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STABILITY INDICATING LC METHOD FOR THE DETERMINATION OF CITICOLINE SODIUM IN INJECTION FORMULATION

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ABSTRACT: A novel stability indicating LC assay method was developed and validated for quantitative determination of citicoline sodium in injection formulation in the presence of degradation products generated from forced degradation studies. An isocratic reverse phase LC method was developed to separate the drug from the degradation products using cosmosil C_{18} (250X4.6mm, 5µm) column and a mobile phase constituted of phosphate buffer and methanol (95:5 % v/v). The wave length of the detection is 276 nm. Citicoline was subjected to stress conditions of hydrolysis (acid, base), oxidation, photolysis and thermal degradations. The validation data showed that the assay is sensitive, specific and reproducible for the determination of citicoline sodium in injection formulation in the presence of stress degradants and impurities. The method is linear from 125µg mL^{-1} to 375µg mL^{-1}. The accuracy of the method was found to be 98.30%. Mean inter and intraday assay relative standard deviation (RSD) were less than 1.0%. The proposed method was found to be suitable and accurate for quantitative determination and the stability study of citicoline sodium in injection formulation.

Key words: Citicoline sodium, method validation, column liquid chromatography and degradation.

1.0 INTRODUCTION

Citicoline is the generic name of synthetic CDP-choline (cytidine diphosphate choline), organic molecule produced endogenously and found in all living cells. CDP-choline a precursor for the synthesis of phospholipids that are essential constituents of cell membranes, including phosphotidyl choline, phosphotidyl serine and phosphotidyl ethanolamine. Because cell membranes have a very high turnover rate, these phospholipids must be continuously synthesized to ensure adequate function of cells. Citicoline is often called as brain nutrient because it increases levels of several neurotransmitters including acetylcholine, dopamine and nor adrenaline; helps maintain the integrity of neuronal cell membranes; and increases energy production in the frontal cortex. The scientific name for citicoline is 5-diphosphocholine. 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].

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 injection 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 injection formulation in the presence of its degradants.

2.0 EXPERIMENTAL

2.1 CHEMICALS AND REAGENTS

All the reagents were of analytical-reagent or HPLC grade unless stated otherwise. Milli-Q-water was used throughout the experiment. Potassium dihydrogen phosphate and hydrogen peroxide (S.D. Fine Chem., Mumbai, India), ortophosphoric acid, tetra butyl ammonium hydroxide and methanol (Spectrochem, Mumbai, India) and sodium hydroxide (Central drug house Pvt. Ltd, Mumbai, India) were used. Citicoline standard was obtained from strides arco laboratories (Bangalore, India).Citicoline sodium injection (250 $mgmL^{-1}$) formulation was purchased from the local pharmacy.

2.2 INSTRUMENTATION

The HPLC system used was an Agilent 1100 series comprised of degasser, quaternary pump, auto injector, column compartment, photo diode array detection and the system was controlled through Chemstation software. Analytical column used for this method is cosmosil 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 phosphate and 0.1M tetra butyl ammonium phosphate. Tetra butyl ammonium phosphate solution was prepared by adjusting the pH of 1.0% potassium dihydrogen phosphate to 4.5 with ortho phosphoric acid.

2.4 STANDARD PREPARATION

Standard stock solution was prepared by dissolving 28 mg of citicoline sodium dihydrate (equivalent to 25mg of citicoline) in sufficient amount of water in a 100 mL volumetric flask and diluted up to the mark with water.

2.5 SAMPLE PREPARATION

5.0 mL of injection was transferred into a 100 mL volumetric flask and diluted up to the mark with water. 2.0 mL of the above solution was further diluted to 100 mL in a volumetric flask.

2.6 CHROMATOGRAPHIC CONDITIONS

Before the mobile phase was delivered into the system, buffer and methanol were filtered through 0.45 µm, PVDF membrane 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 : Cosmosil C_{18} (250 mm x 4.6 mm) 5µm
Wavelength : 276 nm
Injection volume : 20 µl
Flow rate : 1.0 mL min^{-1}
Column temperature: 25^0C
Run time : 25 min

2.6 PROCEDURE FOR FORCED DEGRADATION STUDY OF CITICOLINE SODIUM

2.6.1 ACIDIC DEGRADATION

1.0 mL of citicoline sodium injection was transferred in to a 20 mL volumetric flask, then 2.0 mL of 0.1N HCl was added and kept at 80^0C about 4 hrs in water bath, the solution was allowed to attain room temperature. Then the solution was neutralized by 0.1N NaOH and diluted to volume with water. 2.0mL of the above solution was further diluted to 100 mL with water. Repeated the same with 1.0N HCl.

2.6.2 ALKALI DEGRADATION

1.0 mL of citicoline sodium injection was transferred in to a 20 mL volumetric flask, then 2.0 mL of 0.1N NaOH was added and kept at 80^0C about 4 hrs in water bath, the solution was allowed to attain room temperature. Then the solution was neutralized by 0.1N HCl and diluted to volume with water. 2.0mL of the above solution was further diluted to 100 mL with water. Repeated the same with 1.0N NaOH.

2.6.3 OXIDATIVE DEGRADATION

1.0 mL of citicoline sodium injection was transferred in to a 20 mL volumetric flask, then 2.0 mL of 3% H_2O_2 was added and kept at 80^0C about 4 hrs in water bath, the solution was allowed to attain room temperature. Then the solution was neutralized by 0.1N NaOH and diluted to volume with water. 2.0mL of the above solution was further diluted to 100 mL with water.

2.6.4 THERMAL DEGRADATION

1.0 mL of citicoline sodium injection was transferred in to a 20 mL volumetric flask and kept at

80^0C about 4 hrs and diluted to volume with water. 2.0mL of the above solution was further diluted to 100 mL with water.

2.6.5 UV DEGRADATION

The sample was exposed to UV short (254 nm) and UV long (365 nm) light for 8 hrs. 1.0 mL of UV light exposed sample was transferred in to a 20 mL volumetric flask and made up the volume with water. 2.0mL of the above solution was further diluted to 100 mL with water.

3.0 RESULTS AND DISCUSSION

3.1 OPTIMIZATION OF THE CHROMATOGRAPHIC CONDITIONS

The primary target in developing this stability indicating LC method was to achieve good resolution between citicoline and its degradants. To achieve the separation of degradation products, stationary phase of C_{18} and a combination of mobile phase phosphate buffer with methanol were used. The separation of degradation products and citicoline sodium was achieved on cosmosil C_{18} 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 276 nm. A 20 µl of sample was injected using a fixed loop and the total run time was 25 min. The forced degradation study showed that the method was highly specific and the entire degradation products were well resolved from the main peak. 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 behavior of citicoline sodium in various stress conditions was shown in Fig.1.Peak purity results greater than 990 indicate that the citicoline sodium peak is homogeneous in all stress conditions tested. The results were shown in Table 1.

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 125-375 µg mL^{-1} (Fig 2).Standard solution were prepared at six concentrations (125, 175, 200, 250, 300, 375 µg mL^{-1}) were injected in triplicate. Linear regression of concentration Vs peak area resulted in an average coefficient of determination (R^2) 0.999. The Regression equation is Y= 21.34x+39.54 (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 intermediate precision of the method was also evaluated using two different analysts, different LC systems and two different days in the same laboratory. 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 three concentration levels covering the specified range (50,100,150 µg mL^{-1}). The accuracy of the method ranged from 98.1 to 98.7 %, indicating that this assay is reliable (Table 3).

3.2.4 ROBUSTNESS

To determine the robustness of the developed method, experimental conditions were purposely altered and the resolution between citicoline and base degradation products were evaluated. The flow rate of the mobile phase was 1.0 mL min-^{1}. To study the effect of flow rate it was changed by 0.2 units from 0.8 to 1.2 mL min^{-1}. The effect of column temperature was studied at 20^0C and 30^0C instead of 25^0C, while the other mobile phase components were held constant in chromatographic condition. The RSD was not more than 1% and theoretical plates were not less than 7500 in all conditions (Table 4).

3.2.5 STABILITY IN ANALYTICAL SOLUTION

The stability of the standard and sample solutions were tested at regular intervals. The stability of solutions was determined by comparing results of the assay of freshly prepared standard solutions. The differences in area % values were within 2% up to 9 hrs for standard and 21 hrs for sample.

4.0 CONCLUSION

Forced degradation study on citicoline sodium in injection formulation was carried out under the conditions of hydrolysis, oxidation and photolysis. 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. The resolution of drug and degradation products remained unaffected by change in analytical instrument.

Table 1. Peak purity data for citicoline sodium

S.No	Condition	Purity factor
1	0.1N HCl	999.85
2	1.0N HCl	999.85
3	0.1N NaOH	999.84
4	1.0N NaOH	999.74
5	Sunlight	999.98
6	UV-light	999.98
7	Dry Heat	999.98
8	3% H2O2	999.92

Table 2. Intra and inter day precision for citicoline sodium

S.No	Intraday	Interday
1	10.3.6	105.8
2	103.6	104.6
3	103.2	104.7
4	103.2	105.0
5	103.3	104.9
6	103.1	105.0
Mean	103.3	105.0
% RSD	0.2	0.4

Table 3. Accuracy of the analysis of Citicoline sodium

Percent level	Area [a]	% Recovery [a]	RSD (%) [a]
50	2666.6	98.1	0.3
100	5358.4	98.7	0.3
150	7949.4	98.1	0.1

a : average of three determinations

Table 4. Robustness study of citicoline sodium

Robustness Parameter	Theoretical plate number (n)	RSD (%)
Original condition	8829	0.06
Flow change		
-0.2 mL min^{-1}	9471	0.04
+0.2 mL min^{-1}	7571	0.57
Temperature		
-5.0^0C	7769	0.19
$+5.0^0$C	9354	0.15
pH		
-0.2 units	9471	0.17
+0.2 units	9612	0.06
Organic phase		
-2.0%	9574	0.10
+2.0%	8915	0.09

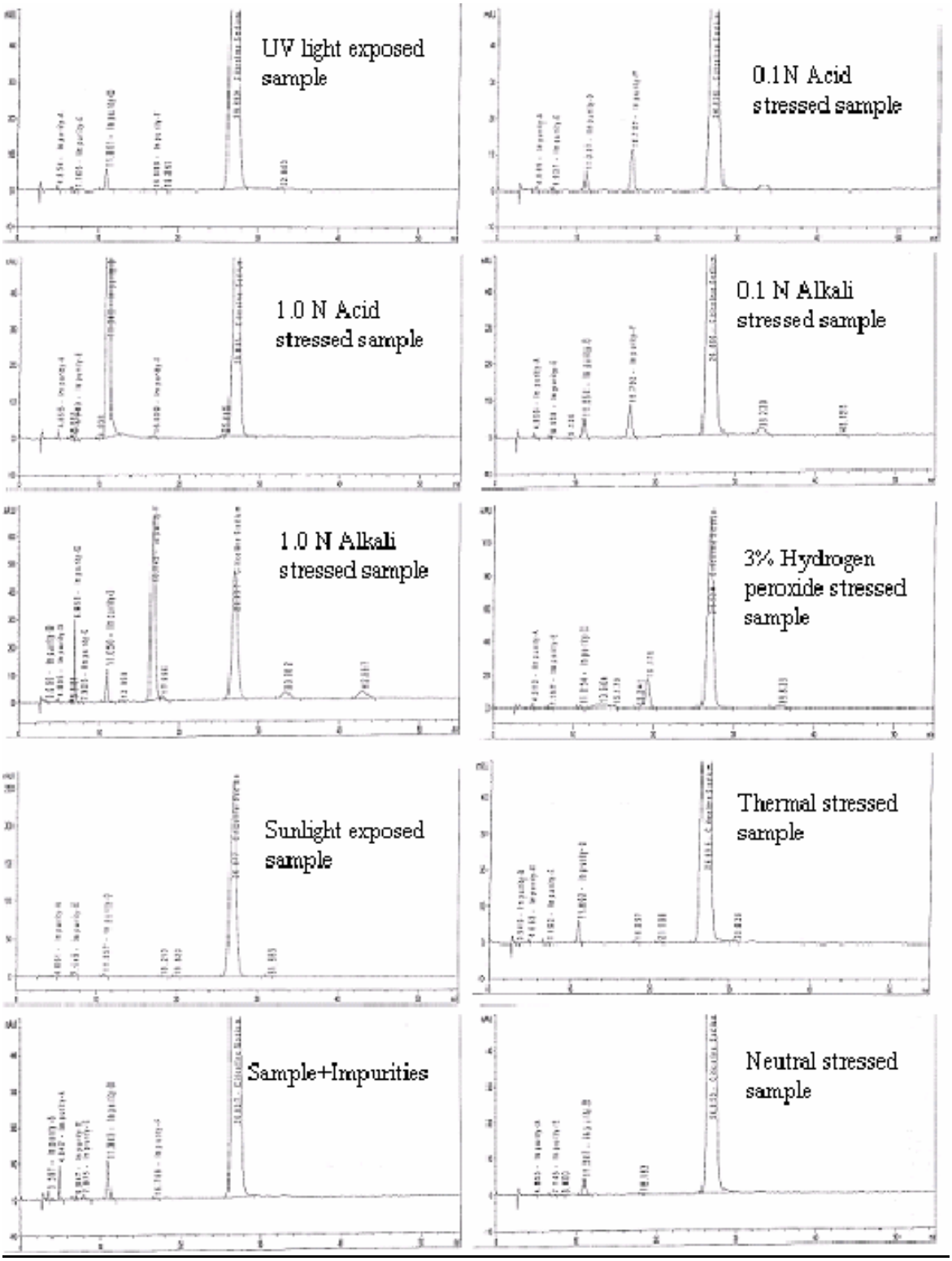

Fig.1 Stress degradation behavior of Citicoline sodium in various stress conditions.

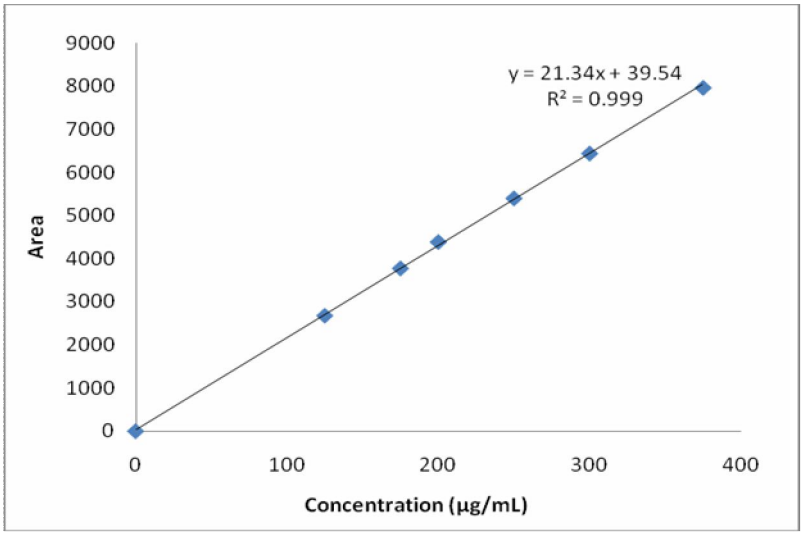

Fig.2: Linearity curve of Citicoline sodium

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