Research Article

Method Development and Validation for the Simultaneous Analysis of Duloxetine HCL and Methylcobalamine by RP-HPLC

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ABSTRACT

A simple, accurate and precise method for simultaneous estimation of Methylcobalamin and Duloxetine by RP-HPLC method has been developed. The chromatographic conditions were successfully developed by using inertsil ODS C18 5µm (4.6*250mm) column, the flow rate was 1ml/min, the mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The retention times were found to be 3.594 min and 5.328 min. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The suggested RP-HPLC method can be used for routine analysis of Methylcobalamin and Duloxetine in API and Pharmaceutical dosage form.

Keywords: Duloxetine, Methylcobalamin, RP-HPLC, Phosphate buffer, orthophosphoric acid.

INTRODUCTION

Duloxetine (DXT) chemical designation is (+)-(S)-N-methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride. The empirical formula is $C_{18}H_{19}NOS \cdot HCl$, which corresponds to a molecular weight of 333.88. The structural formula is:

It is used to treat depression and anxiety, it help relieve nerve pain in people with diabetes or ongoing pain due to a medical condition as arthritis, chronic back pain or fibromyalgia. It is also known as serotonin –nor epinephrine reuptake inhibitor (SNRI). This medication works by helping to restore the balance of certain natural substances in the brain[1,2].

Methylcobalamin (MCB) is a cobalamin (MeB12) used in peripheral neuropathy, diabetic neuropathy etc. It is a form of vitamin B12. This vitamer is one of two active coenzymes used by B-12 dependent enzymes in the body, and is specifically the B-12 form used by 5-methyltetrahydrofolate-homocysteine.

Methyltransferase (MTR), also known as methionine synthase[3,4]. MCB is notable for being one of the few examples in nature of a bona fide organometallic bond. MCB has been studied in conjunction with sleep-wake rhythm disorders, where it appears to yield benefits, but at a low or inconsistent level[5,6]. It is used in treating diseases of vitamin B12 deficiency (such as pernicious anemia), or diseases of effective B12 deficiency, such as vitamin B12 metabolic pathway pathologies. One study suggests that once absorbed, MCB may be retained in the body better than cyanocobalamin[7].

DXT and MCB are recent combination in the market for the treatment of anti-depressant, anti-anxiety. Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Duloxetine Hcl and MCB. The developed method will be validated according to ICH guidelines[8, 9].
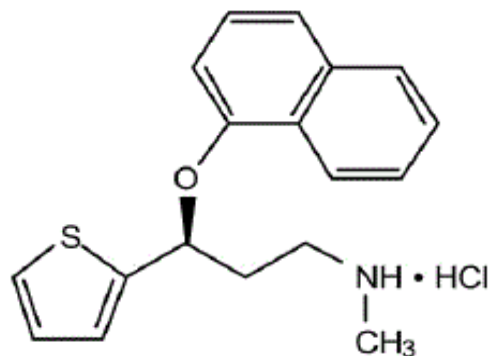
MATERIAL AND METHODS

Chemicals

Pharmaceutical grade Duloxetine and MCB Was Obtained Gift Sample from Pharma Train Rajahmundry, A.P. 10mg of Duloxetine and 10mg of MCB of 10 tablets were purchased in sun Pharma, Vizac, A.P.

Instruments

HPLC Alliance Waters 2695 attached with computer operated software Empower. UV double beam Lab India attached with computer operated software UV Win 5, Ultra sonicator SE60US, Suction pump VE115N were used during the research.

Method Development

Method development for simultaneous estimation of Duloxetine Hcl and MCB in Pharmaceutical dosage forms.

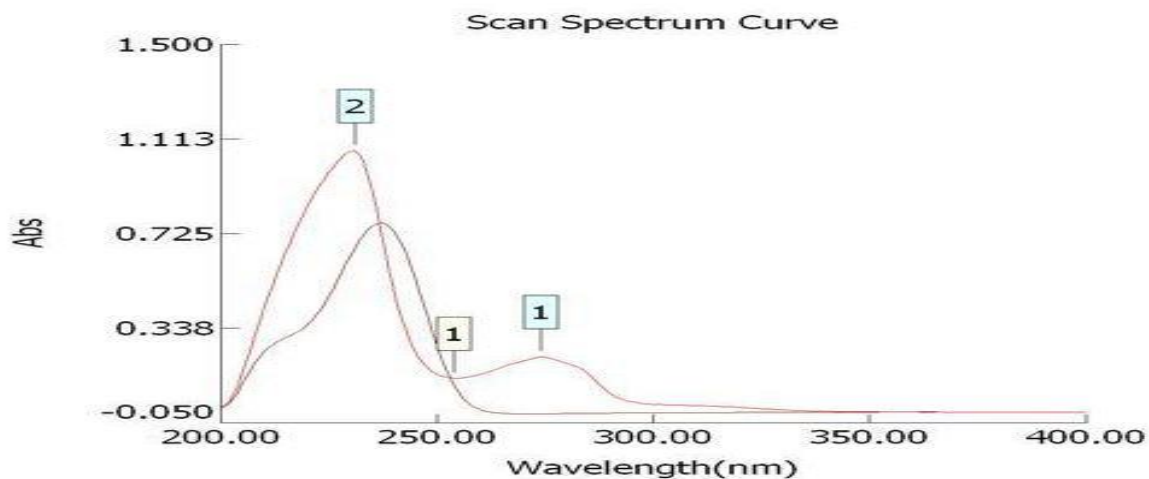*Author for Correspondence: anandpharmacy@cvsr.ac.in*

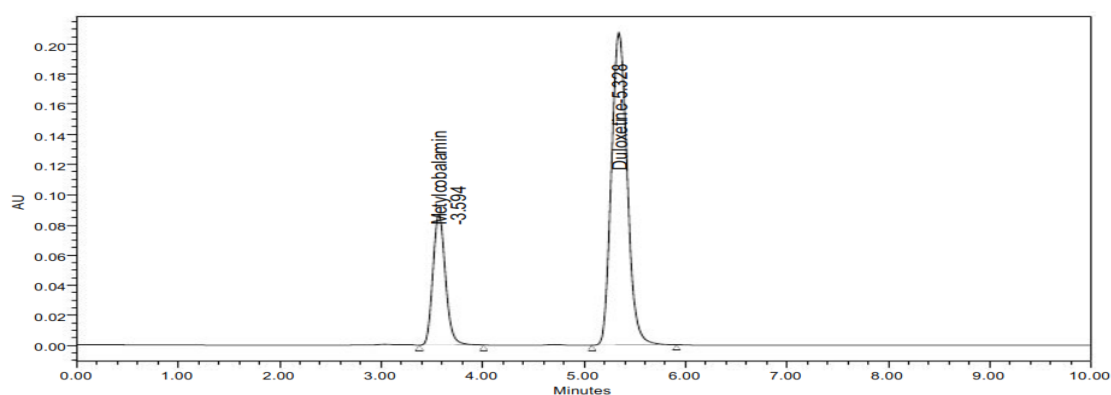Figure 1: Overlay spectrum of DXT and MCB.

Figure 2: Optimized method for MCB and DXT sample solutions.
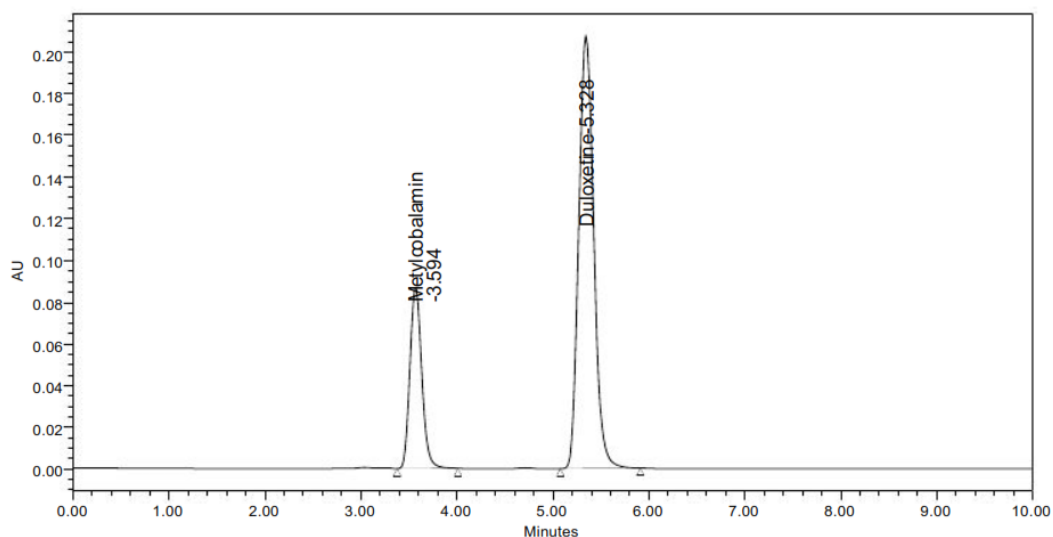

Figure 3: Chromatogram of Trail-5.

Wavelength Detection

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of DXT and MCB Was obtained and the isobestic point of DXT and MCB showed absorbance's maxima at 260 nm.

Optimized Chromatographic conditions

Column : Inertsil C18 5µm (4.6*250mm)
Mobile phase ratio: Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v)
Detection wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 20µl
Column temperature : Ambient

Table 1: Details of Optimized Chromatographic conditions.

S.No	Peak name	R_t	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	MCBe	3.594	946124	155429	5105	1.3	8.1
2	DXT MCBe	5.328	111541	13239	3788	1.4	

Table 2: Accuracy of the developed method

	Added (mg mL^{-1})	Found (mg mL^{-1})	% Recovery	Mean Recovery
DXT	5	5.10	101.8%	100.26
	10	9.99	99.9%	
	15	14.9	99.1%	
MCBe	5	5.0	101.3%	99.96
	10	9.94	99.4%	
	15	14.8	99.2%	

Table 3: Accuracy results of MCB.

% Conc	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
50%	2332744	5	5.10	101.8%	100.5 %
100%	3132697	10	9.99	99.9%	
150%	3918997	15	14.9	99.1%	

The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

Method development for simultaneous estimation of DXT Hcl and MCB in Pharmaceutical dosage forms.

General procedures

Preparation of Phosphate buffer (PH 4.6)

6.8 gm of KH2PO4 was dissolved in 1000ml with MilliQ water and adjusted the pH to 4.6 with ortho phosphoric acid

Preparation of mobile phase

A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of ACN (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

Diluent preparation

Mobile phase is used as Diluent

Preparation of the standard stock solutions

Standard Stock solutions of DXT and MCB were prepared separately by dissolving each of 10 mg in 2 ml of DMF and made up to volume 100 ml with diluent.

Preparation of Sample Solution (Tablet)

Ten tablets were weighed and crushed in mortar and pestle and weight equivalent to 10 mg of MC and DT (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of diluent is added and sonicated to dissolve it completely and make volume up to the mark. 3 ml of above stock solution was pipetted into a 10 ml volumetric flask and diluted with diluent.

20µL of the standard, sample are injected into the chromatographic system and the areas for MCB and DXT peaks were measured and the % Assay was calculated by using the formulae.

Assay calculation:

$$Assay\% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where,

P = Percentage purity of working standard

Lc = label claim of drug in mg/ml.

System Suitability

Tailing factor for the peaks due to MCB and DXT in Standard solution should not be more than 2.0 and Theoretical plates should not be less than 2000.

Analytical method validation

Accuracy

The accuracy of the method was measured by using three concentrations of MCB and DXT at 50, 100 and 150 %. The amount found and amount added for MC and DT and the individual recovery and mean recovery values were calculated. The acceptance criteria is the correlation coefficient should be not less than 0.999.

Precision

Repeatability

The standard solution was injected for five times and the areas for all five injections in HPLC were measured. The % RSD for the area of five replicate injections was found to be within the specified limits. The acceptance criteria should be that % RSD for the area of five standard injections results should not be more than 2.

Intermediate Precision (Ruggedness)

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different columns of same dimensions. The standard solution was injected for five times and the area for all five injections measured in HPLC. The % RSD for the area of five sample injections results should not be more than 2%.

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

LOD and LOQ

LOD and LOQ could be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S).

Linearity

The linearity of the method was determined are concentration levels of 1-5 µg/ml for MCB and 100- 500 µg/ml for DXT

Robustness

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

The flow rate was varied at 0.8ml/min to 1.2 ml/min.

Table 4: Accuracy results of DXT.

%Concentration(at specification level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	353867	5	5.0	101.3%	
100%	4735088	10	9.94	99.4%	100.0%
150%	5911798	15	14.8	99.2%	

Table 5: Repeatability results of MCB.

	Peak name	RT	Area
1	MCB	3.576	750376
2	MCB	3.561	750874
3	MCB	3.597	751087
4	MCB	3.532	751477
5	MCB	3.542	751720
6	MCB	3.539	751365
Mean			751149.8
Std.dev			481.0977
%RSD			0.064048

Standard solution 3ppm of DXT Hcl and 300ppm of MCB was prepared and analyzed using the varied flow rates along with method flow rate.

The organic composition in the mobile phase was varied from 65% to 75 % standard solution 3 µg/ml of DXT Hcl and 300 µg/ml of MCB were prepared and analyzed using the varied mobile phase composition along with the actual mobile phase composition in the method.

RESULTS

Wavelength Detection

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overall spectrum of DXT and MCB was obtained and the isobestic point of DXT and MCB showed absorbance's maxima at 260 nm.

Method Development

The chromatographic method development for the simultaneous estimation of DXT and MCB were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the optimized chromatographic method was selected for the separation and quantification of DT and MC in API and pharmaceutical dosage form by RP-HPLC method.

Optimized Chromatographic conditions

Column : Inertsil C18 5µm (4.6*250mm)
Mobile phase ratio : Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v)
Detection wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 20µl
Column temperature : Ambient

System Suitability

The system suitability parameters for DT and MC such as theoretical plates and tailing factor were found to be 5663, 1.13 and 4019, 1.11. Resolution was 6.8. The %

purity of DXT and MCB in pharmaceutical dosage form was found to be 100.7% and 101.4% respectively.

Method Validation

Accuracy

The accuracy study was performed for 50%, 100% and 150 % for DXT and MCB. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery.

Precision

Repeatability

Intermediate precision (Ruggedness)

Repeatability

The precision study was p e r f o r m e d for five injections of DXT and MCB. Each standard injection was injected in to chromatographic system. The area of each Standard injection was used for calculation of % RSD.

Intermediate precision/Ruggedness

The intermediate precision study was performed for five injections of DXT and MCB. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD.

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The chromatograms are shown in Fig.4.37, 4.38.

The specificity test was performed for DXT and MCB. It was found that there was no interference of impurities in retention time of analytical peak.

Detection of limit

Linearity and Range

The linearity study was performed for the concentration of 100-500 ppm and 1- 5 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The chromatograms are obtained and results are tabulated in Table. 4.26, 4.27 Calibration graph for DXT and MCB are shown in Fig. Limit of detection (LOD) and limit of quantitation (LOQ):

The signal-to-noise ratios of 3:1 and 10:1 were taken as LOD and LOQ, respectively and the values are given in Table:

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

Flow Rate

The robustness was performed for the flow rate variations from 0.8 ml/min to 2ml/min. Standard solution 60 µg/ml of MCB & 30µg/ml of DXT was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of

Table 6: Repeatability results of DXT.

	Peak name	RT	Area
1	DXT	5.351	2249906
2	DXT	5.332	2249724
3	DXT	5.326	2249452
4	DXT	5.370	2249197
5	DXT	5.337	2247457
6	DXT	5.319	2247088
Mean			2248804
Std.dev			1216.077
%RSD			0.054077

the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min.

Mobile Phase: The Organic composition in the Mobile phase was varied from 70% to 60%. Standard solution 300 µg/ml of MCB & 3µg/ml of DXT was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

CONCLUSIONS

A new method was established for simultaneous estimation of MCB and DXT by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of MCB and DXT by using inertsil ODS C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm.

The retention times were found to be 3.594m and 5.328m. The % purity of MCB and DXT was found to be 100.7% and 101.4% respectively. The system suitability parameters for MCB and DXT such as theoretical plates and tailing factor were found to be 1.13, 4019 and 1.12, 5533 the resolution was found to be 6.8.

The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for MCB and DXT was found in concentration range of 10µg/ml-60µg/ml and 20µg/ml-120µg/ml and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.06 and 0.05, % RSD for intermediate precision was 0.02 and 0.03 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of MCB and DXT in API and Pharmaceutical dosage form.

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