Research Article

Development and Validation of HPLC Method for Simultaneous Estimation of Methylcobalamin and Duloxetine Hydrochloride in Capsules

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Received: 05/03/2017
Revised: 14/06/2017
Accepted: 21/06/2017

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Abstract:

An accurate, precise and robust HPLC method has been developed and validated for simultaneous estimation of methylcobalamin and duloxetine hydrochloride in capsules. Both drugs were separated using Phenomenex C18 Column (250mm×4.6mm, 5µm) as stationary phase and mixture of methanol:phosphate buffer(pH-3.5):acetonitrile (60:35:5) as mobile phase with flow rate of 0.8 ml/min. Detection was carried out at 280 nm. The retention times for methylcobalamin and duloxetine hydrochloride were 3.12±0.02 and 6.81±0.02, respectively. The method was validated as per ICH Q2(R1) guideline. Response of drug with respect to peak area was found to be linear for concentration range 15-40µg/ml and 300-800µg/ml for methylcobalamin and duloxetine hydrochloride, respectively. The LOD and LOQ were found to be 1.27µg/ml and 1.91µg/ml for methylcobalamin and 3.85µg/ml and 5.81µg/ml for duloxetine hydrochloride. The percentage recovery of methylcobalamin and duloxetine hydrochloride was found to be 99.54% and 99.57%, respectively. The %RSD values for intra-day precision study and inter-day precision were <2.0%. The proposed method was successfully employed for the simultaneous determination of methylcobalamin and duloxetine hydrochloride in capsules.

Keywords: Methylcobalamin, duloxetine hydrochloride, HPLC, simultaneous determination, validation.

INTRODUCTION

Methylcobalamin (MTH) is chemically carbanide-cobalt(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 (Fig.1). It is a vitamin supplement [1]. Literature review revealed that UV/Visible spectrophotometric [2] and HPLC [3] are reported for estimation of Methylcobalamin from bulk, tablet, capsule. Duloxetine hydrochloride (DUL) is chemically (3S)-N-Methyl-3-(1-naphthyloxy)-3-(2-thienyl)propan-1-amine (Fig.2).It is an antidepressant drug used to treat depression [1]. It is official in United States Pharmacopoeia. Literature review revealed that UV/Visible spectrophotometric [4,5], spectrofluorimetric [6], HPLC method [7], stability indicating RP-HPLC [8], HPTLC [9], UPLC [10] and LC-MS [11] methods are reported for estimation of duloxetine hydrochloride from bulk, tablet, capsule, plasma and other biological fluids.

UV spectrophotometric [12] and HPLC [13, 14] methods are reported for simultaneous estimation of MTH and DUL in pharmaceutical dosage form. It was thought of interest to develop a precise, accurate and robust HPLC method for simultaneous estimation of both drugs in capsules. The method is validated as per ICH Q2 (R1) guidelines [15].

MATERIALS AND METHODS

Materials

Pure drug samples of methylcobalamin and duloxetine hydrochloride were obtained as gift samples from Elite Pharma Pvt. Ltd. Ahmedabad, Gujarat, India. Commercial pharmaceutical capsules (Aristo pharmaceutical Pvt. Limited. Mumbai, Maharastra Gujarat, India) was purchased from local pharmacy.

Methanol, acetonitrile, potassium dihydrogen phosphate, ortho phosphoric acid were purchased from Finar Chemicals, Mumbai, India. The HPLC grade water was prepared in hounse using Milli-Q water system (Millipore, India).

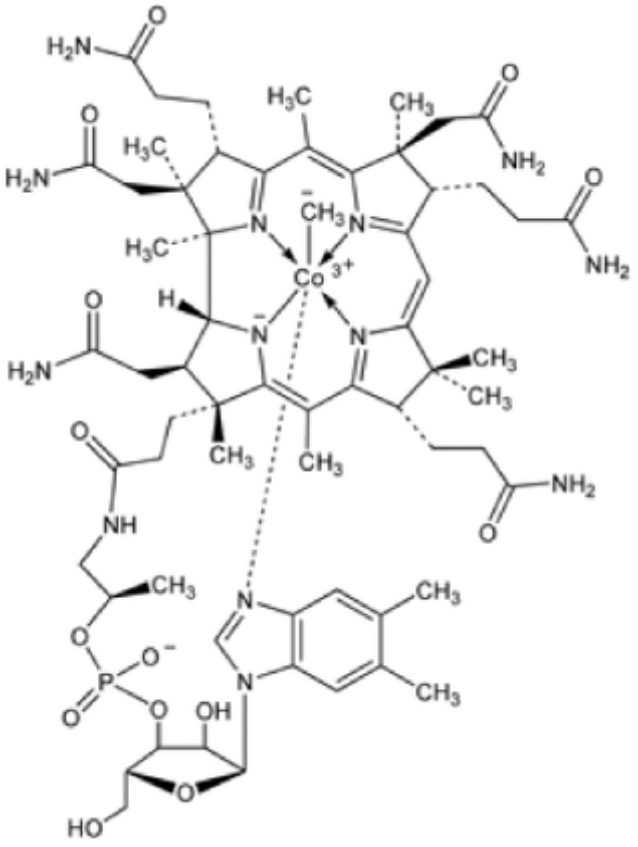

Fig. 1. Chemical structure of methylcobalmin

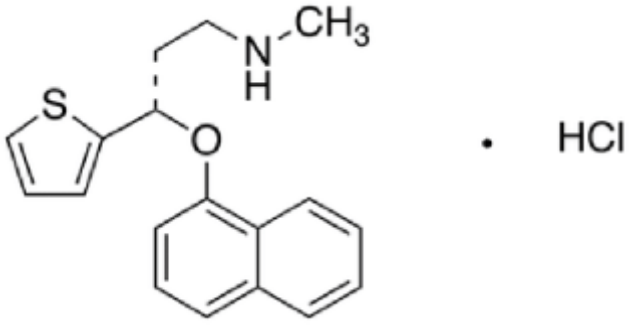

Fig. 2. Chemical structure of duloxetine hydrochloride

Instrumentation and Chromatographic Conditions

The HPLC system (Shimadzu LC-2010, Shimadzu Corporation, Japan) equipped with UV detector was used for method development. Chromatographic separation was carried out using phenomenex C-18 column with 250×4.6mm internal diameter and 5µm particle size as stationary phase and methanol: phosphate buffer (pH-3.5): acetonitrile 60:35:5(v/v/v) as mobile phase at a flow rate of 0.8 ml/min. The detection was carried at 280 nm and runtime was 15 min. The injection volume was 20 µl/min and before injection all the standards, sample solutions as well mobile phase were filtered through membrane filter of 0.45µm then sonicated for 5 min before use to remove dissolved gases. The column was saturated for at least 30min with the mobile phase flowing through the system.

Preparation of standard stock solutions

Accurately weighed standard MTH (10 mg) was transferred into 100 ml volumetric flask, dissolved and diluted up to the mark with methanol to obtain stock solution containing 100µg/ml of MTH. Accurately weighed standard DUL (100 mg) was weighed and transferred to 100 mL volumetric flasks and diluted up to mark with methanol to obtain stock solution containing 1000µg/mL DUL.

Preparation of working standard solution

Aliquots of standard stock solution of MTH (15 mL) and DUL (30 mL) were transferred to a 100 mL volumetric flask and diluted up to the final volume with mobile phase to get the final concentration of MTH and DUL 15µg/mL and 300µg/mL respectively.

Validation of the proposed method

The proposed method was validated in compliance with the ICH guidelines. The specificity of the method was ascertained by comparing standard drug and sample solutions. Linearity of the method was evaluated by linear regression analysis by preparing standard solutions of MTH (15-40 µg/mL) and DUL (300-800 µg/mL) at different concentrations of the drug. The peak area of all solutions was measured and then calibration curve was plotted between peak area versus corresponding concentration of the drug. System suitability test was performed by injecting 20 µL of standard solution, containing 15 µg/mL of MTH and 300 µg/mL of DUL, six times. Resolution (R), column efficiency (N), tailing factor (T) and precision of injection repeatability were analyzed. The intra-day and inter-day precisions (% RSD) were determined by analyzing three concentration levels of MTH (25, 30, 35

µg/mL) and DUL (500, 600, 700 µg/ml) three times on the same day and three different days, respectively. Accuracy was determined by calculating recovery of both the drug by standard addition method at three different concentration levels of drug. LOD and LOQ were calculated from standard deviation of slope and average of intercept from the calibration curve. Robustness of the method was determined by small changes in mobile phase ratio (±2%), flow rate (±0.2ml min^{-1}), wavelength (±2 nm) and pH (± 0.2 unit). The Solution Stability was checked for solution for 24 and 48 hours at room temperature.

Analysis of MTH and DUL in Formulation

Twenty capsules were opened and transferred the contents (each capsule containing 30 mg of DUL and 1.5 mg of MTH) equivalent to 30 mg of DUL and 1.5 mg of MTH into a 100 mL volumetric flask, make up the volume up to mark. Then solution was filtered through 0.45µ membrane filter. So, final solution was found to contain 15µg/mL of MTH and 300µg/mL of DUL. 20µl of this solution was injected in HPLC column followed by detection of both peak at 280 nm. The analysis was repeated for five times.

RESULTS AND DISCUSSION

The HPLC method was optimized for simultaneous estimation of MTH and DUL. The mobile phase system consisting of methanol:50 mM phosphate buffer:acetonitrile (60:35:5 v/v/v) lead to good resolution and sharp peaks of MTH and DUL at retention time 3.12±0.02 and 6.81±0.02, respectively (Fig. 3).

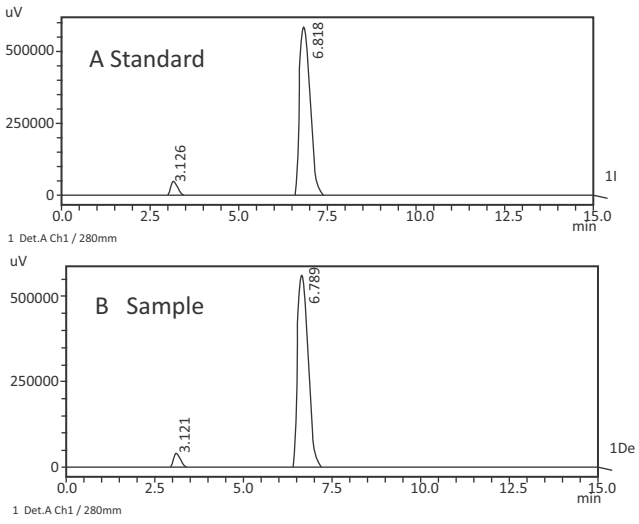

Fig. 3. HPLC chromatograms of (A) standard solution of MTH (25 µg/mL, Rt: 3.126 min) and DUL (500 µg/mL, Rt: 6.818 min), (B) sample solution of MTH (25 µg/mL, Rt: 3.121 min) and DUL (500 µg/mL, Rt: 6.789 min) at 280 nm; mobile phase:methanol:50 mM phosphate buffer:acetonitrile, pH 3.5, adjusted with orthophosphoric acid in ratio of 65:35:5 (v/v/v).

The results of the system suitability test (Table 1) showed that the optimized chromatographic conditions are adequate for simultaneous determination. The resolution between MTH and DUL peaks was found to be 6.84.

Table 1. Results of system suitability test (*n*=6)

Parameters	MTH	DUL
Retention time (min)	3.12	6.81
Resolution	6.84	
Theoratical Plates	8743	17647
Tailing factor	1.30	1.42
Injection repeatability (%RSD)	0.086	0.14

Chromatograms of standard drug solution and sample solution were compared and it showed identical retention times for both drugs (Fig. 3). The peak purity indices for both drugs from standard and sample solution were found to be 0.999.

Linearity data for the calibration curves showed linear relationships between peak area and concentration through in the ranges of 15-40 µg/ml for MTH and 300-800 µg/ml of DUL. Linear equations for the calibration curves were y=19243x+4828.7 and y=21659x+482978 with r^2 being 0.999 and 0.999 for MTH and DUL, respectively.

Table 2. Accuracy data of the proposed method (*n*=3)

Theoretical content		% Recovery		% RSD	
MTH (µg/ml)	DUL (µg/ml)	MTH (µg/ml)	DUL (µg/ml)	MTH (µg/ml)	DUL (µg/ml)
27	540	99.47	99.65	0.61	0.28
30	600	99.93	99.93	1.00	0.26
33	660	99.23	99.04	0.58	0.58

The results of robustness testing revealed that the peak area are not affected upon slight changes in mobile phase composition, pH of mobile phase, mobile phase flow rate and detection wavelength. The stability of working standard solutions of MTH and DUL were evaluated to verify that any spontaneous degradation occur when the samples were prepared. The stability profile for standard solutions of MTH and DUL were studied at 25°C for 48 h. The results were expressed as percentage of drug remaining. The data obtained

showed that sample solutions were stable during 48 h when stored at 25°C with degradation less than 2%.

The proposed method was employed for assay of MTH and DUL from combined pharmaceutical formulation available in the market. The assay results are shown in Table 3.

Table 3. Assay results for marketed formulation containing MTH and DUL

Dosage form	Labelled Claim (mg)		% content of drug (Mean±SD)	
Capsule	MTH	DUL	MTH	DUL
	1.5	30	98.01±1.21	99.05±0.81

CONCLUSIONS

This method was found to be specific, precise, accurate and robust. The proposed method was successfully applied for assay of MTH and DUL from commercially available pharmaceutical dosage form.

ACKNOWLEDGEMENTS

An author acknowledges Charotar University of Science and Technology for providing infrastructural and financial support for completion of the research project.

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