Research Article

Development of a Validated RP-HPLC Method for the Analysis of Citicoline Sodium in Pharmaceutical Dosage Form using Internal Standard Method

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ABSTRACT

Objective: To develop a simple, specific, rapid and validated RP-HPLC method for the determination of citicoline sodium in pharmaceutical dosage form using pyrimethamine as internal standard (IS). **Methods:** The HPLC instrument used was Shimadzu LC- 20AD with reverse phase C_{18} phenomenex (250 × 4.6 mm, 5 µm) column using acetonitrile : phosphate buffer at pH 5.0 (55 : 45 v/v) as mobile phase. The flow rate was maintained at 1.0 ml/min and UV detection was carried at 270 nm. **Results:** The method was validated for linearity, accuracy, precision, specificity and robustness according to ICH guidelines. The retention time was found to be 2.26 ± 0.03 min for citicoline sodium and 4.46 ± 0.025 min for IS. The regression analysis showed good linearity over the concentration range of 5 - 25 µg/ml for citicoline sodium. The recovery studies of the method give good recovery in the range of 99.89 - 100.48% with less than 2% of RSD. **Conclusion:** The method was found to be suitable for the routine analysis of citicoline sodium in presence of excipients in pharmaceutical formulations.

Keywords: Citicoline sodium, RP-HPLC, internal standard, method validation

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INTRODUCTION

Citicoline sodium is chemically cytidine 5'-{Trihydrogen diphosphate}-p'-[2-{trimethyl ammonio}] ethyl ester inner salt. It is a white crystalline hygroscopic powder which is freely soluble in water. It stimulates the biosynthesis of cerebral phosphatidylcholine, main structure component of the phospholipids of the neuronal membrane. It increases the neurotransmission levels due to its dopaminergic agonist action. It behaves like a presynaptic cholinergic agent, favoring the synthesis of acetylcholine. It also improves the neuronal metabolism in those cases where there is a neuronal deterioration due to degenerative, toxic or ischemic cause [1].

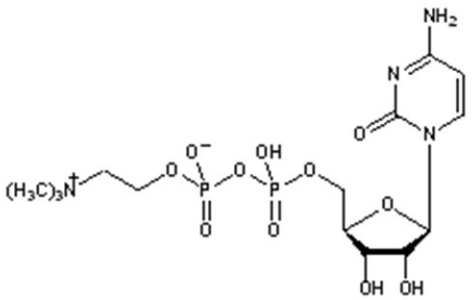

Figure 1: Structure of citicoline sodium

Citicoline sodium is primarily indicated in conditions like cardiac stroke, head trauma, ischemic, head disease, paralysis of lower extremities and also be given in adjunctive therapy as an alternative therapy as an alternative drug of choice in parkinson's disease. It is generally marketed as oral tablets containing 500 mg of citicoline sodium.

The literature survey revealed few methods like UV-spectroscopy, colorimetry and HPLC for the determination of citicoline sodium [2-9]. The aim of the study was to develop a simple, accurate and rapid RP-HPLC method for the determination of citicoline sodium in pure and pharmaceutical dosage form in the presence of an internal standard (pyrimethamine). The internal standard pyrimethamine was structurally similar to citicoline sodium, used to aid in the quantification process. The IS was stable in the mobile phase and should not interfere with the analysis of citicoline sodium. The proposed method was validated for linearity, accuracy, precision, robustness, LOD, LOQ and repeatability according to ICH guidelines and its updated international convention [10].

MATERIALS AND METHODS

Reagents and chemicals

Citicoline sodium pure drug was obtained as gift sample from Pfizer Laboratories, Mumbai, India. The internal standard (IS) Pyrimethamine was obtained from Aristo Pharmaceuticals, Chennai, India. The other chemicals like methanol, acetonitrile and potassium dihydrogen phosphate were of HPLC grade and obtained from Merck Chemicals, Mumbai, India. The tablet dosage forms (labeled to contain 500 mg citicoline sodium) were procured from local pharmacy.

Instrumentation

The HPLC system consist of Shimadzu LC 20AD, with pump LC 10ADVP, Hamilton injector of 20µl capacity and detected by SPD-20A UV detector. The system was controlled by CLASS-VP software. Analytical column consist of reverse phase C_{18} Phenomenex (200×4.6mm id, 5µm) column.

Chromatographic conditions

The mobile phase consist of acetonitrile: phosphate buffer (pH 5.0) in 55: 45 v/v ratio and before applying it was vaccum filtered through 0.45 µ AVIVA SRP15 membrane and degassed by ultra sonication for 10 min. The flow rate was maintained at 1.0 ml/min. After equilibration of the column with the mobile phase, indicated by a stable baseline, aliquots of the samples (20 µl) were injected and the total run was kept at 15 min. The absorbance of the eluent was monitored at 270 nm.

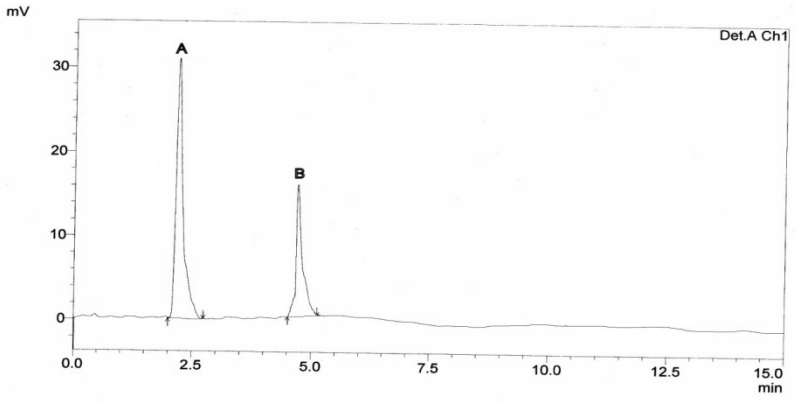

Figure 2: Typical HPLC chromatogram of standard A) citicoline sodium (R_t = 2.26 ± 0.02 min B) IS (R_t = 4.42 ± 0.03 min)

Standard solutions and Calibration curves

The standard stock solutions of the drug and the IS were prepared separately by dissolving 10 mg pure drug in 100 ml acetonitrile. From this stock solution suitable dilutions were made by using acetonitrile, to obtain a combination of solutions containing 5-25 µg/ml of citicoline sodium and 1-5 µg/ml of IS. Each

concentration was injected three times into the column. The calibration graph was constructed by plotting the relative retention factor, RRF value (i.e. ratio between peak area of citicoline sodium and IS) against respective concentration of citicoline sodium.

Sample preparation

Ten tablets of citicoline sodium were weighed and pulverized into fine powder. A mass of powder equivalent to 100 mg of citicoline sodium was accurately weighed and transferred to a volumetric flask containing acetonitrile. The resultant solution was sonicated for 5 min and filtered through nylon filter and the volume was adjusted with acetonitrile. From the resulting sample solution, 100 µg/ml solution was prepared. From this solution (100 µg/ml), 1.5 ml was transferred to 10 ml volumetric flask, added 0.3 ml of IS (100 µg/ml) completed to volume with acetonitrile to have a final concentration of 15 µg/ml of CT and 3 µg/ml of IS. 20µl of this sample solution is then injected into the column and chromatographed in the previously developed chromatographic conditions and detected at 270nm.Quantification was done by using RRF value. The procedure was repeated five times for the same sample solution.

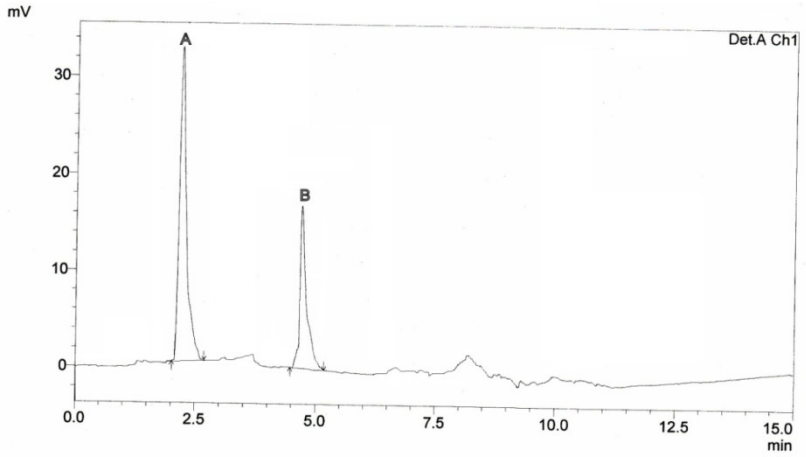

Figure 3: Typical HPLC chromatogram of sample A) Citicoline sodium (R_t = 2.31 ± 0.01 min) B) IS (R_t = 4.7 ± 0.02)

Method Validation

The method was validated according to the ICH guidelines for the following parameters.

Linearity and sensitivity

Standard solutions of citicoline sodium were prepared in the concentration range of 5 - 25 µg/ml. Then 20 µl of each solution were injected three times to the column and developed using above mobile phase. The RRF values were plotted against the corresponding concentration to obtain the calibration graph. The LOD and LOQ were calculated based on the equation:

LOD = 3.3 × S/B and LOQ = 10 × S/B.

where, S is SD of peak areas of the drugs taken as a measure of noise and B is the slope of corresponding calibration curve.

Precision

The interday and intraday precision studies were conducted by using three different concentrations of the standard (initial, medium and final concentrations) in triplicate in a day and on three consecutive days.

Accuracy

The accuracy of the method was examined by performing recovery studies in triplicate using standard addition method (50,100 and 150 %). Accurately known amount of sample were added to a known amount of pre-analyzed tablet powder and was analyzed.

Robustness

Robustness of the method was determined by introducing small changes in the mobile phase composition, change in flow rate, column temperature and detection wavelength. For all changes in conditions, the samples were analyzed in triplicate. The retention factor and resolution between

citicoline sodium and IS were evaluated in each condition.

RESULTS AND DISCUSSION

Optimization of the RP-HPLC method

Various solvent systems were evaluated to obtain an optimum resolution between the drug and IS. Initially, methanol, HPLC grade water, acetate buffer, acetonitrile and phosphate buffer were tried in different ratios. But the resolution was not satisfactory. Finally, the mobile phase consisting of acetonitrile: phosphate buffer (pH 5.0) in the ratio of 55 : 45 v/v gave better resolution and found to be optimum. The chromatographic conditions were finally optimized on Phenomenex C_{18} column using acetonitrile: phosphate buffer of pH 5.0 (55 : 45 v/v) as mobile phase at 1 ml/min flow rate.

Linearity and sensitivity

The linearity was evaluated by determining six working standard solutions containing 5 - 25 µg/ml of citicoline sodium in triplicate. The drug shows good correlation coefficient in the above concentration range. (r = 0.999). The LOD and LOQ was found to be 0.5349 and 2.6515 µg/ml respectively (**Table 1**).

Table 1: Linearity parameters for calibration curve

Parameter	Value
Retention time (Rt)	2.26 ± 0.02
Linearity range (µg/ml)	5 - 25
Regression equation (y = mx+c)	Y = 0.113x+ 0.023
Correlation coefficient (r)	0.999
Limit of detection (µg/ml)	0.5349
Limit of quantification (µg/ml)	2.6515
Regression coefficient (r^2)	0.999
Method precision (RSD%)	0.86

Accuracy and Precision

Recovery of standard drug added was found to be 99.89 - 100.4 % for citicoline sodium with less than 2% of RSD values, indicating that the proposed method was accurate (**Table 2**). **Table 3** shows the precision study results. The RSD values for intraday and interday precision were not more than 2 %, indicating the repeatability and reproducibility of the method.

Robustness

The %RSD values of all robust parameters were examined and found to be within the limit of less than 2%, showed that the proposed method was robust (**Table 4**).

Analysis of Marketed formulations

Experimental results of amount of citicoline sodium in tablets were in good agreement with the expressed label claim, suggesting that there was no interference from any of the excipients in the tablets. The low %RSD value confirmed the suitability of the method for routine analysis of citicoline sodium in pharmaceutical dosage forms (**Table 5**).

Table 2: Accuracy of the proposed method

Drug	Level (%)	Theoretical concentration (µg/ml)	Observed concentration (µg/ml) ± SD[a]	Mean recovery (%)	SEM	% RSD
CT	50	7.5	7.5106 ± 0.021	100.14	0.01	0.27
	100	10.0	10.0479 ± 0.125	100.48	0.056	1.22
	150	12.5	12.4873 ± 0.066	99.89	0.029	0.52

[a]Mean of three replicates

Table 3: Precision study

Drug	Initial amount (µg/ml)	Amount found (µg/ml) ± SD[a]	% Recovery	% RSD
	Intra day			
	5.0	5.062 ± 0.0673	101.23	1.19
CT	15.0	15.096 ± 0.1569	100.64	1.04
	25.0	24.963 ± 0.0870	99.85	0.35
	Inter day			
	5.0	4.998 ± 0.0374	99.96	0.75
CT	15.0	15.08 ± 0.0536	100.56	0.36
	25.0	24.896 ± 0.438	99.58	1.76

[a]Mean of three replicates

Table 4: Results of robustness study

Parameter	R_t	Resolution	%RSD
Change pH of mobile phase			
pH 4.8	2.35	3.53	0.33
pH 5.0	2.26	3.64	0.74
pH 5.2	2.30	3.68	0.47
Change in temperature			
20° C	2.24	3.54	0.26
25° C	2.26	3.58	0.68
30° C	2.26	3.43	0.53
Change in flow rate			
0.8 ml/min	2.45	3.52	1.03
1.0 ml/min	2.27	3.51	1.31
1.2 ml/min	2.03	3.51	0.94
Change in wavelength			
275 nm	2.42	3.33	0.64
272 nm	2.28	3.53	0.96

Table 5: Results from HPLC quantification of citicoline in tablets

Sample	Label claim (gm)	Amount present (gm)	SD[a]	%RSD
Brand I	0.500	0.4998	0.0011	0.22
Brand II	0.500	0.5008	0.0033	0.67

[a]Mean of five replicates

CONCLUSION

The proposed RP - HPLC method provide simple, accurate and reproducible quantitative analysis of citicoline sodium in formulations. The method was validated as per ICH guidelines. The statistical analysis indicates that the method was suitable for the routine analysis of citicoline sodium in formulations.

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