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Original Article

A NEW STABILITY INDICATING UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DULOXETINE AND MECOBALAMIN IN BULK AND IN ITS DOSAGE FORMS

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ABSTRACT

Objective: The objective of the work is to develop and validate a stability indicating, new, simple, highly sensitive RP-UPLC method for simultaneous estimation of Duloxetine and Mecobalamin in bulk and in its dosage forms.

Methods: The Method was developed and excellent chromatographic separation was obtained on a reversed-phase Acquity UPLC BEH C_{18} (1.0 × 100 mm, 1.7 µm) column using an isocratic elution mode by the mobile phase using Methanol: Water (55:45). The flow rate of the mobile phase was 1.0mL/min and the total run time was 10 minutes. UV-Spectroscopic detection at a wavelength of 320 nm was performed and the column oven temperature was maintained at 40^{0}C. The analytical procedure was validated by assessing the specificity, linearity, precision, robustness, ruggedness, limit of detection, limit of quantification and accuracy as per ICH guidelines.

Results: The retention times in the standard solution having the concentration of 3 µg/ml and 75 µg/ml of Duloxetine & Mecobalamin were found to be around 4.320 and 5.320 min respectively. The percentage purity values are 99.71 % w/v and 99.02% w/v. Calibration plots were linear (r^2 > 0.999) over the concentration range of 1.5 – 5.25 µg/ml and 37.5 – 131.25 µg/ml, the percentage recovery was found to be 99.74% and 99.82% for Duloxetine & Mecobalamin respectively. %RSD for system precision, method precision, robustness and ruggedness was found to be with in 2. The LOD 0.04791 µg/mL for Duloxetine and 0.4496 µg/mL for Mecobalamin. The LOQ was found to be 0.1452 µg/mL for Duloxetine and 1.3625 µg/mL for Mecobalamin.

Conclusion: The method represents a fast analytical procedure for the simultaneous quantification of Duloxetine & Mecobalamin. The developed method requires lesser analysis time and less mobile phase consumption. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that the method is specific, rapid, reliable, accurate, robust and reproducible. The method is amenable to the routine analysis of large numbers of samples with good precision and accuracy. The most striking feature of this method is its simplicity and rapidity, non-requiring-consuming sample preparation such as extraction with solvents, heating, degassing which are needed for HPLC procedure.

Keywords: RP-UPLC, ICH, Duloxetine, Mecobalamin, UV-Spectroscopy, LOD, LOQ.

INTRODUCTION

UPLC system was designed to provide the highest analysis speed and resolution and at the same time keep system pressure at a minimum. It is fastest, most efficient and most flexible LC system in the world [1, 2]. It is up to 20 times faster with same or better performance, 60% higher, resolution, 10% more sensitive, peak capacity is 1.4 %, reduced runtime from 60 to 20 min (related substances) and cut cost by 60% when compared to HPLC [3].

Duloxetine [4-7], with the chemical name (+)-(S)-N-Methyl-3-(napthalen-1-yloxy)-3-(thiophen-2-yl) propan-1- amine Hydrochloride [I], having the structure as shown in (**fig 1)**. is a selective serotonin and nor epinephrine reuptake inhibitor (SNRI) used for treating depression[8], anxiety disorder, and pain associated with diabetic peripheral neuropathy or fibromyalgia. It prevents the reuptake of serotonin and epinephrine by nerves after they have been released. Since uptake is an important mechanism for removing released neurotransmitters and thus increasing the effect of serotonin and norepinephrine in the brain.

Mecobalamin [9-11] with chemical name Cobinamide, Co-methyl derivative, hydroxide, dihydrogen phosphate (ester), inner salt, 3'-ester with 5, 6-dimethyl-1-α-D-ribofuranosyl-1H-benzimidazole, is a vitamin B-12 co-enzyme[II] having the structure as shown in (**fig 2)**. That occurs in blood and cerebrospinal fluid. It is taken by nervous tissues more actively and accelerates the synthesis of nucleic acid in bone marrow, it increases erythrocyte production by promoting nucleic acid synthesis in the bone marrow and by promoting maturation and division of erythrocytes.

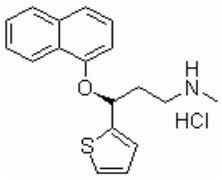

Fig. 1: Chemical Structure of Duloxetine

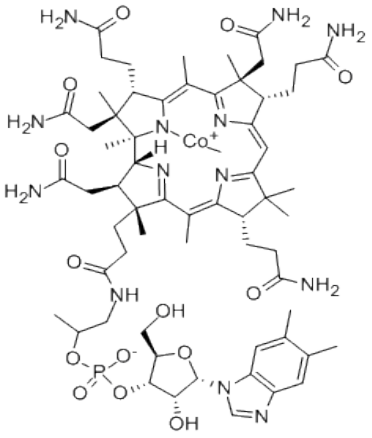

Fig. 2: Chemical Structure of Mecobalamine

There are many reported methods to determine either Duloxetine or Mecobalamin alone or in combination with other drugs in dosage forms. So far there is no stability indicating method reported in any of the Pharmacopoeia or in the literature [12-14] for the simultaneous determination of Duloxetine and Mecobalamin by RP-UPLC method. This paper aims to describe the development and validation of the Stability indicating RP-UPLC method for the simultaneous determination of Duloxetine and Mecobalamin in bulk and in its pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals and reagents

Pure samples of Duloxetine and Mecobalmine were obtained from Orchid Pharmaceuticals, Chennai. The commercial samples of the tablets Dulane-M containing Duloxetine HCl-30 mg and Mecobalamin-750 mg were provided by Sun Pharma. HPLC grade water and Methanol were provided by Merck Ltd. All the other used reagents were of analytical grade.

Chromatographic conditions

Chromatographic separation was achieved by using a Waters e2695 Separation Module UPLC system with PDA detector. The chromatographic column utilized in these studies was an Acquity UPLC BEH C_{18} (1.0 × 100 mm, 1.7 µm). The mobile phase consists of 550mL (55 %) of Methanol and 450mL of water (45 %). It is degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum filtration. The flow rate selected was 1.0mL/min with wave length of 320 nm. All the determinations were performed at column oven temperature 40^0 C and an injector volume is 10µl. The spectra was obtained from the PDA detector.

Preparation of standard solution of duloxetine and mecobalamin

Accurately 30 mg and 750 mg of Duloxetine & Mecobalamin were weighed and transferred into 100mL volumetric flask, about 20mL of diluent was added and sonicated for 20 minutes to dissolve it. The volume was made up with diluent. From this 1mL of the solution was pipetted out and transferred into 100mL the volumetric flask and made up with diluent to give concentrations of 3 µg/mL and 75 µg/mL of Duloxetine & Mecobalamin respectively.

Preparation of sample solution of duloxetine and mecobalamin

Dulane-M tablets containing 30mg Duloxetine and 750mg Mecobalamin were weighed and the average weight was calculated. The tablets were powdered in a mortar and an amount of powder of 1190 mg equivalent to 30 mg of Duloxetine and 750 mg of Mecobalamin were weighed accurately and transferred into a 100mL volumetric flask containing 25mL of mobile phase and sonicated for 30 min., diluted to 100mL with mobile phase, then the solution was filtered through 0.45 µm membrane filter and 1mL of filtrate taken into 100mL volumetric flask and made up to the volume with mobile phase, having a concentration of 3µg/mL of Duloxetine and 75µg/mL of Mecobalamin respectively.

Assay

10 µl of standard and sample solutions were injected into the injector of UPLC, and the peak areas of the drugs in standard and sample were compared and assay was performed.

Method development

The RP-UPLC method development for the estimation of Duloxetine and Mecobalamin involves Development of suitable mobile phase, Optimization of the chromatographic conditions and selection of suitable detection wavelength.

Selection of detector wavelength

Since both the compounds shown maximum absorbance at 320 nm, it was selected for the experiment.

Optimized chromatographic conditions

As the drug was polar in nature, RP-UPLC method was preferred. Five trials were performed by changing the mobile phase compositions for better resolution with less tailing factor. As seen from the chromatogram **(fig 3)**, the final trial was found to be optimized method. The R_t were observed at 4.320 (Duloxetine) and 5.320 (Mecobalamin). The peaks are sharply resolved with less tailing and hence the final trial method is optimized for analysis.

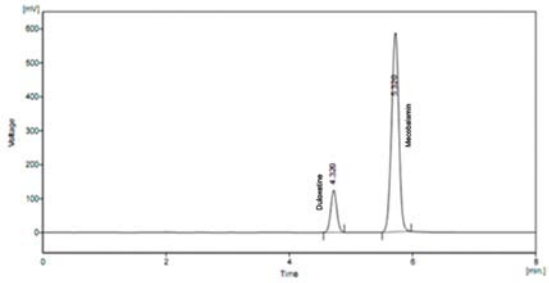

Fig. 3: Chromatogram showing optimized method for duloxetine and mecobalamin

Method validation

The proposed method was validated with the aspect of system suitability test, specificity, linearity and range, accuracy, precision, LOD, LOQ, robustness and ruggedness according to ICH guidelines.

System suitability test

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated and the parameters like tailing factor, retention time, theoretical plates per unit, resolution factor are determined and the results are tabulated.

For system suitability, six replicates of standard solutions of Duloxetine and Mecobalamin were injected and studied for the suitability parameters like Plate number (N), Resolution (R) and Relative retention time (α), and Peak symmetry of samples (As) were studied with the help of standard chromatograms.

Preparation of standard solution for system suitability

Accurately 30 mg and 750 mg of Duloxetine & Mecobalamin respectively were weighed and transferred into 100mL volumetric flask, about 20mL of diluent was added and sonicated for 20 minutes to dissolve it. The volume was made up with diluent.

From this 1mL of solution was pipetted out and transferred into 100mL volumetric flask and the volume was made up with diluent to give a concentrations of 3 µg/mL and 75 µg/mL of Duloxetine & Mecobalamin respectively.

Inject 10 µl of the standard solution into the chromatographic system and measure the area for of Duloxetine & Mecobalamin peaks.

Specificity

Specificity is the ability to assess unequally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix etc.

Procedure

Preparation of placebo

Placebo was prepared by mixing all the excipients without active ingredients. Accurately 410 mg of placebo was weighed and transferred into 100mL volumetric flask, about 20mL of diluent was added and sonicated for 20 minutes to dissolve it. The volume was made up with diluent. The solution was filtered through 0.45 µm membrane filter (Stock solution).

From this 1mL of solution was pipetted out and transferred into 100mL volumetric flask and the volume was made up with diluent to give concentrations of 3 µg/mL and 75 µg/mL of Duloxetine and Mecobalamin respectively. Inject 10 µl of standard solution for six times into the system. The chromatograms were studied and checked for any interference.

Preparation of standard solution for specificity

Accurately 30 mg and 750 mg of Duloxetine and Mecobalamin were weighed and transferred into 100mL volumetric flask, about 20mL of diluent was added and sonicated for 20 minutes to dissolve it. The volume was made up with diluent. The solution was filtered through 0.45 µm membrane filter (Stock solution).

From this 1mL of the solution was pipetted out and transferred into 100mL the volumetric flask and the volume was made up with diluent to give concentrations of 3 µg/mL and 75 µg/mL of Duloxetine and Mecobalamin respectively. Inject 10 µl of Standard solution for six times into the system. The chromatograms were studied and checked for any interference.

Preparation of standard solution + placebo for specificity

Accurately 30 mg and 750 mg of Duloxetine and Mecobalamin were weighed and transferred into 100mL volumetric flask. To this add 410 mg of Placebo, about 20mL of diluent was added and sonicated for 20 minutes to dissolve it. The volume was made up with diluent. The solution was filtered through 0.45 µm membrane filter (Stock solution). From this 1mL of the solution was pipetted out and transferred into 100mL the volumetric flask and the volume was made up with diluent to give concentrations of 3 µg/mL and 75 µg/mL of Duloxetine and Mecobalamin respectively. Inject 10 µl of Standard solution for six times into the system.

Linearity and range

Preparation of standard stock solution

Accurately 30 mg and 750 mg of Duloxetine & Mecobalamin respectively were weighed and transferred into 100mL volumetric flask, about 20mL of diluent was added and sonicated for 20 minutes to dissolve it. The volume was made up with diluent. The solution was filtered through 0.45 µm membrane filter (Stock solution). From the above stock solution pipette out 0.5, 0.75, 1.0, 1.25 1.5mL and 1.75mL respectively into individual 100mL of volumetric flasks and diluted up to the mark with diluent to prepare the 37.5, 56.25, 75.0, 93.75, 112.5, 131.25 µg/mL solutions of Mecobalamin and 1.5, 2.25, 3.0, 3.75, 4.5, 5.25 µg/mL solutions of Duloxetine. Mix well and filter through 0.45 µm filter. Inject 10 µl of blank solution and each linearity level standard solutions into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

$$Correl(X,Y) = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{\sum (x-\bar{x})^2 \sum (y-\bar{y})^2}}$$

Precision

System precision

Accurately 30 mg and 750 mg of Duloxetine and Mecobalamin respectively were weighed and transferred into 100mL volumetric flask, about 20mL of diluent was added and sonicated for 20 minutes to dissolve it. The volume was made up with diluent. The solution was filtered through 0.45 µm membrane filter (Stock solution). From this 1mL of the solution was pipetted out and transferred into 100mL volumetric flask and the volume was made up with diluent to give a concentrations of 3µg/mL and 75 µg/mL of duloxetine and Mecobalamin respectively. Inject 10 µl of Standard solution for six times into the system. The chromatograms were studied and checked.

Method precision

Accurately Transfer the 1190 mg of the sample into a 100mL volumetric flask and 20mL of diluent was added and sonicate for 20 min and made up to the mark with diluent. From this 1mL of solution was pipetted out and transferred into 100mL volumetric flask and the volume was made up with diluent to give a concentrations of 3 µg/mL and 75 µg/mL of Duloxetine and Mecobalamin respectively. Inject 10 µl of the six replicate injections of sample solution of duloxetine and mecobalamin 10 µg/mL for six times and calculate the %RSD for the area of six replicate injections.

Accuracy

Accuracy expresses the closeness of agreement between the value, which was accepted either as conventional true value or and accepted reference value (International standard e. g pharmaceutical standard) and the value found (mean value) obtained by applying the test procedure a number of times. To study reliability, suitability and accuracy of the method, recovery studies were carried out, by adding a known quantity of the standard to the pre analysed sample and recovery study were done. The recovery was carried out at 80%, 100%, 120% level and the contents were determined from the respective chromatogram. From the results obtained we conclude that the method was accurate.

Preparation of 80 %, 100% and 120% solutions

Accurately 30 mg and 750 mg of Duloxetine and Mecobalamin were weighed and transferred into 100mL volumetric flask. To this add 410 mg of Placebo, about 20mL of diluent was added and sonicated for 20 minutes to dissolve it.

The volume was made up with diluent. The solution was filtered through 0.45 µm membrane filter (Stock solution). From this 0.8, 1 and 1.2mL of solution was pipetted out, transfer it in to three different 100mL volumetric flask and the volume was made up with diluent to give a concentrations of 2.4 g/ml and 60 µg/mL; 3 µg/mL and 75 µg/mL; 3.6µg/mL and 90 µg/mL of Duloxetine & Mecobalamin respectively. Inject 10 µl of placebo and solutions of Accuracy – 80 %, Accuracy -100 % and Accuracy - 120 % solutions into system. Now calculate the amount obtained and the amount added (API) for Duloxetine & Mecobalamin samples. Calculate the concentration in µg/ml in the spiked placebo in all the above cases by comparing with the standard solution. Calculate the individual recovery and mean recovery values.

Formula

% recovery = 100 X (Amount recovered) /(Actual amount added)

Robustness

It is a measure of ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the developed method experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes in chromatographic conditions and allow routine analysis of the sample. Effect of column temperature, and effect of change in flow rate was carried out and standard was injected. There was no change in system suitability parameters.

Preparation of standard solution for robustness

Accurately 30 mg and 750 mg of Duloxetine and Mecobalamin were weighed and transferred into 100mL volumetric flask, about 20mL of diluent was added and sonicated for 20 minutes to dissolve it. The volume was made up with diluent. The solution was filtered through 0.45 µm membrane filter (Stock solution). From this 1mL of solution was pipetted out and transferred into 100mL the volumetric flask and the volume was made up with diluent to give a concentrations of 3 µg/mL and 75 µg/mL of Duloxetine and Mecobalamin respectively.

A) Temperature variation

10 µl of the standard solution of robustness was injected for six times and analysed using variable temperature $40^0C \pm 5^0C$.

B) Flow rate variation

10 µl of the standard solution of robustness was injected for six times and analysed using varied flow rates (0.8mL, 1.2mL) along with method flow.

Ruggedness

Ruggedness was determined by carrying out the assay under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. There was reproducibility of test results under normal, expected operational conditions from laboratory and from analyst to analyst. The method is rugged and does not show variations in the results on slight variations of parameters.

Limit of detection

Limit of detection is the least concentration of the analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. It is determined based on the standard deviation of response and the slope. The detection limit may be expressed as

$$LOD = \frac{3.3\,\sigma}{S}$$

Where:

σ = standard deviaton of standard

S = Slope of calibration curve

Limit of quantification

Limit of quantitation is the least concentration of the analyte in a sample that can be estimated quantitatively by injecting decreasing amount of drug with acceptable precision and accuracy under the stated experimental conditions of the method. Based on the standard deviation of the response and the slope. Limit of Quantification (LOQ) may be expressed as

$$LOQ = \frac{10\,\sigma}{S}$$

Where:

σ = standard deviation of standard

S = Slope of calibration curve

Degradation studies

Degradation studies were carried out as per ICH guidelines. The sample solutions were subjected to acidic, basic, peroxide, water and light.

Standard stock solution preparation for degradation studies

Accurately weigh and transfer about 30 mg of Duloxetine and 750 mg of Mecobalamin standard into a 100mL dry volumetric flask add about 70mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Hydrolytic degradation under acidic condition

1.0mL of of the above standard stock solution, add 3mL of 0.1N HCl was added in 10mL of the volumetric flask. The volumetric flask was kept at normal condition for 90 minutes and then neutralized with 0.1 N NaOH and make up to 10mL with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition

1.0mL of of the above standard stock solution, add 3mL of 0.1N NaOH was added in 10mL the volumetric flask. The volumetric flask was kept at normal condition for 90 minutes and then neutralized with 0.1 N HCL and make up to 10mL with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Thermal induced degradation

1.0mL of the above standard stock solution, add 3mL of diluent was added in 10mL volumetric flask. The volumetric flask was kept at reflex condition for 60 minutes and make up to 10mL with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Oxidative degradation

1.0mL of of the above standard stock solution add 1mL of 3 % w/v of hydrogen peroxide added in 10mL of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photolytic degradation

1.0mL of the above standard stock solution was taken in 10mL of volumetric flask and the volume was made up to the mark with diluents. The volumetric flask was then exposed to light for 12 hr. Filter the solution with 0.45 microns syringe filters and place in vials.

RESULTS AND DISCUSSION

The result from development activity is that a suitable, easy, less time-consuming validated method has been developed for Duloxetine & Mecobalamin. The retention times of Duloxetine & Mecobalamin in the standard solution having the concentration of 3µg/ml and 75µg/ml of Duloxetine & Mecobalamin were found to be around 4.320 min and 5.320 respectively. Duloxetine & Mecobalamin shows the percentage purity values are 99.71 % w/v and 99.02% w/v respectively.

System suitability test

System suitability parameters such as resolution, tailing factor, no. of theoretical plates were calculated. The acceptance criteria was less than 2% relative standard deviations (*RSD*) for peak areas, not less than 2000 column plates, and not less than 2.0 tailing factor. The tailing factor values for duloxetine and mecobalamine indicating the symmetrical nature of the peak.

The no. of theoretical plates was high indicating the efficient performance of the column. The results obtained were all within the acceptable limits. The results are reported in Table-1. A typical chromatogram for the system suitability test is shown in fig. 4.

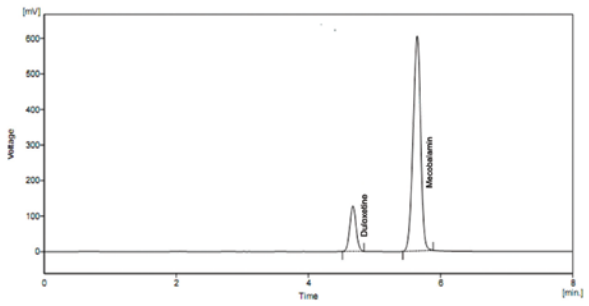

Fig. 4: Chromatogram of the system suitability test solution

Table 1: System suitability data for Duloxetine and Mecobalamin

System Suitability Parameters	Duloxetine	Mecobalamin
Resolution	3.746	
Tailing Factor	1.0402	0.918
Number of theoretical Plates	3594	3334
Retention time	4.348	5.342

Specificity

There was no shift in wavelength interference due to placebo. This confirms the specificity of the proposed method**.** There is no peak in the blank and Placebo solution run at the retention time corresponding to Duloxetine and Mecobalamin as in standard run as shown in (fig 5).

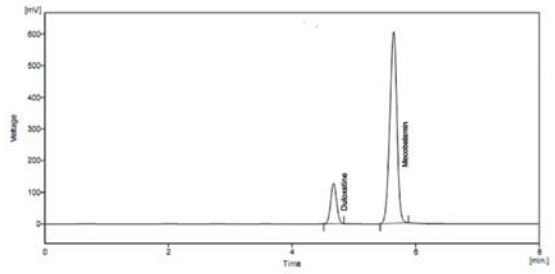

Fig. 5: Chromatogram showing specificity of Duloxetine and Mecobalamin

Linearity and range

Linearity was evaluated by visual inspection of plot of peak area as a function of analyte concentrations for Duloxetine and Mecobalamin. The linearity was checked by analyzing six working solutions of Duloxetine over the concentrations range 1.5-5.25 µg/mL (1.5, 2.25, 3.0, 3.75, 4.5, 5.25 µg/mL) and 37.5-131.25 µg/mL (37.5, 56.25, 75.0, 93.75, 112.5, 131.25 µg/mL for Mecobalamin.

The following results were obtained: slope=180.4 r^2= 0.999 for Duloxetine, and slope= 39.07 r^2= 0.999 for Mecobalamin. The results indicated that the method was linear over the concentration range study. The results are reported in Table-2. The calibration curves are shown in (fig. 6 & 7).

Table 2: Linearity Results for Duloxetine and Mecobalamin

Parameters	Duloxetine	Mecobalamin
Linear Dynamic Range	1.5 – 5.25 µg/ml	37.5 – 131.25 µg/ml
Correlation Coefficient	0.999	0.999
Slope (m)	180.4	39.07

Table 3: system precision data for Duloxetine

	Sample Name	Name	Peak Area
1	Sys pre	Duloxetine	804.202
2	Sys pre	Duloxetine	806.602
3	Sys pre	Duloxetine	816.968
4	Sys pre	Duloxetine	817.332
5	Sys pre	Duloxetine	815.694
6	Sys pre	Duloxetine	804.202
Mean			810.833
Std. Dev			6.470645
%RSD			0.798024

Table 4: system precision data for Mecobalamin

	Sample Name	Name	Peak Area
1	Sys pre	Mecobalamin	4664.222
2	Sys pre	Mecobalamin	4665.943
3	Sys pre	Mecobalamin	4562.585
4	Sys pre	Mecobalamin	4663.066
5	Sys pre	Mecobalamin	4664.558
6	Sys pre	Mecobalamin	4664.222
Mean			4647.433
Std. Dev			41.57692
%RSD			0.89462

Table 5: Method precision data for Duloxetine

S. No.	Sample Name	Name	Peak Area
1.	Method Precision	Duloxetine	833.187
2.	Method Precision	Duloxetine	826.814
3.	Method Precision	Duloxetine	828.435
4.	Method Precision	Duloxetine	811.505
5.	Method Precision	Duloxetine	822.505
6.	Method Precision	Duloxetine	811.505
Mean			822.325
SD			9.05144
% RSD			1.100711

Table 6: Method precision data for Mecobalamin

S. No.	Sample Name	Name	Peak area
1	Method Precision	Mecobalamin	4565.943
2	Method Precision	Mecobalamin	4664.222
3	Method Precision	Mecobalamin	4572.585
4	Method Precision	Mecobalamin	4663.066
5	Method Precision	Mecobalamin	4654.558
6	Method Precision	Mecobalamin	4663.066
Mean			4628.74
SD			46.75883
% RSD			1.010184

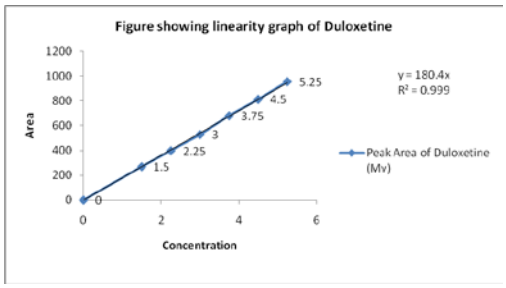

Fig. 6: Linearity of Duloxetine

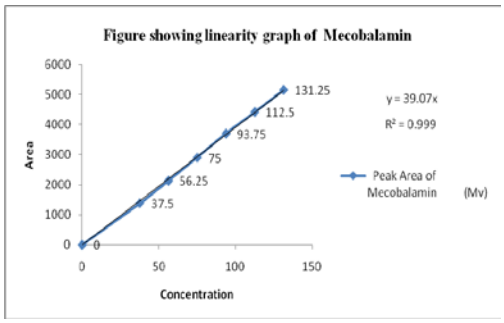

Fig. 7: Linearity of Mecobalamin

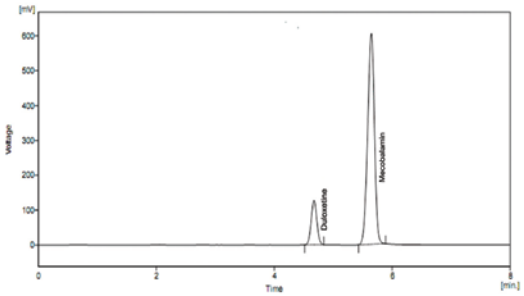

Fig. 8: Chromatogram showing system precision

Precision

System precision

The precision of the system was determined by six replicate injections of mixed standard solution. The % R. S. D of Area and retention time are within the acceptance criteria of 2 %. The results are reported in Table-3 & 4. The chromatograms are shown in (fig 8).

Method precision

The precision of the method was determined by six replicate injections of the sample solution. The % R. S. D of Area and retention time and assay are present within the acceptance criteria of 2 %. The results are reported in Table-5 & 6. The chromatograms are shown in (fig.9).

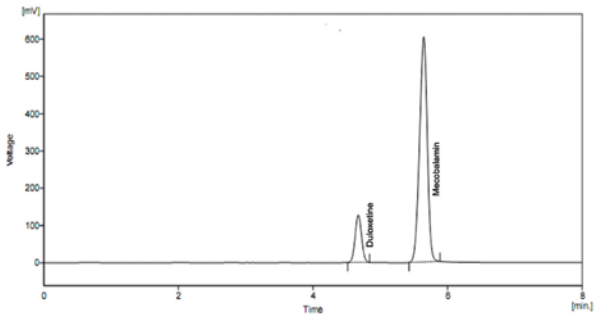

Fig. 9: Chromatogram showing method precision
Thus the proposed method was found to be high degree of precision and reproducibility.

Accuracy

The validation of the proposed method was further verified by recovery studies. Acceptance criteria are 98 - 102 % w/v. The results are reported in table -7 and 8.

Robustness

Robustness was determined by carrying out the assay during which flow rate and temperature were altered slightly. The results are reported in Table- 9.

Table 7: Recovery studies for Duloxetine and Mecobalamin

S. No.	Inj. Sample	Spike level	Amount Present	Amount Recovered	% Recovered
1		80 %	2.4mcg	2.3976	99.765%
2	**Duloxetine**	100 %	3.0mcg	2.971	99.77%
3		120 %	3.6mcg	3.598	99.70%
4.		80 %	60mcg	59.968	99.60%
5	**Mecobalamin**	100 %	75 mcg	74.951	99.99%
6		120 %	90mcg	89.872	99.89%

Table 8: Mean average recovery of duloxetine and mecobalamin for accuracy

Accuracy level	Mean Recovery of Duloxetine	Mean Recovery of Mecobalamin
Accuracy 80%	99.76	99.6
Accuracy 100%	99.77	99.99
Accuracy 120%	99.70	99.89

This serves as a good index of the accuracy and reproducibility of the proposed method.

Table 9: Robustness data for Duloxetine and Mecobalamin

Parameters	% RSD Duloxetine	% RSD Mecobalamin
Flow rate	0.003	0.003
Temperature	0.008	0.007

% RSD values for robustness indicated that the method is robust and does not show variations in the results on slight variations in flow rate and temperature.

Table 10: Ruggedness data for Duloxetine and Mecobalamin

Parameters	% RSD Duloxetine	% RSD Mecobalamin
Analyst 1	0.004	0.008
Analyst 2	0.008	0.007

% RSD values for ruggedness indicated that the method is rugged and does not show variations in the results when performed by different analysts.

Ruggedness

% RSD values for ruggedness indicated that the method is rugged and does not show variations in the results when performed by different analysts. The results are reported in table - 10.

Limit of detection

The limit of detection of Duloxetine and Mecobalamin was calculated and found to be 0.04791µg/mL and 0.4496µg/mL respectively.

Limit of quantification

The limit of quantification of Duloxetine and Mecobalamin was calculated and found to be 0.1452 µg/mL and 1.3625 µg/mL respectively.

Degradation studies

Degradation studies were carried out as per ICH guidelines. The sample solutions were subjected to acidic, basic, peroxide, water and light.

Where as in acidic, basic the % degradations were found to be -8%, -6 % and -7 %, -8 % for Duloxetine and mecobalamin respectively. The % degradation by peroxide was found to be -7 % and -6 %. The % degradation by water was found to be -6 % and -8 %.

The solid sample was subjected to light for 7 days and then the % degradation was found to be -4 % and -5 %. The results are reported in Table-11.

Table 11: Results of Degradation studies for Duloxetine and mecobalamin

S. No.	Name	Sample weight	Sample area-Duloxetine	Sample area-Mecobalamin	% Assay-Duloxetine	% Assay-Mecobalamin	% DEG-Duloxetine	% DEG-Mecobalamin
1	Acid	1190	788.04	4602.12	91	93	-8	-6
2	Base	1190	796.08	4624.38	92	91	-7	-8
3	Peroxide	1191	796.56	4624.56	92	93	-7	-6
4	Water	1191	800.12	4645.32	93	91	-6	-8
5	Light	1190	802.22	4653.24	95	94	-4	-5

CONCLUSION

A new stability indicating analytical method is developed and validated for Simultaneous Estimation of Duloxetine and Mecobalamine by RP-UPLC technique. The sample preparation is simple, consumes less amount of mobile phase and the required time for analysis is very short, the information presented in the study could be very useful for the quality monitoring of Duloxetine and Mecobalamine in combined pharmaceutical dosage forms and can be used to check drug quality during stability testing. The analytical procedure is validated as per ICH guidelines and shown to be accurate, precise and specific. This method represents a fast analytical procedure for the simultaneous quantification of Duloxetine and Mecobalamine. The method is amenable for the routine analysis of large numbers of samples with good precision and accuracy. The short chromatographic time makes this method suitable for processing of multiple samples in a limited amount of time.

CONFLICT OF INTERESTS

Declared None

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