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Original Article

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Duloxetine Hydrochloride and Methylcobalamin

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Abstract

This work is concerned with application of simple, accurate, precise and highly selective reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of duloxetine hydrochloride (DUL)and methylcobalamin (MCB) in combined dosage form. chromatographi separation was achieved by using a reverse phase C18 coloum (Hypersil BDS 250X 4.6X5µm). The mobile phase composed methanol : phosphate buffer in ratio 70:30 at flow rate of 1ml/min .The ph was adjusted to 4.5 with .1N NaOH. Detection was carried out using a UV-vis detector at 215 nm. The mean retention time of duloxetine hydrochloride (DUL)and methylcobalamin(MCB) was found to be 3.3 min 6.7 min respectively. The method was found to be linear in the range of 30 - 90 µg/ml and 2.5-6.75 µg/ml for DUL and MCB respectively, with mean recovery of 98.88 % DUL and 100.22% for MCB the correlation coefficient for both DUL and MCB were closed to one. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribe values thus proposed method was successfully applied for simultaneous determination of DUL and MCB in routine analysis of formulation.

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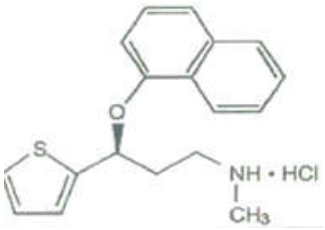
Introduction

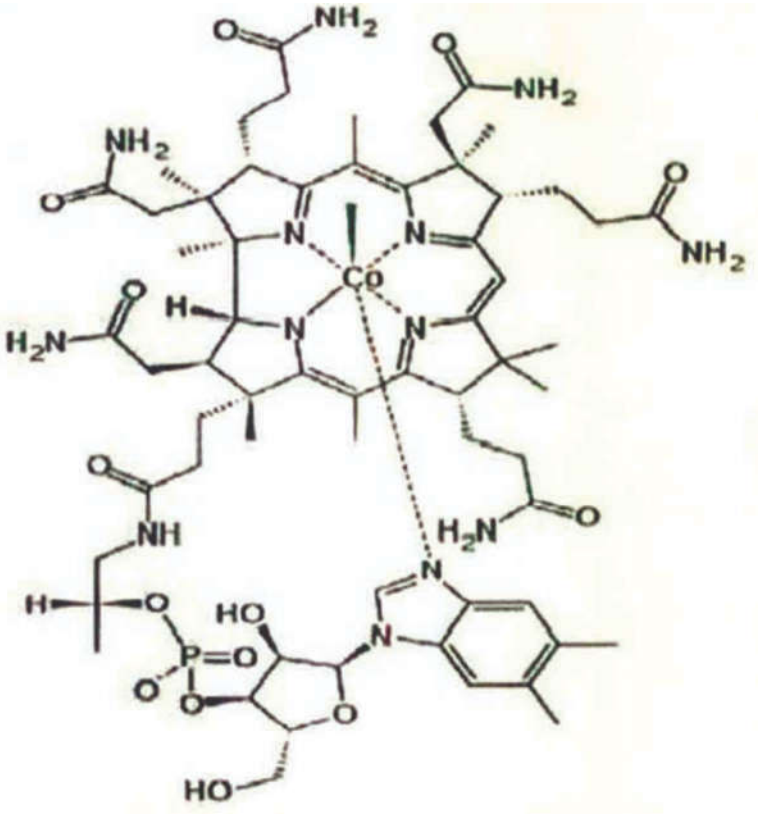
Duloxetine is used for major depression disorders and anxiety. It is a potent inhibitor of neuronal serotonin and nor epinephrine reuptake [1,2]. It produces its pain inhibitory action by potentiation of serotonergic and Noradrenergic [3,4]. Duloxetine hydrochloride (DUL) is chemically (3S)-N-methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propan-1-amine hydrochloride [5,6].



Duloxetine Hydrochloride

Methylcobalamin used in the treatment of diabetic neuropathy, trigeminal neuralgia, megaloplastic anemia, and facial paralysis in Bell's palsy syndrome. It is chemically Coα-[α-(5,6-dimethylbenz-1H-imidazolyl)] Coâmethylcobamide.Chemically it is known as carbanidecobalt(3+)[5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2(hydroxymethyl)oxolan-3-yl] 1-[3-[(4Z, 9Z,14 Z)-2,13,18-tris (2-amino-2-oxoethyl)-7,12,17-tris (3-amino-3-oxopropyl)-3, 5, 8, 8, 13, 15, 18, 19-octamethyl-2, 7,12, 17 tetrahydro-1H-corrin-21-id-3-yl] propanoylamino propan-2-yl phosphate and have

molecular formula.C63H91CoN13O14P[4]. It is a dark red crystalline powder soluble in water and ethanol[5]. Vitamin B12 is used in the body in two forms such as Methylcobalamin and 5-deoxyadenosyl cobalamin [7-12].



Methylcobalamin

The combined dosage forms of these drugs are used for the treatment of neuropathic pain associated with peripheral neuropathy especially diabetic polyneuropathy. It restores the balance of neurotransmitters in the brain like serotonin and norepinephrine [13-17].

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. The present work describes simple, specific, rapid, accurate and precise chromatographic method based on Absorption correction method for simultaneous estimation of both drugs in their combined tablet dosage forms. Several methods has been reported for estimation for individual drugs. Dulo can be quantified by spectrophotometric method [14],RP-HPLC including solid phase extraction , GC-NPD and GC-MS [15-21], Similarly literature are available for the quantification of methylcobalamin by HPLC [22-32].

Experimental

Chemicals & Reagents

DUL and MCB were obtained as gift samples from Lupin pharmaceutical Ankeleshwar India. All chemicals and reagents were of analytical grade. HPLC grade methanol,water from Loba chem. A commercial sample of tablet of containing DUL and MCB in ratio of 20mg:1500mcg respectively was procured from local market.

Instrumentation and Chromatographic Conditions

The HPLC system consisted of a thermo separation products quaternary gradient system equipped with HPLC pump specter system Stationary phase used Hypersil BDS C-18 (250 x 4.6 mm), 5)l column . the data was acquired by a chromatographic module connected to a personal computer and processing was performed running DATA ACE software . The chromatographic conditions were optimized by varying the concentration and Ph of water and the percentage of organic solvent . The mobile phase consisted of Methanol: Phosphate Buffer (70/30 v/v) pH of mobile phase was adjusted to 4.5 using O.1N

NaOH ,Flow rate: 1.0 ml/min. The mean retention time for DUL and MCB was as 3.3min and 6.7min respectively.

Preparation of Standard Solution

Accurately weighed Duloxetine Hydrochloride (60 mg) was transferred to 100 ml volumetric flask and dissolved in Methanol: Phosphate Buffer (PH 4.5) and diluted up to the mark to give a stock solution having strength 1mg/ml (600 ug/ml).60 ug/ml of DUL working standard solution was prepared by diluting 1 ml of stock solution to 10 ml with Methanol: Phosphate Buffer (pH 4.5) Accurately weighed Methy1cobalamin (45 mg) was transferred to 100 ml volumetric flask and dissolved in Methanol: Phosphate Buffer (pH 4.5) and diluted up to the mark to give a stock solution having strength 1mg/ml (450ug/ml), Take 1 ml of secondary stock solution and diluted it up to 10ml with Methanol: Phosphate Buffer (pH 4.5) to Produce a stock solution having strength 1mg/ml (45ug/ml). 4.5ug/ml of Methylcobalamin working standard solution was prepared by diluting 1 ml of stock solution to 10 ml with Methanol: Phosphate Buffer (pH 4.5)

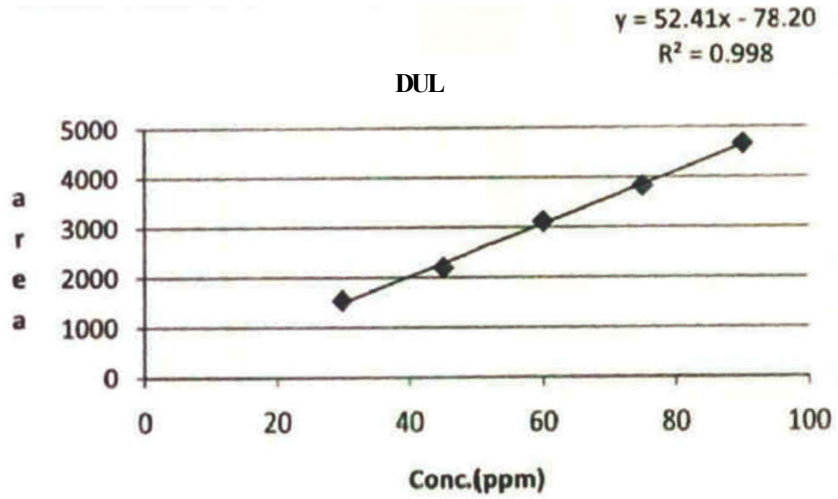


Fig. 1: Standard calibration curve for duloxetine hydrochloride

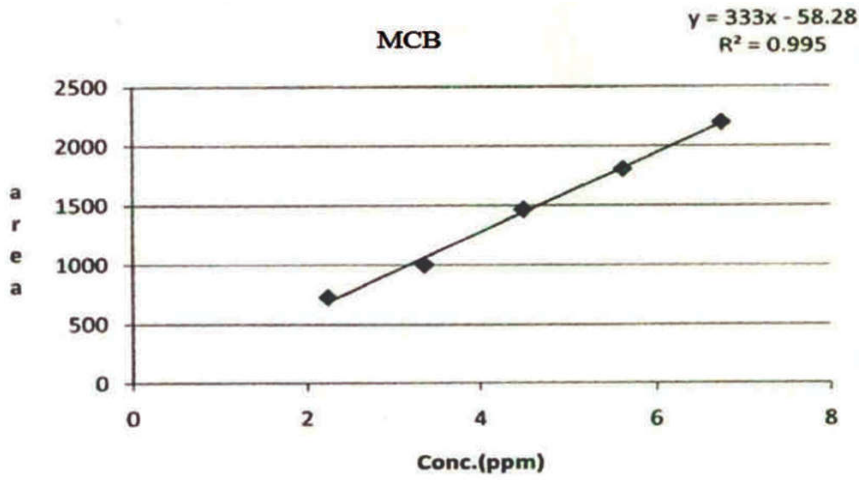


Fig. 2: Standard calibration curve for methylcobalamin

Sample Preparation

Accurately 20 tablets were weighed to determine average weight of tablets. Then tablets were finely crushed and tablet powder equivalent to 20 mg Duloxetine hydrochloride and 1500 mcg Methylcobalamin was transferred into 100 ml volumetric flask. Then 50 ml diluents was added to flask and sonicated for few minutes with intermittent shaking. Make up volume up to 100 ml. than solution was filtered and the final concentration of test sample solution was made up to 60ug/ml of Duloxetine Hydrochloride and 4.5 ug/ml of Methylcobalamin.

Results and Discussion

Method Development and Optimization of Chromatographic Condition

Chromatographic condition was achieved on Hypersil BDS C-18 (250 x 4.6 mm), 5) 1 column by varying the concentration of organic phase and water simultaneously pH was varied. Mobile phase was optimized for method advantage by making use of methanol instead of water hence in the process od optimization success was achieved by making use of Methanol: Phosphate Buffer (70/30 v/v).The pH of mobile phase was adjusted to 4.5 using O.1N NaOH , flow rate was 1.0 ml/min, Temperature: 25 ± 2^0C , Wavelength: 215nm , Run time: 20 min ,both DUL and MCB were will separated from each other with mean retention time for DUL and MCB as3.3min and 6.7min respectively.

Method Validation

Linearity

The method was linear in the range of 30 -90µg/ml to 2.25 to 6.75 µg/ml for DUL and MCB. Linear regression date was given in Table 1.

Precision

For the precision study ,repeatability study was carried out for 0.5, 1.0 and 1.5 ml of DUL & 0.5, 1.0 and 1.5 ml of MCB of combined working standard solutions (600 ug/ml of DUL and 45ug/rnl of MCB) were transferred into a 10 ml volumetric flasks and diluted up to mark with Methanol: Phosphate Buffer (pH 4.5) to get 30, 60 and 90µg/ml of DUL and 2.5, 4.5 and *6.75µg/ml* of MCB. Each concentration was prepared in triplicate. The absorbance of the each solution was measured at selected wavelengths and %C.V. was calculated The Intraday %C.V. for DUL and MCB were found to be 0.949-1.742 and0.611-1.348 respectively. Interday %C.V. for DUL and MCB were found to be 0.761-1.65 and1.135-2.426 respectively. from the date obtained the developed RP-HPLC Method was found to be precise.

Accuracy

Accuracy was determined over the range 50% to 150% of the sample concentration. Calculated amount of DUL and MCB was added in placebo to attain 80%, 100% and 120% of sample concentration. Each sample was prepared in triplicate at each level and each preparation was injected in duplicate. Blank and standard preparations were injected and the chromatograms were recorded. Mean % recovery for Duloxetine Hydrochloride and Methylcobalamin were found to be 99.88% and 100.22% respectively.

Table 1: Linear regression data for calibration curve

Parameter	Duloxetine Hydrochloride	Methylcobalamin
Linearity range (µg/ml)	30-90	2.25-6.75
Correlation coefficient	0.998	0.995
Intercept	78.20	58.28

Table 2: Repeatability data of duloxetine hydrochloride and methylcobalamine

Sr. No.	Statistical data	Duloxetine Hydrochloride	Methylcobalamin
1	Mean	3100.65	1455
2	S.D.	12.55	23.78
3	%C.V.	1.10	1.47

Table 3: Summary of precision data for duloxetine hydrochloride and methylcobalamine

Parameter	Statistical data	Duloxetine Hydrochloride			Methylcobalamin		
	Concentration(µg/ml)	30	60	90	2.25	4.5	6.75
Intra- day	Mean	1524.4	3092	4625.6	458.14	856.1	1259.02
	SD	17.26	10.33	14.6	2.90	7.56	16.98
	%C.V.	1.021	1.742	0.949	0.611	0.884	1.348
Inter-day	Mean	1528	3090	4631	463.01	857.42	1254.15
	SD	7.96	8.46	13.6	11.23	9.74	14.23
	%C.V.	1.650	0.761	0.828	2.426	1.136	1.135

Conclusion

RP-HPLC method was developed for estimation of Duloxetine Hcl and Methylcobalamin using methanol: phosphate buffer pH 4.5 (70:30 v/v) as a mobile phase. The plot of area versus respective concentrations of DUL and MCB were found to be linear in the concentration range of 30- 90lµg/ml and 2.25-6.75 µg/ml respectively with correlation coefficient 0.998 and 0.995. Repeatability was found to be 1.10 for DUL and 1.47 for MCB. Precision was calculated as interday and intraday variations and % CV was found to be less than 3% for both the drugs. The mean % recovery ranges were found to be 98.78-100.63% and 98.98-101.23% for DUL and MCB respectively.

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