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SIMULTANEOUS ESTIMATION OF DULOXETINE HYDROCHLORIDE AND METHYLCOBALAMIN BY UV SPECTROSCOPIC METHOD

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ABSTRACT

A simple, accurate and precise UV Spectroscopic method was developed for the simultaneous estimation of Duloxetine Hydrochloride and Methylcoblamin. The overlay spectra of Duloxetine hydrochloride and Methylcoblamin exhibit λ max of 291 nm and 350 nm for Duloxetine hydrochloride and Methylcoblamin in Double distilled water respectively. The drugs obeyed the Beer's law in the range of 8-28µg/ml and 0.6-15µg/ml for Duloxetine hydrochloride and Methylcoblamin with correlation coefficients of 0.998 and 0.999 respectively and it has showed good linearity. The results of analysis were validated by recovery studies. The % recovery was found to be 99.99-100.96 % for Duloxetine hydrochloride and 100.55-101.02 % for Methylcoblamin. LOD and LOQ were found to be 1.278µg/ml, 2.330µg/ml for Duloxetine hydrochloride and 0.949µg/ml, 2.857µg/ml for Methylcoblamin respectively. The %RSD values were less than 2. The method was found to be simple, accurate, precise, economical and reproducible.

Keywords: Duloxetine hydrochloride, Methylcoblamin, Simultaneous Equation Method.

INTRODUCTION

Duloxetine hydrochloride is a white to slightly brownish white solid powder (Fig.1a). It is chemically, (+)-N-Methyl-3-(1-naphthalenyloxy)-3-(2thienyl) propanamine hydrochloride used in the treatment of diabetic complications like neuropathy, retinopathy and nephropathy [1,2,3]. Methylcoblamin is a dark red crystalline powder and it has been referred for neurological illness, diabetic neuropathy, hearing loss and Alzheimer's disease (Fig.1b). Methylcoblamin is designated as Coα-[α-(5,6-dimethylbenz-1H-imidazolyl)]-Coβmethylcobamide [1,2]. From the literature survey conducted it was found that there are some methods reported for estimation of Methylcoblamin and Duloxetine Hydrochloride individually or combination with other drugs & also in biological fluids by HPLC method and most of works reported are done in RP-HPLC, GC-MS, HPTLC[4-9]. There is no analytical method reported for the simultaneous estimation of Duloxetine Hydrochloride and Methylcoblamin in bulk drug and their combined tablet dosage form, so it was felt that there is a need to develop a simple, reliable, rapid, sensitive, and accurate analytical method for simultaneous estimation of Duloxetine Hydrochloride & Methylcoblamin by using UV spectroscopy.

MATERIALS AND METHODS:

Materials: The Pure drug of Duloxetine Hydrochloride and Methylcoblamin were kindly gifted by FDC Pvt. Ltd., Mumbai, India and Meyer Organics Pvt. Ltd., Mumbai, India. Tablet samples each containing 20 mg of Duloxetine Hydrochloride and 1.5 mg of Methylcobalamin was purchased from local pharmacy (Torrent Pharmaceutical Pvt. Ltd.).

Instrument: A Double beam UV-Visible Spectrophotometer, JASCO was used to measure absorbance of resulting solutions, Ultrasonicator.

Selection of solvent: Different solvents were selected for developing spectral characteristics of drugs. The selection was made after assessing the solubility of drug in different solvents and on the basis of following parameters.

The ideal properties of the solvent to be used in UV-visible Spectrophotometry include:
1. Both drugs should show solubility in the solvent used.
2. Both drugs should show stability in the selected solvent.
3. Both drugs should obey linearity over an appropriate range of analytical concentrations.
4. The solvent should be to the extent possible economic.
After taking above factors into consideration, Double Distilled Water was selected as a solvent for preparation of stock solution.

Selection of λmax: **[11]** Each Standard drug solution was scanned between the range 200-400nm in 1 cm cell against blank. After examining the overlay spectrum, two drugs have different λmax and both the drugs showed the absorbance at each other's λmax. Duloxetine hydrochloride (DULO) and Methylcoblamin (MC) showed absorbance maxima at 291nm (λ_1) **(Figure2a)** and 350nm (λ_2) **(Figure2b)** respectively. The overlay spectrum showed λ max of both drugs and also isoabsorptive points at 328nm.**(Figure3)**

Preparation of standard solutions of Duloxetine hydrochloride and Methylcoblamin:

Standard stock solution: Accurately weighed quantity 20 mg of Duloxetine hydrochloride and 1.5 mg of Methylcoblamin was transferred to two different 100.0 ml volumetric flasks. Each drug was dissolved in 20 ml of double distilled water and then sonicated for 10 minutes. Later the volume was made up to the mark with same to make 200 ppm concentration of Duloxetine hydrochloride and 15 ppm of Methylcoblamin. The working standard solution was prepared by diluting 1 ml in to 10 ml with double distilled water for both the drugs to obtain 20µg/ml of DULO and 1.5µg/ml of MC.These solutions were separately scanned.

Preparation of mixed standard stock: Different mixtures of the two drugs were prepared by diluting different volumes of Duloxetine hydrochloride and Methylcoblamin with double distilled water. The concentrations of the Duloxetine hydrochloride and Methylcobalamin were determined by measuring the absorbance of the prepared mixtures at 291 nm and 350 nm. From these absorbance values, the concentrations of Duloxetine hydrochloride and Methylcoblamin were determined using Simultaneous equation method **[10].**

$$C_X = \frac{A_2\, a_{Y1} - A_1\, a_{Y2}}{a_{X2}\, a_{Y1} - a_{X1}\, a_{Y2}} \quad (1)$$

$$C_Y = \frac{A_1\, a_{X2} - A_2\, a_{X1}}{a_{X2}\, a_{Y1} - a_{X1}\, a_{Y2}} \quad (2)$$

Where,

A_1 and A_2 :	Absorbances of mixture at 291 nm and 350 nm respectively.
ax_1 and ax_2 :	Absorptivities of DULO at λ_1 and $\lambda_2$respectively.
$ay_1$and ay_2 :	Absorptivities of MC at λ_1 and λ_2 respectively.
Cx and Cy :	Concentrations of DULO and MC respectively.

Preparation of sample solution: Marketed tablet formulation (SYMBAL M) containing 20 mg of Duloxetine hydrochloride and 1.5 mg of Methylcoblamin were used for preparation of sample solution.

Analysis of marketed tablet formulation: Twenty tablets were weighed accurately, finely powdered and powder equivalent to 20 mg of Duloxetine hydrochloride and 1.5 mg of Methylcoblamin was transferred into 100 ml volumetric flask, dissolved the mixture in 25 ml of diluent and sonicated for 10-15 minutes. The final volume of the solution was made up to 100 ml with diluent, and the solution was filtered through Whatmann filter paper no. 41.The solutions were scanned separately in the range of 200-400nm.The result of analysis of marketed tablet formulation shown in **Table 5**

ANALYTICAL METHOD VALIDATION: **[12,13]**

The method was validated according to ICH Q_2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte.

Linearity: Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Duloxetine hydrochloridewas found to be 8-28 µg/ml and for Methylcoblamin was found to be 0.6-15 µg/ml. Standard solutions of drugs containing Duloxetine hydrochloride and

Methylcoblamin was prepared and scanned for absorbance at 291 nm and 350 nm respectively.

Preparation of test solution for Duloxetine hydrochloride:

Stock solution I: Accurately weighed quantity 20 mg of Duloxetine hydrochloride was transferred to 100.0 ml volumetric flask, 20 ml of double distilled water was added and then sonicated for 10 minutes. Finally the volume was made up to the mark with same.Appropriate aliquots were pipetted out separately from standard stock solution to get a set of solutions for Duloxetine hydrochloride having concentration range 8µg/ml -28µg/ml.

Stock solution II: 0.4 ml-1.4 ml of the above solution was pipetted into 10 ml volumetric flasks and the volumes were made up with double distilled water.

Preparation of Test solution for Methylcoblamin:
Stock solution I: Accurately weighed quantity 1.5 mg of Methylcoblamin was transferred to 100.0 ml volumetric flask; 20 ml of double distilled waterwas added and then sonicated for 10 minutes. Finally the volume was made up to the mark with same. Appropriate aliquots were pipetted out separately from standard stock solution to get a set of solutions for Methylcoblamin having concentration range 0.6 µg/ml –2.1µg/ml.

Stock solution II: 0.4 ml-1.4 ml of the above solution was pipetted into 10 ml volumetric flasks and the volumes were made up with double distilled water. By analyzing different concentration solutions was measured at 291nm and 350nm against blank. The calibration curve is produced and was shown in **Figure 4**and **5.** The data for calibration curve is given in **Table 1**and **2** for Duloxetine hydrochloride and Methylcoblamin respectively. The calibration parameters were shown in **Table 3.**

Accuracy: To check the degree of accuracy of the proposed method, recovery studies were carried at three different levels (80%, 100% and 120%) and percentage recovery was calculated. Percent recovery for DULO and MC was found in the range of 99.99% to 101.02%. Results of recovery studies are shown in **Table 6**

Precision: Precision was studied to find out intra and inter-day variations in the test method of DULO and MC. Calibration curves prepared in medium were run in triplicate in same day and for three days. %RSD were calculated which is less than 2 % which complies ICH norms. The results are tabulated in **Table 7**

Repeatability: Repeatability of analytical method is the precision of the procedure when repeated by same analyst under the same operating conditions (same reagents, equipments, settings and laboratory) over a short interval of time. The standard solutions were prepared and absorbance was measured. The absorbance of the same concentration solution was measured and standard deviation was calculated and presented in **Table 8**

Robustness: Robustness is the measure of its capacity to remain unaffected by small, but deliberate variations in method conditions and its indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of method, the experimental conditions were deliberately altered. The %assay and %R.S.D. were calculated and their values are given in **Table 9**

Limit of Detection and Limit of Quantitation: The limit of detection and limit of quantitation were calculated from the standard deviation and slope. The results obtains were given in **Table 10**.

RESULT AND DISCUSSION

The overlay spectra of DULO and MC exhibit λ max of 291 nm and 350 nm for DULO and MC respectively which are quite distinctly separated from each other. Additionally one isoabsorptive point was observed at 328 nm. Linearity was determined at different concentration; DULO and MC were showed linearity in the concentration range of 8-28µg/ml and 0.6-15 µg/ml with correlation coefficient of 0.998 and 0.999 respectively. The accuracy of the method was confirmed by recovery studies at three different levels of standard additions; recovery in the range of 98 – 102% justifies the accuracy of method. Result of analysis of formulation showed % relative standard deviation values in the range which indicates good repeatability of the method. The reproducibility of sample was expressed in terms of ±SD and % RSD. There was no interference from the common excipients present in tablets. The results i.e. % RSD < 2 signifies the precision of the method. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by standard deviation of response and slope of calibration curve. LOD and LOQ were found to be 1.278µg/ml, 2.330µg/ml for DULO and 0.949µg/ml, 2.857µg/ml for MC respectively.

CONCLUSION

The proposed method was validated as per International Conference on Harmonization (ICH Q_2B) Guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of Duloxetine hydrochloride and Methylcoblamin. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

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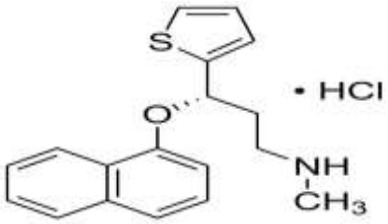

Fig.1a. Structure of Duloxetine hydrochloride

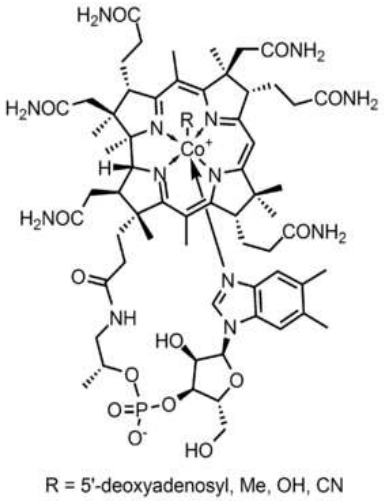

Fig.1b. Structure of Methylcoblamin

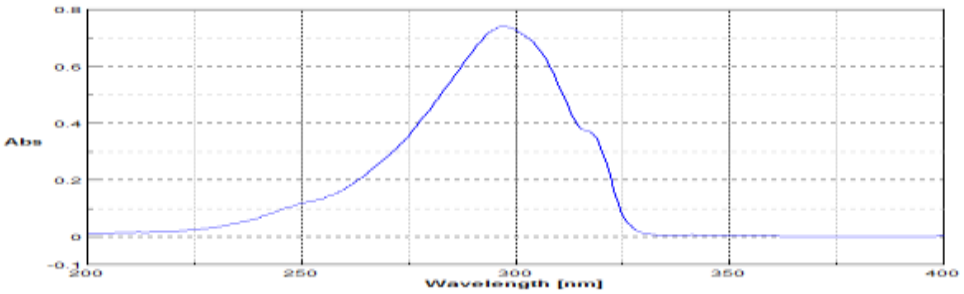

Figure: 2a. UV scan of Duloxetine Hydrochloride (DULO) in distilled water.

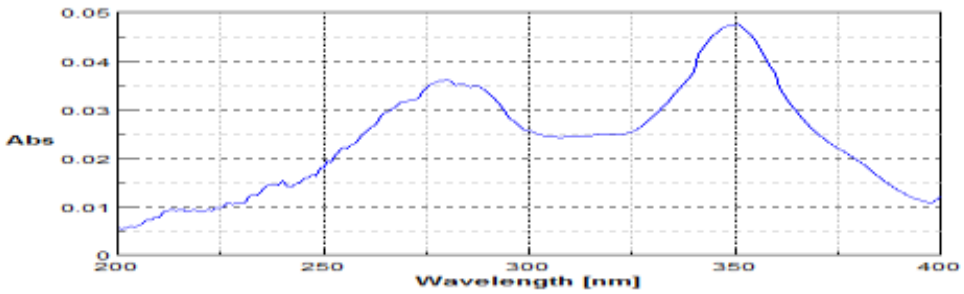

Figure: 2b.UV scan of Methylcobalamin (MC) in double distilled water.

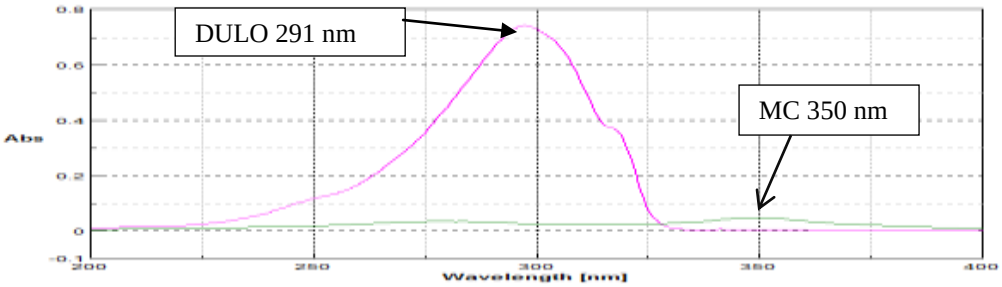

Figure: 3 Overlay spectrum of Duloxetine Hydrochloride (DULO) and Methylcobalamin (MC)

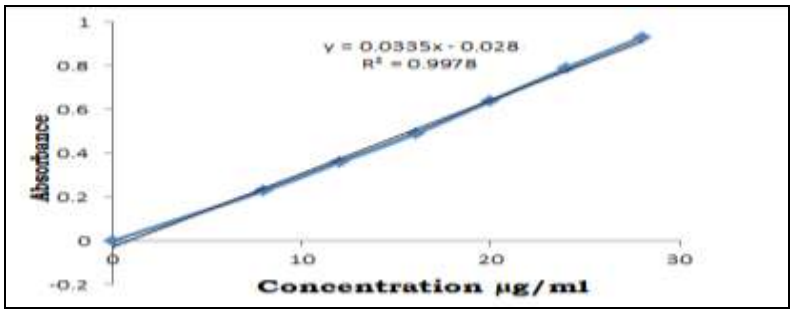

Figure: 4 Calibration curve for DULO at 291.0 nm

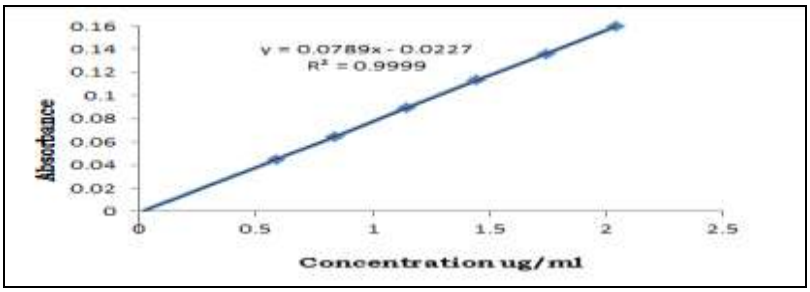

Figure: 5 Calibration curve for MC at 350.0 nm

Table 1: Linearity data for DULO at 291.0 nm

Concentration(µg/ml)	Absorbance
8	0.2297
12	0.3494
16	0.4791
20	0.6388
24	0.7885
28	0.9282

Table 2: Linearity data for MC at 350.0 nm

Concentration (µg/ml)	Absorbance
0.6	0.02481
0.9	0.04762
1.2	0.07243
1.5	0.09624
1.8	0.11905
2.1	0.14286

Table 3: Linear Regression data for calibration curve of DULO and MC

Drug	DULO	MC
Slope	0.0335	0.0789
Intercept	0.028	0.0227
Correlation Coefficient(r^2)	0.998	0.999

Table 4: Linear regression analysis of calibration curves with their respective absorptivity values.

Parameter	DULO	MC
λmax(nm)	291	350
Beer's law limit (µg/ml)	8-28	0.6-15
Molar absorptivity (lit/mole/cm)	11232.724	7611.462
Regression equation	y = 0.0335x -0.028	y = 0.0789x-0.0227
Slope	0.0335	0.0789
Intercept	0.028	0.0227
Correlation Coefficient(r^2)	0.998	0.999

Table 5: Results of analysis of tablet formulation.

Drug	Label Claim(mg)	Amount Found(mg)	%R. S. D.	% Recovery*
DULO	20	20.06	0.1531	100.34
MC	1.5	1.5	0.1077	100.82

** Average of three determinations; R.S.D.; Relative Standard Deviation.*

Table 6:Recovery study data

Drugs	Level of % Recovery	% Recovery* ± S.D.	%R.S.D
DULO	80	100.96 ± 0.961	0.284
	100	99.99 ± 0.430	0.128
	120	100.07 ± 0.162	0.048
MC	80	100.55 ± 0.270	0.118
	100	100.91 ± 0.090	0.049
	120	101.02 ± 0.213	0.088

** Average of six determinations; S.D. Standard deviation, %R.S.D. Relative Standard Deviation*

Table 7:Result of precision study

Day	% Label claim estimated* (Mean ± % R.S.D.)	
	DULO	MC
Intraday	101.39± 0.459	100.42 ± 0.329
Interday	100.33 ± 0.165	100.60 ± 0.469

**Average of six determinations; %R.S.D. Relative Standard Deviation.*

Table 8: Results of repeatability

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S.D.)	%R.S.D.
DULO	20	101.45 ± 0.06	0.018
MC	1.5	99.67 ± 0.75	0.329

** Average of six determinations; S.D. Standard Deviation, R.S.D. Relative Standard Deviation.*

Table 9: Results of robustness (Analysis using methanol [10%])

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S.D.)	%R.S.D.
DULO	20	100.13 ± 0.691	0.207
MC	1.5	100.58 ± 0.270	0.119

** Average of six determinations; S.D. Standard Deviation, R.S.D. Relative Standard Deviation.*

Table 10:LOD and LOQ values

Parameter	Analyte	
	DULO	MC
L.O.D. (µg / ml)*	1.278	0.949
L.O.Q. (µg / ml)*	2.330	2.857

** Average of six determinations; L.O.D. Limit of Detection, L.O.Q. Limit of Quantitation.*

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