Research Article

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DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CITICOLINE AND METHYLCOBALAMIN

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ABSTRACT

A simple, accurate, precise and stability-indicating method was developed and validated for the quantitative determination of citicoline and methylcobalmin in pharmaceutical dosage form. The method was based on RP-HPLC. Chromatographic separation was performed on HPLC system (Waters with Empower2 Software) containing C_{18} (YMC, 250 x 4.6mm, 5µ) column with UV- PDA detection using a mobile phase consisting of a mixture of the mobile phase consisted of potassium dihydrogen phosphate: acetonitrile (adjusted to pH 3.0 using orthophosphoric acid) in the ratio of 50:50 v/v. The following system conditions were maintained throughout development and validation i.e., flow rate 0.8 mL/min, column was maintained at ambient temperature and the detected by a PDA detector at wave length 295nm. The citicoline and methylcobalmin was well resolved on the stationary phase and the retention time was 3.2 min and 4.1min. The linearity of the method was found to be within the concentration range of 500-1500 µg/ml solutions of citicoline and 0.75-2.25µg/ml solutions of methylcobalmin , the correlation coefficient (r^2) for the drug was 0.999. The precision, accuracy, specificity, ruggedness, robustness and forced degradation studies were determined to validate the method.

Keywords: Citicoline, methylcobalmin, RP-HPLC, chromatography.

1. INTRODUCTION:

Citicoline[1] is chemically 5'-O-[hydroxy({hydroxyl [2-(trimethylammonio) ethoxy] phosphoryl}oxy) phosphoryl] cytidine. It is also known as cytidine diphosphate-choline (CDP-Choline) & cytidine 5'-diphosphocholine is a psychostimulant / nootropic. It is an intermediate in the generation of phosphatidyl choline from choline. It is used as neuroprotective and memory disorders. Methylcobalmin[2] is a form of vitamin B_{12}. It features octahedral cobalt (III) at centre. It used in the treatment of peripheral neuropathy, diabetic neuropathy, and as a preliminary treatment for amyotrophic lateral sclerosis. The review of literature revealed that various analytical methods involving spectrophotometry, HPLC have been reported for citicoline in single form and in combination with other drugs [3-8].

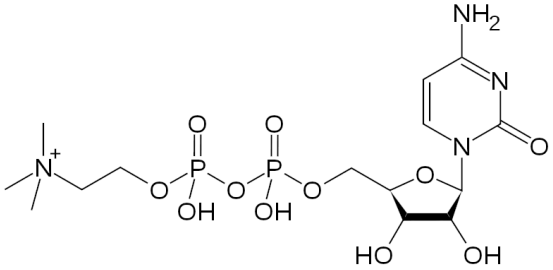

Fig.1: The molecular structure of Citicoline

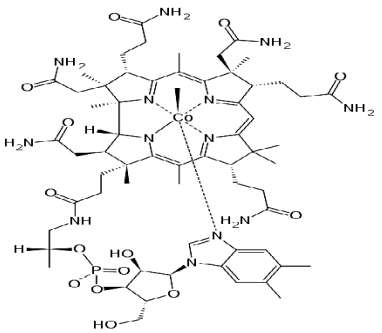

Fig.2: The molecular structure of methylcobalmine

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Several analytical methods have been reported for methylcobalmine in single form and in combination with other drugs including spectrophotometry, HPLC [9-11].

There is no research work carried on simultaneous estimation of citicoline and methylcobalmin by HPLC in bulk drug and in pharmaceutical dosage forms. In this present investigation developed and validated HPLC method for the estimation of citicoline and methylcobalmin in bulk drug and pharmaceutical dosage forms. The proposed method is validated as per ICH guidelines.

2. EXPERIMENTAL:

2.1 Materials and reagents:

Analytically pure citicoline and methylcobalmin were obtained from Lara Laboratory, Hyderabad as gift samples. HPLC grade acetonitrile was purchased from Merck & Co. Triple distilled water is used for all purpose.

2.2 Instrumentation:

HPLC system (Waters with Empower2 Software) containing C_{18} (YMC, 250 x 4.6mm, 5µ) column with UV- PDA detection.

3. Method development:

3.1 Optimization of the chromatographic conditions:

The mobile phase consisted of potassium dihydrogen phosphate: acetonitrile (adjusted to pH 3.0 using orthophosphoric acid) in the ratio of 50:50 v/v. The contents of the mobile phase were filtered before use through a 0.45µ membrane and degassed for 10 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.8 ml/min and the injection volume was 20µL. The column temperature was maintained at ambient temperature. The eluents were monitored at 295 nm.

3.2 Preparation of standard stock solutions:

Accurately weighed 500mg of citicoline and 0.75mg methylcobalmine standard were transferred to separate 100 ml volumetric flask and dissolved in 10 ml methanol. The flasks were shaken and volume was made up to the mark with distilled water to give solutions containing 5000 µg/ml citicoline and 7.5 µg/ml methylcobalmine. From this solution 5ml was transferred to volumetric flask of 25 ml capacity. Volume was made up to the mark to give a solution containing 1000µg/ml of citicoline and 1.5µg/ml methylcobalmine.

3.3 Calibration of standards:

The standard calibration curve was constructed for citicoline and methylcobalmin. Different volumes of stock solutions of each were accurately Transferred in to 10mL volumetric flasks and diluted to mark to yield a concentration range of 500-1500 µg/ml solutions of citicoline and 0.75-2.25µg/ml solutions of methylcobalmin. The calibration line was obtained by plotting the peak area against concentration of drug.

3.4 Determination of citicoline and methylcobalmin in their combined Dosage:

Sample preparation:

A powder quantity equivalent to 500 mg citicoline and 0.75 mg methylcobalmin was accurately weighed and transferred to volumetric flask of 100 ml capacity. 50ml of distilled water was transferred to this volumetric flask and sonicated for 15 min. The flask was shaken and volume was made up to the mark with distilled water. The above solution was filtered through whatmann filter paper (0.45µ). From this solution 5ml was transferred to volumetric flask of 25ml capacity. Volume was made up to the mark to give a solution containing 1000µg/ml of citicoline and 1.5µg/ml of methylcobalmin. The resulting solution was analyzed by proposed method. The quantification was carried out by keeping these values to the straight line equation of calibration curve.

4. RESULTS AND DISCUSSION:

4.1 Method validation:

The proposed method has been extensively validated in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproducibility. After validation, the developed methods have been applied to pharmaceutical dosage form.

System suitability criteria:

The system suitability was assessed by five replicate analyses of the drugs at concentrations of 1000µg mL^{-1} of citicoline and

1.5µg mL^{-1} of methylcobalmin and for this, parameters like plate number (n), tailing factor, retention time, resolution, similarity of samples were measured.

4.2 Validation parameters:

Method was validated as per ICH (Q2B) guidelines with respect to linearity, accuracy, precision, specificity, and robustness, limit of detection (LOD) and limit of quantification (LOQ).

4.2.1 Specificity:

Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of drugs. Specificity of the method was shown by quantifying the analyte of interest in the presence of matrix and other components. Blank injections have shown no peaks at retention time of 3.2 min and 4.1min, the proposed method was specific for the detection of citicoline and methylcobalmin respectively.

4.2.2 Linearity:

Linearity found to be 500-1500µg/ml for citicoline and 0.75-2.25µg/ml for methylcobalmin.

4.2.3 Accuracy:

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the prequantified placebo preparation at 3 different concentration levels 50, 100 and 150 %, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured

4.2.4 Precision:

The repeatability was evaluated by assaying 6 times of sample solution prepared for assay determination. The intra and inter-day precision study of citicoline and methylcobalmin was carried out at 100% concentrations of citicoline and methylcobalmin, 3 times on the same day and on 3 different days (first, second, third).

4.2.5 Robustness:

The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying the HPLC pump flow rate (±0.1ml) and temperature (±1°C). None of the alterations caused a significant change in peak area, retention time, USP tailing factor.

4.2.6 LOD and LOQ:

LOD and LOQ were 29.88, 90.54 for citicoline LOD and LOQ were 0-05, 0.15 for methylcobalmin

5. CONCLUSION:

The present study represents an accurate, precise and specific HPLC method for routine analysis of citicoline and methylcobalmin combination in tablet dosage form. In addition to assay it may be used to detect related substance or other impurities which are formed during storage conditions and the analyte of interest could be estimated without any interferences. The use of C_{18} column in the present work has shown better elution of analytes with good resolution, improved plate count, capacity factor, and reduced tailing. So the C_{18} column can be used to achieve high specificity in shorter time of analysis of citicoline to methylcobalmin in tablet as per ICH Q2B guidelines.

The developed RP-HPLC method for simultaneous determination of citicoline and methylcobalmin in combined pharmaceutical dosage form is simple and reliable. From the study of validation parameters namely accuracy, precision (SD and RSD), (inter-day, intraday), specificity, linearity and range, it was observed that the method is specific, accurate, precise and reproducible. Hence the method can be employed for routine analysis of dosage form.

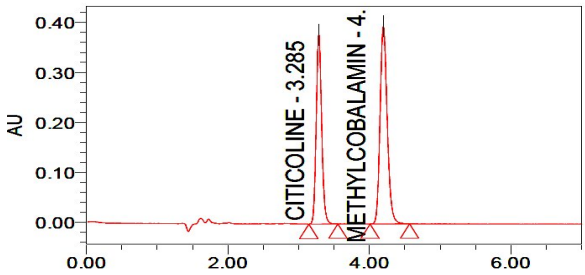

Fig. 3: Optimized chromatogram of citicoline and methylcobalmin

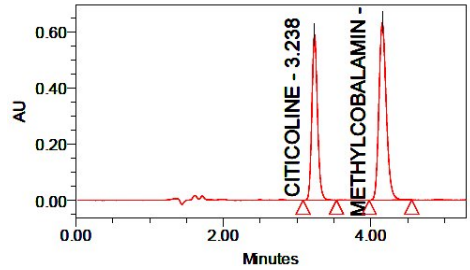

Fig.4: Assay chromatogram for citicoline and methylcobalamin in combined tablet dosage form.

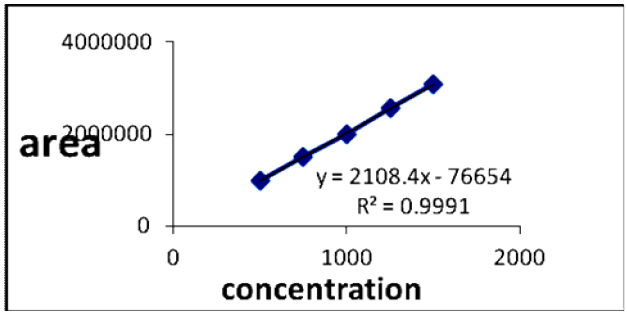

Fig.5: Linearity for citicoline

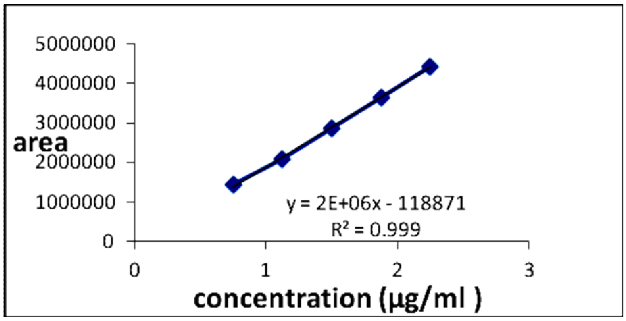

Fig.6: Linearity for methylcobalmin

Table 1: Optimized chromatographic conditions of citicoline and methylcobalamin

S. No	Parameters	Specification
1	Mobile phase	KH_2PO_4 : acetonitrile (50:50 v/v)
2	Flow rate (mL/min)	0.8 ml/min
3	Run time (min)	10min
4	Column Temperature (℃)	ambient
5	Volume of injection (µL)	20 µl
6	Detection wavelength (nm)	295 nm

Table 2: Recovery report of citicoline and methylcobalamin

Drug	Amount taken (µg/ml)	Recovery level (%)	Amount of drug added	Amount of drug found	% RSD	% Recovery
CIT	1000	50	500	1507.65	1.1	100.51
		100	1000	1978.33	1.0	98.91
		150	2500	2523.78	0.7	100.95
MTC	1.5	50	0.75	2.27	1.3	100.88
		100	1.5	2.97	0.8	99
		150	2.25	3.80	0.7	101.33

Table 3: Precision (intraday and inter-day Precision)
Intraday precision:

S. No	Rt of CIT (min)	Peak area of CIT	Rt of MTC (min)	Peak area of MTC	%RSD	
					CIT	MTC
1	3.288	2038642	4.203	2803798	0.809	0.598
2	3.292	2067066	4.207	2835554	0.804	0.600
3	3.290	2035515	4.207	2802533	0.801	0.608
Average					0.804	0.602

Inter-day precision:

S. No	Rt of CIT (min)	Peak area of CIT	Rt of MTC (min)	Peak area of MTC	%RSD	
					CIT	MTC
1	3.289	2042324	4.204	2821512	0.810	0.600
2	3.284	2024464	4.200	2798886	0.817	0.594
3	3.274	2016862	4.194	2795275	0.791	0.597
Average					0.806	0.597

Table 4: Robustness studies

S. No	Parameter	Modification	Retention time		Tailing factor	
			CIT	MTC	CIT	MTC
1	Flow rate	0.9	3.233	4.168	1.153	1.197
		0.7	3.229	4.165	1.151	1.201
2	temperature	31.9 C°	3.229	4.167	1.156	1.204
		27.9 C°	3.232	4.159	1.152	1.207

Table 5: LOD and LOQ data

S. No	Parameter	Citicoline	Methylcobalmin
1	LOD	29.88	0.05
2	LOQ	90.54	0.15

Table 6: System suitability parameters

Parameter	Value obtained (n=6)		Acceptance criteria
	Citicoline	Methylcobalmin	
Plate count	8973	8482	>2000
Tailing factor	1.124	1.183	≤2.0
Resolution	-	5.544	>2
Similarity	1.0	1.0	0.98 – 1.02
Rt (min)	3.277	4.186	-
%RSD	0.8	0.6	≤2.0

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