

DEVELOPMENT AND VALIDATION OF RP- HPLC METHOD FOR DETERMINATION OF CITICOLINE MONOSODIUM IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT:

A Simple, selective, rapid, precise and economical reverse phase HPLC method was developed and validated for the Quantitative Estimation of Citicoline Monosodium in pharmaceutical preparation. Isocratic separation was accomplished using C18 column (250 mm x 4.6 mm, 5 μ m particle size) with mobile phase consisting of Tetra butyl ammonium hydrogen sulphate Buffer (pH 6): Methanol (95:05, v/v), flow rate was 1.0 ml/min. and the detection wavelength was 270 nm. The proposed method has permitted the quantification of Citicoline Monosodium in the linearity range of 20- 100 μ g/ml with coefficient of correlation (r^2) 0.9999. The column was maintained at ambient temperature and analytical run time of approximately 10 min and it was eluting at approximately 6.3 \pm 0.5 min. The percentage recovery was found to be in between 97.85-99.75 % and the % RSD of Precision was found to be 0.187 \pm 0.18. The percentage amount of marketed formulation of Citicoline Monosodium was found to be 99.54 %. The method was validated for linearity, accuracy, precision, specificity, Robustness, solution stability, the assay may be applied to a routine analysis in industries.

Keyword: Citicoline Monosodium; RP-HPLC; Assay Method; Quantitative Estimation; Method Validation.

1. Introduction:

Citicoline Monosodium is Cytidine 5' (Trihydrogen diphosphate) P' [2-trimethylammonio) ethyl] ester inner salt Monosodium salt belongs to medicine known as cerebral vasodilator¹. Citicoline Monosodium is primarily used in pharmacotherapy of brain Insufficiency and other related neurological disorders viz., as stroke, brain trauma and Parkinsonism disease². It is a white crystalline powder, freely soluble in water but insoluble in ethanol, acetone and chloroform³. There was no method based on Tetra butyl ammonium hydrogen sulphate Buffer (pH 6): Methanol (95:05, v/v), the proposed method is a simple, specific, rapid, and accurate for quantitative estimation of Citicoline Monosodium. The results obtained have been statistically validated in accordance with the ICH guidelines⁴ and therefore can be effectively used in quality control of Citicoline Monosodium for bulk as well as pharmaceutical dosage form. The functional recovery, promote nerve regeneration, and reduce postoperative scarring after peripheral nerve surgery in rats used a topical application of 0.4 mL of saline or 0.4 mL of Citicoline, respectively⁵. A number of Methods for the determination of Citicoline were reported in the literature⁶⁻⁹. In the present study attempts

were made to develop a rapid, economical, precise and accurate method for the estimation of Citicoline Monosodium in Tablet dosage form.

2. Experimental:

2.1 Chemicals and reagents: All the reagents were of analytical-reagent or HPLC grade unless stated otherwise. Citicoline Monosodium was kindly supplied by Torrent Pharmaceutical Limited, (India), Methanol (Merck Ltd., Rankem, RFCL Ltd), Tetra Butyl Ammonium hydrogen sulphate (CDH Ltd), Acetic acid (Sd.Fine Chem. Ltd) Triethylamine (Sd.Fine Chem. Ltd) and Triple distilled water (In-House).

2.2 Instrumentation: HPLC analysis were performed on YoungLin system equipped with quaternary SP930D gradient pump, a vacuum degasser & mixer, an UV730D UV/VIS detector and a rheodyne injector holding 20 μ l loop. The signals were acquired and analyzed using Windows XP based YoungLin Autochro-3000 software.

2.3 Preparation of Stock solutions: Tetra butyl ammonium hydrogen sulphate Buffer prepared by dissolves 1.697gm of Tetra Butyl Ammonium Hydrogen Sulfate Dissolve in 1000ml of water, add 2.5 ml Triethylamine and adjust pH 6.0 with diluted acetic acid.

2.4 Preparation of Stock solutions: Accurately weighed 50 mg Citicoline Monosodium was transferred into 50 ml volumetric flask and dissolved in Mobile phase, then volume was made up to 50 ml with mobile phase to get a concentration of 1000 µg/ml (Stock-A). 10 ml of stock-A was taken in 25 ml volumetric and diluted up to 25 ml to get concentration of 400 µg/ml (Stock-B). Finally from stock-B solution different of, 20, 40, 60, 80 and 100 µg/ml were prepared for analysis. Linearity was observed by the linear regression equation (Fig.3) and correlation coefficient was found to be 0.9999

2.5 Chromatographic Conditions: Before the mobile phase was delivered into the system, mobile phase were filtered through 0.45 µm filter and degassed using vacuum. For analysis of samples, the homogeneity was expressed in terms of peak purity and was obtained directly from the special analysis report obtained using the above mentioned software. The chromatographic conditions used for the analysis were given below. The separation of the compound was made on a nucleosil-C 18 column (250 mm x 4.6 mm, 5 µm particle size) using isocratic elution. Wavelength: 270 nm, Injection volume: 20 µl, Flow rate: 1.0 ml/min, Column temperature: 25°C, Run time: 10 min [Fig.2]

2.6 System Suitability Parameters: Separation variables were set and column was allowed with the mobile phase at a flow rate of 1.0 ml/min. After complete saturation of column, six replicates of standard of Citicoline Monosodium (50 µg/ml) were injected. Peak report and column performance are given in Table1 for Citicoline Monosodium.

3. Results and Discussion:

In order to confirm the validity of the method, laboratory and Tablet samples containing Citicoline Monosodium were prepared in the range of 20 µg/ml – 100 µg/ml. The amount of drug present in the standard and Test solution was calculated by using the selected linearity equation and the results are tabulated in the Table 2.

3.1 Method Validation: The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, robustness and system suitability in accordance with ICH guidelines

3.1.1 Linearity: The curve proved to be linear over a concentration range of 20-100 µg mL⁻¹ (Fig 3). Standard solution were prepared at five concentrations (20, 40, 60, 80, 100 µg mL⁻¹)

were injected in triplicate. Linear regression of concentration Vs peak area resulted in an average coefficient of determination (R²) 0.9999. The Regression equation is $Y = 53.442X + 7.4158$ (Fig.3). For establishing the linearity range samples of five concentration of Citicoline Monosodium in the range of 20-100 µg mL⁻¹ was prepared and analyzed the response ratio for each concentration. The Bar Graph was plotted between response ratios Vs. Concentration. (Fig 4).

3.1.2 Precision: The precision of the method was evaluated by carrying out six independent assays of test samples of Citicoline Monosodium. The precision of the method was also evaluated in same day for repeatability of precision and in different days. The results shown in Table.3, indicates that the method is reproducible.

3.1.3 Accuracy: Accuracy was calculated as the percentage recovery of the known added amount of Citicoline Monosodium reference substance in the sample solutions using three concentration levels (50 %, 100 %, and 150 %). covering the specified range (20, 40, and 60 µg mL⁻¹). The accuracy of the method ranged from 97.85-99.75 % indicating that this assay is reliable (Table 4).

3.1.4 Robustness: To determine the robustness of the developed method, experimental conditions were purposely altered. The ratio of mobile phase was change 95:05 by ± 2 to 97:03 and 93:07 (Tetra butyl ammonium hydrogen sulphate Buffer (pH 6): MeOH v/v) and Changed flow rate by ± 0.1 ml/minute [use flow rate 0.9 ml and 1. 1 ml]. While the other parameters were held constant in chromatographic condition. The % RSD was not more than 2 % in both conditions (Table 5).

3.2 Stability in Analytical Solution: Sample and standard solution were prepared and injected and assay value calculated. After storing at 25°C it was run against the freshly prepared standard solution at Variable Time Period. The % RSD was not more than 2 %.

3.3 Analysis of Tablet Formulation: Tablet formulation that was used for analysis (Strolin) contains 500 mg Citicoline Monosodium per tablet. For analysis, 20 tablets were taken, accurately weighed there average weight was determined and crushed into powder. From the crushed mass, powder equivalent to 50 mg of Citicoline Monosodium was accurately weighed and transferred to a 50 ml volumetric flask and made up to the mark with the Mobile Phase. This solution was sonicated for 20 min and filtered through whatman filter paper (41

numbers) to get a solution of 1000µg/ml. Further diluted samples in the range of 20 µg – 100 µg/ml were prepared and injected to the HPLC system after filtering through 0.22 µ syringe filter. Corresponding peak areas of chromatograms obtained by UV/VIS detection at 270 nm were used to calculate the amount of drug present in the sample solution by using the selected linearity equation and the results.

4. Conclusion:

A simple, rapid, accurate and precise RP-HPLC method has been developed and validated for the routine analysis of Citicoline monosodium in API and tablet dosage forms. The RP-HPLC method is suitable for the determination of Citicoline monosodium in formulations. The developed method is recommended for routine and quality control analysis of the investigated drug in pharmaceutical preparation

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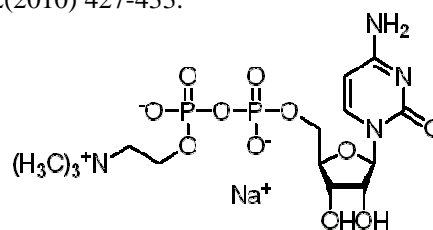


Fig.1Molecular Structure of Citicoline Monosodium

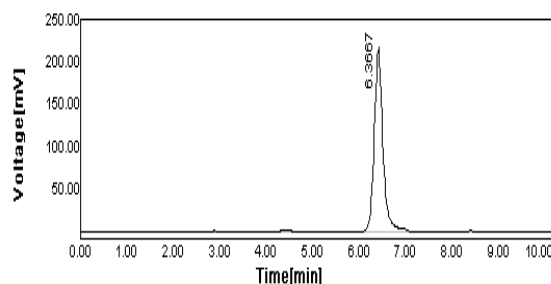


Fig.2 Chromatogram of Citicoline Monosodium

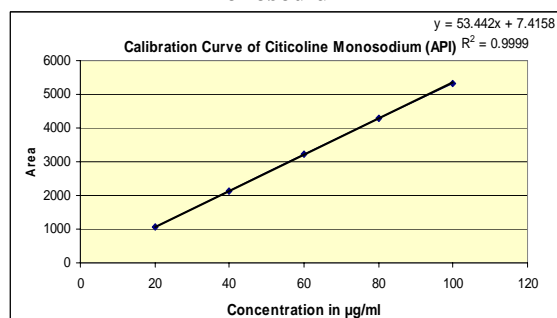


Fig.3 Calibration Curve of Citicoline Monosodium

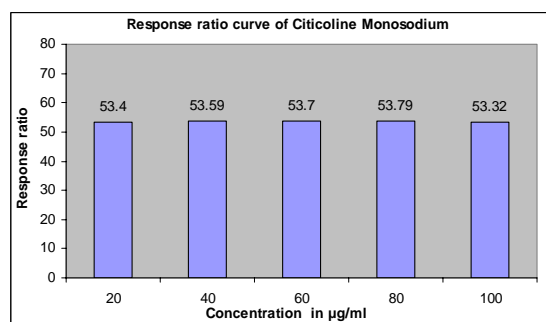


Fig. 4 Response ratio of Citicoline Monosodium

Table 1 System suitability parameters

System suitability Parameter →	RT	AUC	Tailing factor
Mean	6.37	2670.87	1.17
S.D.	0.0671	1.095	0.014
% R.S.D.	1.053	0.041	1.197

Table 2. Results of Laboratory samples & Tablet Formulation

Parameters	Laboratory samples	Tablet Formulation
% Mean Found	99.74	99.54
S.D.	0.196	0.410
%RSD	0.197	0.413
Acceptance criteria	NMT 2.0%	NMT 2.0%

Table 3. Results of Precision

Precision	% Found	SD	% RSD	Acceptance criteria
Repeatability	99.62	0.185	0.186	NMT 2.0%
Intermediate Precision	99.89	0.130	0.13	NMT 2.0%

Table 4. Results of Recovery Study

Percentage Level	% Recovery	SD	RSD (%)	Acceptance criteria
50	99.01	1.021	1.041	NMT 2.0%
100	99.45	0.0750	0.113	NMT 2.0%
150	99.75	0.1708	0.286	NMT 2.0%

Table 5. Results of Robustness study

Robustness study when Mobile Phase Composition Change			
Parameters	95: 05	97: 03	93: 07
S.D.	0.8215	0.8729	0.8617
%RSD	0.038	0.041	0.040
Robustness study when Flow rate Change			
Parameters	1.0 ml/min	0.9 ml/min	1.1 ml/min
S.D.	0.9530	0.9880	0.9465
%RSD	0.045	0.046	0.044