Abs.* at 351 nm
1.	10	0.281	1.5	0.035
2.	20	0.588	3.0	0.69
3.	30	0.966	4.5	0.107
4.	40	1.289	6.0	0.149
5.	50	1.616	7.5	0.185
6.	60	1.929	9.0	0.220

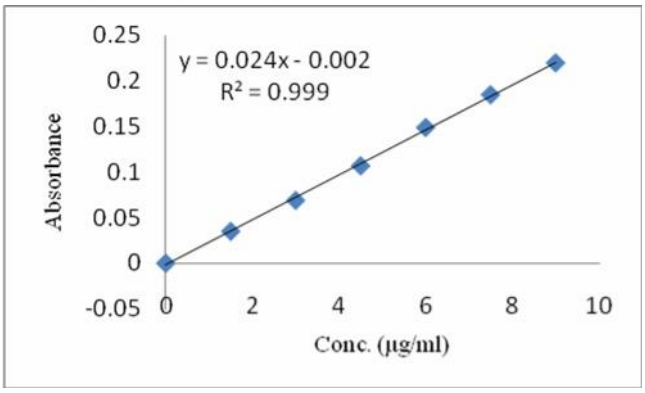

Fig. No. 7: Calibration curve of MTH

Table No 4: Optical characteristics and other parameters

Parameters	DUL	MTH
Working wavelength (nm)	289	351
Linearity range (µg/ml)	10-60	1.5-9.0
Molar absorptivity	29.12	44.97
Limit of detection (µg/ml)	0.05	0.145
Limit of quantitation (µg/ml)	0.166	0.484
Y= mx + c		
Slope	0.032	0.024
Intercept	-0.028	-0.002
Regression Coefficient	0.999	0.999

Analysis of Market Formulation

Twenty capsules were weighed accurately; the average weight was determined and then triturated to a fine powder. A quantity equivalent to 20 mg of DUL and 1.5 mg of MTH was weighed and transferred to a 100 ml volumetric flask. The content was shaken with 70 ml water to dissolve the capsule powder and the volume was made up to 100 ml with water and filtered through Whatman filter paper no. 41 to give the stock solution containing 200 µg/ml of DUL and 15 µg/ml of MTH. Various dilutions of the capsule stock solutions were scanned and the absorbance of these solutions were measured at 289 nm and 351 nm respectively. The analysis procedure was repeated six times. The results of marketed capsule formulation are given in Table No. 5.

Precision of method

Precision of the method was verified by using stock solutions in the ratio of 1:13.33 containing 40 µg/ml DUL and 3.0 µg/ml of MTH. System repeatability was done by repeating the assay three times of six replicate dilutions of the same concentration after every two hours on the same day for intraday precision. Interday precision was carried out by performing the assay of six sample sets after 24 hours and 48 hours. The results of intermediate precision are given in Table No. 6.

Accuracy (Recovery study):

To check the accuracy of the proposed method, recovery studies were carried out at 80, 100 and 120 % of the test concentration as per ICH guidelines. As per the label claim, capsule contains 20 mg of DUL and 1.5 mg of MTH. For recovery studies different levels of the standard concentration according to 80%, 100% and 120% are made and % mean recoveries are calculated. The results of recovery study are presented in Table no.7.

Table No. 5: Results of marketed capsule formulation

Sr. No.	Label Claim (mg/tab)		Amount Found (mg/tab)		% of Label Claim	
	DUL	MTH	DUL	MTH	DUL	MTH
1	20	1.50	19.99	1.49	99.95	99.98
2	20	1.50	20.03	1.49	100.15	99.97
3	20	1.50	19.97	150	99.86	100.02
4	20	1.50	19.92	1.49	99.61	99.91
5	20	1.50	19.94	1.50	99.72	100.00
6	20	1.50	19.96	1.49	99.80	99.99
				Mean	99.84	99.97
				SD	0.1881	0.0376
				% RSD	0.1884	0.0376

Table No. 6: Results of intermediate precision

Formulation	Parameter	Intra-day precision*	Inter-day precision*
DUL	% Mean	99.81	99.93
	SD	0.0709	0.1126
	RSD	0.0710	0.1126
MTH	% Mean	99.72	99.91
	SD	0.1563	0.1255
	RSD	0.1567	0.1256

*Each value is a mean of six observations

Table No. 7: Results of recovery studies

Level of (%) Recovery	Amount present (mg/tab)		Amount of standard added (mg)		Total amount recovered (mg)		% Recovery*	
	DUL	MTH	DUL	MTH	DUL	MTH	DUL	MTH
80	20	1.50	16	1.20	19.93	1.49	99.68	99.51
100	20	1.50	20	1.50	19.94	1.49	99.71	99.90
120	20	1.50	24	1.80	99.99	1.50	99.98	100.20
						Mean	99.79	99.87
						SD	0.1652	0.3459
						% RSD	0.1655	0.3463

*Each value is the mean of three observations

*In the proposed method for the analysis of DUL and MTH in combined dosage form, method employed two wavelengths for analysis of drugs which were 289 nm (DUL) and 351 nm(MTH).

The assay result of method was in good agreement with the labelled claim. The developed method was validated in terms of accuracy, precision and linearity range studies. The method was found to be accurate with mean percent of 99.51 to 100.2. The precision of two methods were confirmed by low standard deviation (S.D.) values.

Conclusion

The proposed method was fast, accurate, precise and economical for the determination of Duloxetine and Methylcobalamin in combined capsule. Hence it can be successfully applied for routine estimation for Duloxetine and Methylcobalamin in quality control laboratories.

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