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Stability indicating LC Method for Citicoline Sustained Release Tablet

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Abstract: A Reverse phase liquid chromatography (RP-LC) assay method was developed for quantitative determination of citicoline from citicoline sustained release tablet. The chromatographic separation was achieved on Waters 2487 Hypersil BDS C18 (250×4.6mm) 5µ column, and mobile phase comprising Buffer : Methanol (100:2) The flow rate was 1.0 ml/min and the detection wavelength was 280 nm and injection volume was 20 µl .The method was validated for linearity, accuracy, precision, specificity, Robustness, Ruggedness, solution stability, The Tablet was subjected to stress condition of hydrolysis, heat degradation, acid degradation, base degradation. No interference of degradation product was found at the retention time of principle peak.
Key words: Citicoline sodium, method validation, column liquid chromatography and degradation.

INTRODUCTION:

Citicoline sodium Cytidine 5'(trihydrogen diphosphate) P'[2-trimethylammonio) ethyl] ester inner salt monosodium salt belongs to medicine known as cerebral vasodilator.

Citicoline, a form of essential nutrient choline, shows promise of clinical efficacy in elderly patients with cognitive deficits, inefficient memory and early stage Alzheimer's disease. Citicoline has also been investigated as a therapy in stroke patient.

Citicoline serve as a choline donor in the metabolic pathways for biosynthesis of acetyl choline & neuronal membrane phospholipids, chiefly phosphotidylcholine. The principal component of Citicoline, choline and cytidine are radialy absorbed in GI tract and easily cross BBB. Citicoline is complex organic molecule that functions as an intermediate in the biosynthesis of cell membrane phospholipids. Citicoline is also known as CDP- choline and cytidine diphosphate choline ,cdp choline belongs to the group of biomolecules in living system known as "nucleotides" that play important role in cellular metabolism. Citicoline is degraded to uridine and choline during intestinal absorption [1]. These two compounds then pass through the blood brain barrier to reconstitute citicoline in the brain [2].

Citicoline available in market as conventional tablet and as injection dosage form.A liquid chromatography method for the determination of citicoline sodium and its injection was reported in the literature [3]. A rapid and sensitive high performance liquid chromatography assay method for citicoline in formulation dosage form was also reported in literature [4]. So far to our knowledge none of the reported analytical procedures describe a method for the determination of citicoline sodium in sustained release tablet formulation in the presence of degradation products generated from forced degradation studies. In the present study attempts were made to develop a rapid, economical, precise and accurate method for the estimation of citicoline sodium in sustained release tablet in the presence of its degradants.

EXPERIMENTAL

2.1 Chemicals and Reagents

All the reagents were of analytical-reagent or HPLC grade unless stated otherwise. Water for injection (M.J.Biopharma) was used throughout the experiment.

Potassium dihydrogen orthophosphate and Tetra butyl ammonium hydroxide (Merck Labortories), Acetonitrile (Rankem), Methanol (S.D. Fine chem) Citicoline standard was obtained from Probio sint, Italy Citicoline sodium sustained release tablet (1000 mg) formulation was purchased from the local pharmacy.

2.2 Instrumentation

The HPLC system used was a Waters 2487 series comprised of degasser, auto injector, column compartment, photo diode array detection and the system was controlled through Empower software. Analytical column used for this method is Hypersil BDS C_{18} (250 mm x 4.6 mm, 5µm).

2.3 Buffer Preparation

Buffer solution was prepared by mixing equal Volumes of 0.1M potassium dihydrogen orthophosphate and 0.01M tetra butyl ammonium hydroxide in 1000ml of water.

2.4 Standard Preparation

Standard stock solution was prepared by dissolving 210 mg of citicoline sodium
(Equivalent to 200 mg of citicoline) in sufficient amount of water in a 100 mL volumetric flask and sonicated to dissolve and centrifuged for 5 min. Further diluted up to the mark with water, and filtered through 0.45u membrane filter. (Nylon 66)

2.5 Sample Preparation

Equivalent to 1000mg of Citicoline from 20 crushed tablets was accurately weighed and transferred into a 500ml of volumetric flask. 300ml. of water added, it was sonicated to dissolve and centrifuged for 5 min. Further 5ml. of clear supernatant solution was diluted to 100ml with water, and filtered through 0.45u membrane filter. (Nylon 66)

2.6 Chromatographic Conditions

Before the mobile phase was delivered into the system, buffer and methanol were filtered through 0.45mm, Nylon 66 membrane 0.45 µm filter and degassed using vacuum. For analysis of forced degradation samples, the photo diode array detection was used in scan mode with a scan range of 200-400 nm. The peak 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.
Column: Hypersil BDS C18 (250 mm x 4.6 mm) 5µm
Wavelength: 280 nm
Injection volume: 20 µl
Flow rate: 1.0 mL min^{-1}
Column temperature: 25ºC
Run time: 7 min

2.6 Procedure For Forced Degradation Study Of Citicoline Sodium

2.6.1 Acid Degradation

Accurately weighed equivalent to 1000 mg Citicoline from 20 crushed tablets transferred into a 500 ml volumetric flask. 20 ml of 0.1N Hydrochloric acid added and it kept for 1 hours at 25 ± 1° and volume was made up to the mark with water, mixed and centrifuged for 5 minutes. Further diluted 5 ml of clear supernatant solution to 100 ml with water. Filtered solution through 0.45µ membrane filter.
(Citicoline conc.0.1 mg/ml).

2.6.2 Alkali Degradation

Accurately weighed equivalent to 1000 mg Citicoline from 20 crushed tablets transferred into a 500 ml of volumetric flask, 20 ml of 0.1N sodium hydroxide added and solution kept for 1 hours at 25 ± 1° and volume was made up to the mark with water and centrifuged for 5 minutes. Further diluted 5ml of clear supernatant solution to 100 ml with water. Filtered solution through 0.45µ membrane filter.
(Citicoline conc.0.1 mg/ml).

2.6.3 Oxidative Degradation

Accurately weighed equivalent to 1000 mg Citicoline from 20 crushed tablets transferred into a 500 ml volumetric flask, 10 ml of 6% hydrogen peroxide added and solution kept for 1 hours at 25 ± 1° and volume was made up to the mark with water, mixed and centrifuged for 5 minutes. Further diluted 5 ml of clear supernatant solution to 100 ml with water. Filtered solution through 0.45µ membrane filter.
(Citicoline conc.0.1 mg/ml).

2.6.4 Thermal Degradation:

Accurately weighed equivalent to 1000 mg Citicoline from 20 crushed tablets transferred into a 500 ml volumetric flask.300 ml of water added and dissolved. Heated up to boiling and cooled at room temperature and volume was made up to the mark with water, mixed and centrifuged for 5 minutes. Further 5 ml of clear supernatant solution diluted to 100 ml with water. Solution was filtered through 0.45µ membrane filter. (Citicoline conc. 0.1 mg/ml).

3.0 RESULTS AND DISCUSSION

3.1 Optimization Of The Chromatographic Conditions

The primary target in developing this stability indicating LC method was to Developed accurate, precise, specific, economic method for determination of citicoline in various formulation and good resolution between citicoline and its degradants. To achieve the

Separation stationary phase of C18 and a combination of mobile phase phosphate

buffer with methanol were used. The separation of degradation products and citicoline sodium was achieved on Hypersil BDS C18 column and phosphate buffer: methanol (95:5%/v/v) as a mobile phase. Mobile phase flow rate was maintained at 1.0 mL min^{-1} and eluent were monitored at 280 nm. A 20 µl of sample was injected using a fixed loop and the total run time was 7 min. The forced degradation study showed that the method was highly specific and No interference of degradation product at the retention time of principle peak was found. The developed method was found to be specific and validated as per ICH guidelines.

3.2 Results Of Forced Degradation Experiments

Degradation of citicoline sodium was observed almost in all conditions. The degradation behaviour of citicoline sodium in various stress conditions was shown in Fig.1.Purity angle was found to be smaller than purity threshold of citicoline sodium main peak at initial and after the stressed experimental condition. Results indicate that the citicoline sodium peak is stable and homogeneous in all stress conditions tested. The results were shown in Table 1.

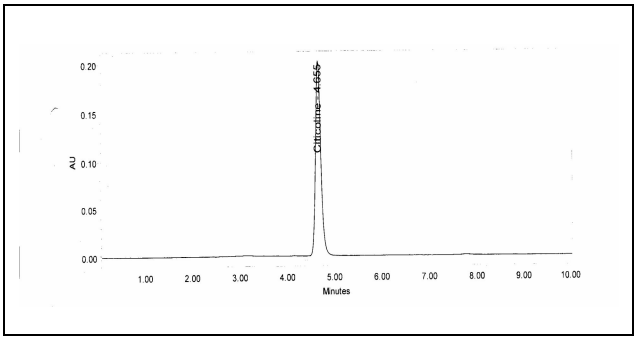

Fig A: Thermal stress sample

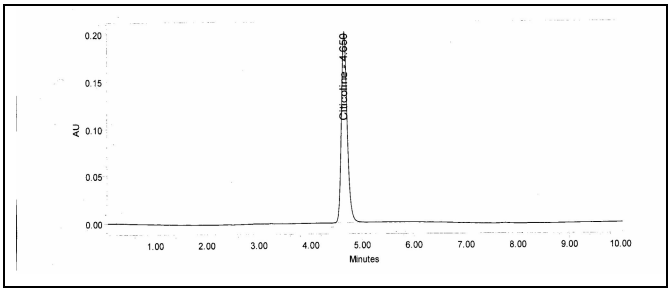

Fig C: Alkali stressed sample

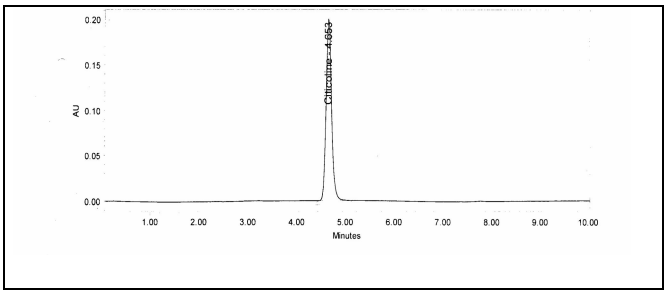

Fig B: Acid stressed sample

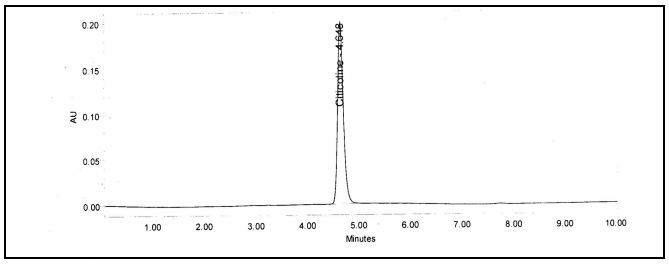

Fig D:Peroxide stressed sample

Fig.1 A,B,C,D- Stress degradation behaviour of Citicoline sodium in various stress conditions.

Table 1. Peak purity data for Citicoline sodium

Sr. No.	Parameters	Acid Degradation	Base Degradation	Peroxide Degradation	Thermal Degradation
1	Wt of sample	1650.45	1650.08	1650.75	1650.02
2	Area Response	1556225	1567558	1553439	1583592
3	Chromatographic Purity	100.00	100.00	100.00	100.00
4	Purity angle	0.042	0.042	0.047	0.045
5	Purity Threshold	0.273	0.259	0.271	0.278

3.2 Method Validation

The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, robustness and system suitability in accordance with ICH guidelines Q2A (R1).

3.2.1 Linearity

The curve proved to be linear over a concentration range of 20-180 µg mL^{-1} (Fig2). Standard solution were prepared at six concentrations (20,40,60,80,100,120,140,160,180 µg mL^{-1}) were injected in triplicate. Linear regression of concentration Vs peak area resulted in an average coefficient of determination (R2) 0.999. The Regression equation is Y= 15188X (Fig.2).

3.2.2 Precision

The precision of the method was evaluated by carrying out six independent assays of test samples of citicoline sodium. The precision of the method was also evaluated using two different analysts, different LC systems. The results shown in Table.2, indicates that the method is reproducible.

3.2.3 Accuracy

Accuracy was calculated as the percentage recovery of the known added amount of citicoline sodium reference substance in the sample solutions using five concentration levels covering the specified range (50,75, 100, 125, 150 µg mL^{-1}). The accuracy of the method ranged from 100.22 to 101.32% indicating that this assay is reliable (Table 3).

Table 2. Method precision for citicoline sodium

Sr. No.	Sample wt. (mg)	Area of rep1	Area of rep2	Mean area	Mg/tab	% assay
1	1649.82	1570645	1570946	1570796	1007.93	100.80
2	1648.95	1567274	1568814	1568044	1006.69	100.70
3	1648.06	1568779	1571478	1570129	1008.58	100.90
4	1649.74	1575358	1569272	1572315	1008.95	100.90
5	1648.96	1581694	1574249	1577972	1013.06	101.30
6	1649.08	1578417	1581147	1579782	1014.15	101.00
Avg.						101.00
SD						0.28
RSD						0.28

Table 3. Accuracy of the analysis of Citicoline sodium

Percentage Level	Area	% Recovery	RSD(%)
50	769043	101.32	0.23
75	1138773	100.22	0.24
100	1529802	100.90	0.11
125	1907256	100.73	0.36
150	2281051	100.37	0.15

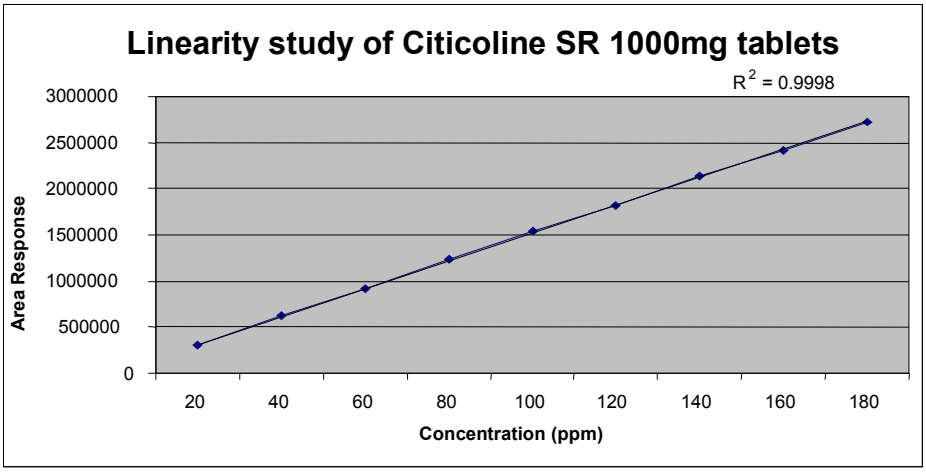

Fig.2: Linearity curve of Citicoline sodium

Table 4. Robustness study of citicoline sodium

		Observation	% RSD	Acceptance criteria
Effect of Wavelength	Wavelength 278 nm	The cumulative % RSD of assay values For 1000 mg/tab	0.01	NMT 2.0%
	Wavelength 282 nm	The cumulative % RSD of assay values For 1000 mg/tab	0.00	NMT 2.0%

3.2.4 Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered. The effect of wavelength change at 278nm and 282nm instead of 280 nm have studied. While the other parameters were held constant in chromatographic condition. The RSD was not more than 1% in both conditions (Table 4).

3.2.5 Stability In Analytical Solution

Sample and standard solution were prepared and injected and assay value calculated. After storing at 25ºc it was run against the freshly prepared standard solution at 4 hrs, 8 hrs, and 12 hrs. The % RSD was not more than 2%.

4.0 CONCLUSION

Forced degradation study on citicoline sodium in tablet formulation was carried out under the conditions of hydrolysis, oxidation, acid, base and thermal degradation. Based on the information generated by forced degradation, a stability-indicating assay method was developed and validated. The method was found sufficiently linear, precise, accurate, sensitive and specific to the drug. Study of various robustness parameters revealed the method to be robust. No interference of degradation product at the retention time of principle peak was found in degradation study.

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