Original Article

Stress degradation studies on citicoline sodium and development of a validated stability-indicating HPLC assay

Abstract

Aim: High performance liquid chromatography presents a useful aspect for the development of stability indicating assays. Objective of the current study was to establish inherent stability of citicoline sodium through stress studies under a variety of international conference on harmonization recommended test conditions and to develop a stability-indicating assay. **Materials and Methods:** Thermal stability studies were performed in a hot air oven (Hicon) and photo stability studies were carried out under the sunlight. The chromatographic separations were carried out on a reverse phase phenomenox C18 (250 mm×4.6 mm i.d., particle size 5 µm) column. The chromatographic method was fine tuned using the sample generated from forced degradation studies. Mobile phase consists of mixture of buffer (potassium dihydrogen orthophosphate, tetra butyl ammonium, triethyl amine; pH 5.5; 0.002M) and methanol (95:5, v/v). **Results:** Good resolution between the peaks corresponds to degradation products and the analyte was achieved on 5 µ, 250 mm×4.6 mm i.d., C18 column (Luna, phenomenox, USA). The method was validated for accuracy, precision, linearity, range, and selectivity. **Conclusion:** The study shows that citicoline sodium is a labile molecule in acid, oxidative, and alkali conditions. It is stable to light and dry heat. A stability-indicating method was developed and is recommended for analysis of the drug and degradation products in stability samples.

Key words:
Assay, citicoline sodium, degradation, stress testing, validation

Introduction

The parent drug stability test guideline Q1A (R2) issued by International Conference on Harmonization (ICH)[1] suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products, and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and they should be fully validated.

Accordingly, the aims of the present study were to establish inherent stability of citicoline sodium through stress studies under a variety of ICH recommended test conditions,[2] and to develop a stability-indicating assay. The drug is chemically 5′-O-[hydroxy({hydroxy[2-(trimethylammonio)ethoxy] phosphoryl}oxy)phosphroyl]cytidine [Figure 1]. It is an amorphous, somewhat hygroscopic powder having molecular weight 489.332 g/mol and pKa value of 4.4. It is soluble readily in water to form acidic solution, practically insoluble in most organic solvents.[3] It is a psychotherapeutic agent used as psychostimulant, nootropics and neurotonics.[4] It exerts its action by activating the biosynthesis of structural phospholipids in the neuronal membrane, increases cerebral metabolism, and increases the levels of various neurotransmitters, including acetylcholine and dopamine.[5] Citicoline sodium is primarily indicated in conditions like cardiac stroke, head trauma, ischemic heart disease, and paralysis of lower extremities and can also be

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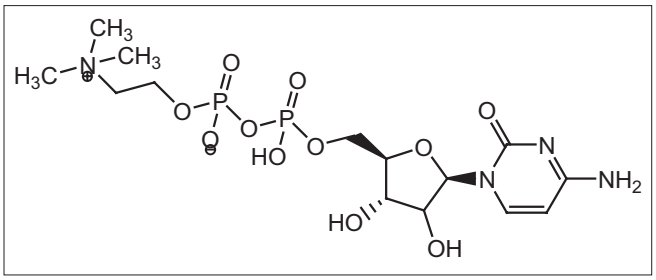

Figure 1: Structure of citicoline sodium

given in adjunctive therapy as an alternative drug of choice in Parkinson's disease.[6] It is generally prescribed as an oral tablet containing 500 mg drug.

Literature survey revealed High performance liquid chromatography (HPLC) method for determination of citicoline sodium in pharmaceutical preparation and in biological fluids, the active principle as well as its metabolites have been determined by HPLC through UV detection,[7] proton-decoupled phosphorus magnetic resonance spectroscopy,[8] and diffusion-weighted magnetic resonance imaging.[9] The latter report includes limited stress testing. More intensive stress studies in our laboratory showed various degradation products under different stress conditions. Accordingly, a stability-indicating method was developed, which could separate various degradation products in only 10 min analysis run time.

Materials and Methods

Materials

Citicoline sodium was procured as gift sample from Torrent Research Center, Gandhinagar, India. Tetra butyl ammonium as an ion-pairing reagent was provided by Lambda therapeutics research Limited, Ahmedabad, India. Acetonitrile, methanol, and water (HPLC grade) were purchased from Finar Ahmedabad, India.

Instrumentation

Thermal stability studies were performed in a hot air oven (Hicon) and photo stability studies were carried out under the sunlight. Sunlight intensity during the studies was tested using a Lux meter (ELM 201, Escon, New Delhi, India). The HPLC system consisted of an on-line degasser (DGU-14A), low-pressure gradient flow control valve (FCV-10ALVP), solvent delivery module (LC-10ATVP), auto injector (SIL-10ADVP), column oven (CTO-10ASVP), UV-visible dual-wavelength detector (SPD-10AVP), system controller (SCL-10AVP), and CLASS-VP software (all from Shimadzu, Kyoto, Japan). The chromatographic separations were carried out on a reverse phase phenomenox C18 (250 mm×4.6 mm i.d., particle size 5 µm) column.

Separation studies

Separations were achieved by isocratic elution using buffer (0.002 M potassium dihydrogen orthophosphate, 0.15% tetra butyl ammonium, 0.2% triethyl amine, pH adjusted to 5.5 with orthophosphoric acid) - Methanol (95:5, v/v) as the mobile phase. The mobile phase was filtered through 0.45-µm nylon membrane and degassed before use. The injection volume was 20 µl and the mobile phase flow rate was kept constant at 1 ml min^{-1}. The analyses were carried out at 280 nm.

Degradation studies

All stress decomposition studies were performed at an initial drug concentration of 1 mg ml^{-1} in water. Acid hydrolysis was performed in 0.1 M HCl at 100°C for 2 hrs. The study in alkaline condition was carried out in 0.1 M NaOH at 100°C for 2 hrs. Oxidative studies were carried out at 80°C in 20% hydrogen peroxide for 2 hrs. Photo degradation studies were performed by exposing drug powder to sun light for 7 days. Additionally, the drug powder was exposed to dry heat at 100°C for 2 days. Samples were withdrawn at appropriate time and subjected to HPLC analysis after suitable dilution.

Preparation of citicoline sodium standard stock solution

A stock solution of the drug was prepared at strength of 1 mg ml^{-1}. It was diluted to prepare solutions containing 20–100 µg ml^{-1} of the drug.

Preparation of sample solution

Twenty tablets of citicoline sodium were weighed and analyzed as follows: A mass of tablet powder equivalent to 100 mg citicoline sodium was weighed and transferred in a 100 mL volumetric flask mixed with water (60 mL), and sonicated for 10 min. The solution was filtered through Whatman filter paper No. 41, and the residue was washed thoroughly with water. The filtrate and washings were combined in a 100 mL volumetric flask and diluted to the mark with water. This solution was suitably diluted with water to obtain a working solution of citicoline sodium (50 µg ml^{-1}).

Validation of the method

Linearity and range

Calibration curves were constructed by plotting peak areas versus concentration of citicoline sodium and the regression equations were calculated. The calibration curves were plotted over the concentration range 20–100 µg ml^{-1}.The solutions were injected into the HPLC column, keeping the injection volume constant (20 µl).

Precision

Six injections of three different concentrations (40, 60, and 80 µgml^{-1}), were given on the same day and the values of relative standard deviation (RSD) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

Accuracy

Accuracy was evaluated by calculating recoveries of citicoline sodium by the standard addition method. Known amounts of standard solutions of citicoline sodium (50, 100, and 150 %) were added to prequantified sample solutions of tablets. The amounts of citicoline sodium were determined by applying these values to the regression equation of the calibration curve.

Specificity and selectivity

The specificity of the method was established through study of resolution factors of the drug peak from the nearest resolving peak, and also among all other peaks.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the effect on resolution was recorded. The flow rate of mobile phase was changed by 0.1 units from 0.9 to 1.1 mL min^{-1}. The effect of mobile phase composition was studied by varying the percentage of two components. Changes in the column oven temperature and pH of mobile phase were studied in the same way.

Stability of solutions

The solution stability of citicoline sodium in the assay method was studied by leaving both the test solutions of sample and reference standard in tightly capped volumetric flask at room temperature for 24 hrs. The same sample solutions were assayed at 0 hrs, 12 hrs, and up to study period. The mobile phase stability was also carried out by assaying freshly prepared sample solutions against freshly prepared reference standard solution for 12 hrs interval up to 24 hrs. Mobile phase preparation was kept constant during the study period. The RSD for the assay of citicoline sodium was calculated during solution stability experiments.

Results and Discussion

Degradation behavior

HPLC studies on citicoline sodium under different stress conditions suggested the following degradation behavior:

Acidic condition

The drug gradually decreased with time on heating at 100°C in 0.1 M HCl, forming degradation products at relative retention time (RRT) 0.82 and 1.25. The rate of hydrolysis in acid was higher as compared to that of alkali or water [Figure 2].

Degradation in alkali

The drug gradually decreased with time on heating at 100°C in 0.1 M NaOH, forming degradation products at RRT 0.82, 1.07, and 1.25. The rate of hydrolysis in alkali was lesser as compared to that of acidic or oxidative condition [Figure 3].

Oxidative conditions

Major degradation product was observed after exposure of drug solution in 20% hydrogen peroxide at 80°C for 2 hrs, forming degradation products at RRT 0.72 and 0.92 [Figure 4].

Photolytic conditions

The photolytic studies showed that citicoline sodium was stable to the effect of light. When the drug powder was exposed to sun light for 7 days, no decomposition of the drug was seen [Figure 5].

Solid-state study

The solid-state studies showed that citicoline sodium was stable to the effect of temperature. When the drug powder was exposed to dry heat at 100°C for 2 days, no decomposition of the drug was seen [Figure 6].

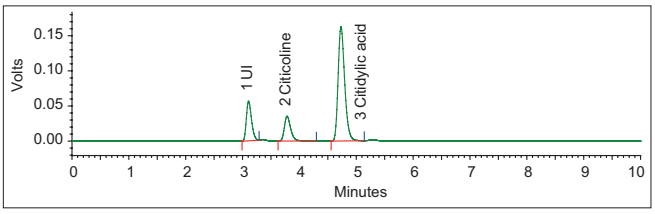

Figure 2: Chromatogram showing degradation in acidic condition (0.1 N HCL)

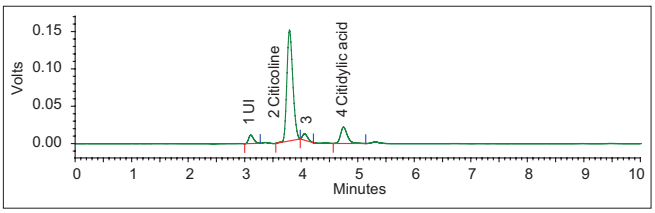

Figure 3: Chromatogram showing degradation in alkaline condition (0.1 M NaOH)

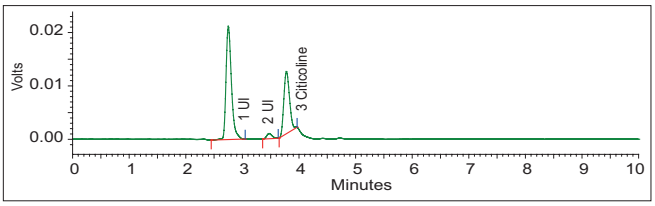

Figure 4: Chromatogram showing degradation in oxidative condition (20% H2O2)

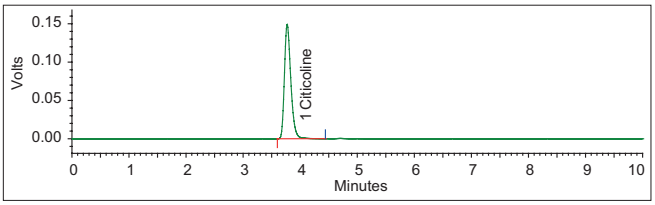

Figure 5: Chromatogram after exposure of drug to photolytic condition

Development and optimization of the stability-indicating method

The method was optimized to separate major degradation products formed under various conditions. Resolution was also checked on the degradation solutions to confirm the separation behavior. The resulting chromatogram is shown in Figures 2-4. It indicates that the isocratic method was successful in separation of drug and all degradation products.

It may be pertinent to add here that the product at RRT 1.25 [Figures 2 and 3] formed was isolated and characterized as citidylic acid through mass studies [Figure 7] and probable degradation pathway is represented in Figure 8.

Validation of developed stability-indicating method

The response for the drug was strictly linear in the concentration range between 20 and 100 μg ml^{-1}. The mean (±%RSD) values of slope, intercept and correlation coefficient were 12290 (±1.123), 4292 (±0.945) and 0.999 (±0.005), respectively [Figures 9 and 10].

The data obtained from precision experiments are given in Tables 1 and 2 for intra and inter day precision studies. The %RSD values for intra-day precision study were <1.0% and for inter-day study were <2.0%, confirming that the method was sufficiently precise. Percentage recovery was calculated from differences between the peak areas obtained for fortified and unfortified solutions. As shown from the data in Table 3, excellent recoveries were made at each added concentration. The data obtained from robustness are given in Table 4 for changes in flow rate, ratio of mobile phase components, column oven temperature, and pH of mobile phase.

The RSD values of the assay of citicoline sodium during solution stability experiments were within 1%. No significant changes were observed in the content of solution during stability experiments. The solution stability experimental data confirmed that the sample solutions and mobile phases used during assay and related substance determination were stable for at least 1 day [Table 5].

Figures 2-4 show that the method was sufficiently specific to the drug. The resolution factor for the drug peak was >3 from the nearest resolving peak. Good separations were always achieved, indicating that the method remained selective for all components under the tested conditions.

Analysis of a formulation

The proposed method was applied for the determination of citicoline sodium in tablet (strolin). A typical chromatogram obtained during the assay of tablets is depicted in Figure 11. The result of these assays were 100.12% (RSD=0.56%) of the label claim for the formulation. The result of the assay indicates that the method is selective for assay of citicoline sodium without interference from excipients used in the tablet.

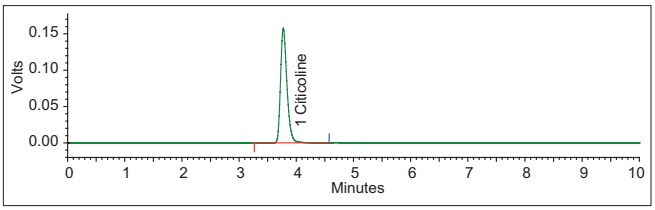

Figure 6: Chromatogram after exposure of drug to thermal condition

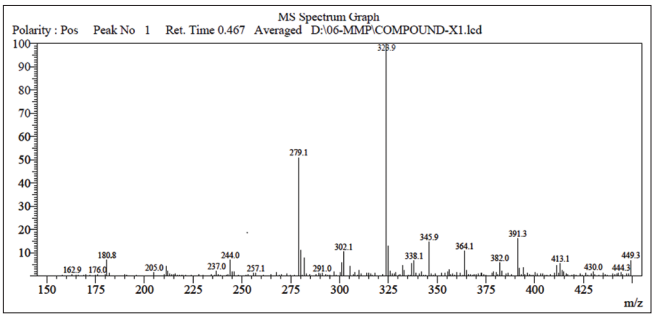

Figure 7: Mass spectrum graph of citidylic acid

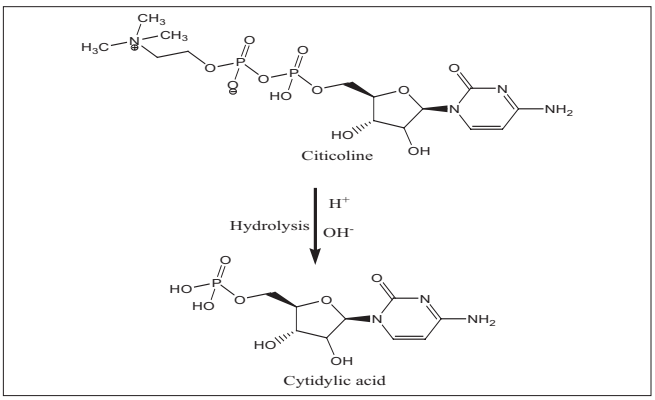

Figure 8: Probable mechanism of degradation

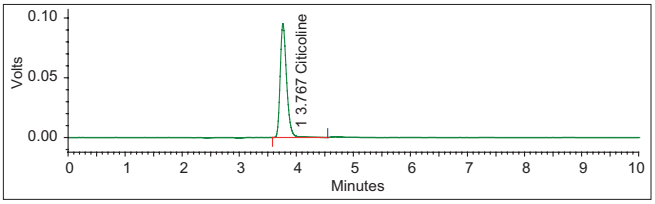

Figure 9: Chromatogram of standard citicoline sodium

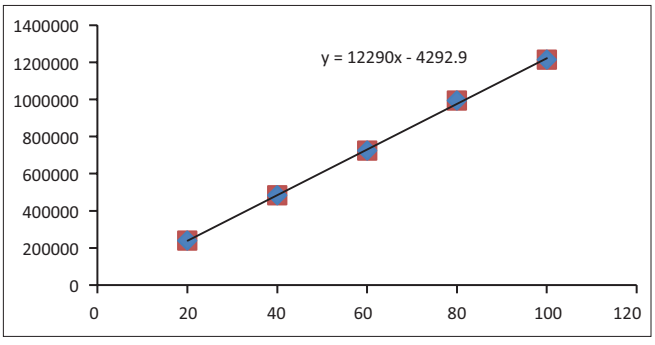

Figure 10: Calibration data for analysis of citicoline sodium

Table 1: Intra-day precision

Actual concentration (μgml−1)	Intra-day measured concentration (n=6)		
	Amount measured (μg ml-1)	SD	%RSD
40	41.15	0.12	0.29
60	58.89	0.35	0.59
80	77.12	0.72	0.93

Table 2: Inter-day precision

Actual concentration (μg ml−1)	Inter-day measured concentration (n=6)		
	Amount measured (μg ml-1)	SD	%RSD
40	41.10	0.24	0.58
60	58.32	0.68	1.16
80	76.32	1.21	1.58

Table 3: Recovery studies

Formulation citicoline sodium, (μg ml^{-1})	Amount added (μg ml^{-1})	Calculated concentration (mg)±SD.; %RSD (n=3)	Recovery (%)
40	20	60.12±0.32;0.53	100.2
40	40	80.47±0.49;0.60	100.5
40	60	101.32±0.73;0.72	101.3

Table 4: Robustness data for citicoline sodium

Parameter	Modification	Recovery, %±SD	%RSD
Flow rate, 1 mL/min	1±0.1	100.6±0.84	0.83
Mobile Phase composition	95±5	100.6±0.76	0.75
	96±4	98.7±0.91	0.92
	94±6	99.3±0.62	0.62
Column temperature, 25°C	25±5	100.4±0.59	0.58
pH of mobile phase, 5.5	5.5±0.2	100.1±0.83	0.82

Table 5: Solution stability

Time interval h	Standard solution±SD	%RSD (n=3)	Sample solution±SD	%RSD (n=3)
0	100.3±0.67	0.66	99.8±0.48	0.48
12	99.6±0.73	0.73	98.7±0.64	0.64
24	98.1±0.58	0.59	97.5±0.81	0.83

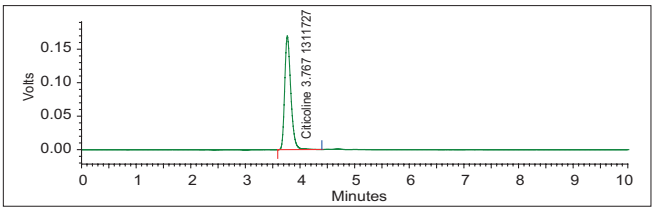

Figure 11: Chromatogram of citicoline sodium tablet

Conclusions

The study shows that citicoline sodium is a labile molecule in acid, oxidative, and alkali conditions. It is stable to light and dry heat. A stability-indicating method was developed, which separates all the degradation products formed under variety of conditions. The method proved to be simple, accurate, precise, specific, and selective. Hence, it is recommended for analysis of the drug and degradation products in stability samples by the industry.

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