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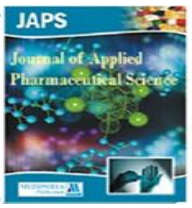

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Rapid analytical procedure for Citicoline in bulk and pharmaceutical dosage form by UV Spectrophotometer

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ABSTRACT

A simple, rapid, accurate, precise, and inexpensive method for the determination of citicoline has been developed using double beam UV spectrophotometer. Ultraviolet spectrophotometric analysis was carried out on a Shimadzu UV 1800 (Shimadzu, Japan) spectrophotometer, in a 1cm quartz cuvette. Citicoline has absorption maxima at 272 nm and the measurements were obtained against distilled water. Beer Lambert's law was obeyed in the concentration range of 5-50µg/ml with correlation coefficient (r^2) 0.9998. The analytical method was successfully validated in order to verify its proper selectivity, linearity, accuracy and precision for the goal intended and its further implementation for the quantification of the active compound in the pharmaceutical speciality for quality control.

Key words: Citicoline, UV Spectrophotometer, Calibration curve.

INTRODUCTION

Citicoline (Cytidine-5'-diphosphocholine or CDP-choline) is a complex organic molecule that functions as an intermediate in the biosynthesis of phosphatidylcholine and chemically it is Cytidine 5'-{trihydrogen diphosphate} p'-[2-{trimethylammonio}ethyl] ester inner salt (figure 1). CDP-choline belongs to the group of biomolecules in living systems known as "nucleotides" that play important roles in cellular metabolism (Conant et al., 2004). Citicoline is readily absorbed in the GI tract and widely distributed throughout the body, crosses the blood-brain barrier and reaches the central nervous system (CNS), where it is incorporated into the membrane and microsomal phospholipid fraction. Citicoline activates biosynthesis of structural phospholipids of neuronal membranes, increases brain metabolism, and acts upon the levels of different neurotransmitters (Secades et al., 2006). It has a variety of beneficial effects in CNS injury models and cognitive enhancing, neuroprotective, and neurological disorders of the brain such as stroke, brain, trauma, Alzheimer's and Parkinson's disease (Nitta et al., 1994, Leon-Carrion et al., 2000, Baskaya et al., 2000, Clark et al., 2001, Adibhatla et al., 2002 and Mirakor et al., 2007). In cocaine-addicted individuals, citicoline has been shown to increase brain dopamine levels and reduce cravings (Killgore et al., 2009).

Literature survey revealed, much work has been published which described physico-chemical properties, pharmacokinetics, toxicity, bioavailability, CNS injury models and neurodegenerative diseases. An extensive literature survey also revealed citicoline monograph is not available in any official pharmacopoeias (IP, BP and USP).

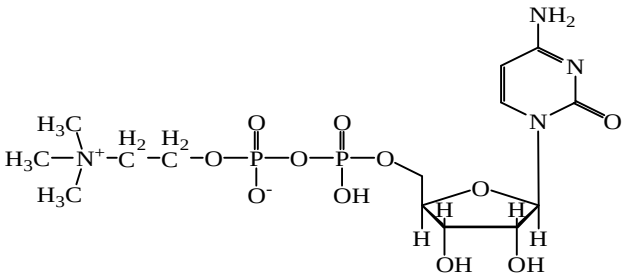

Fig. 1 Chemical Structure of Citicoline.

One analytical method based on HPLC method (Mirakor et al., 2007) and one spectrophotometric method adapting colour reaction in visible range (Malipatil et al., 2010) for quantitative estimation has been published. But no study has been reported for spectrophotometric method in UV range. Thus increasing utilization of this drug in many countries demands the development of new, economic and alternative methods to successfully determine citicoline in raw material and pharmaceutical dosage forms. Therefore, purpose of this study was the development of an analytical methodology for the determination of citicoline in bulk and pharmaceutical preparation.

EXPERIMENTAL

Materials and instruments

A Shimadzu model 1800 (Japan) double beam UV-Visible Spectrophotometer with a pair of 1cm matched quartz cells was used to measure absorbance of the analytical solutions. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample. An ultrasonic bath (model SN-24, India) was used for sonicating the tablet sample solution. Pure sample (reference standard) of citicoline was procured from Cipla Ltd., Baddi as gift sample. Tablet formulation (PREXARON-500) containing citicoline (Lyka Labs Ltd, Maharashtra, India) was purchased from local market and double distilled water was used for preparation of solutions.

Preparation of stock and working standard solutions

An accurately weighed 10mg of citicoline reference standard was transferred to a 50ml volumetric flask. It was dissolved in distilled water and then volume was adjusted to 50ml with distilled water. The final concentration of stock solution was 200µg/ml of citicoline. Working standard solutions of citicoline were prepared by diluting different volumes of stock solution (200µg/ml) in a 10ml volumetric flask to have a series of concentrations *viz.* 5, 10, 20, 30, 40 and 50µg/ml using distilled water.

UV detection

Wavelength was selected by scanning standard drug over the wide range of wavelength 200nm to 400nm. Drug shows reasonably good response at 272nm, is shown in figure 2.

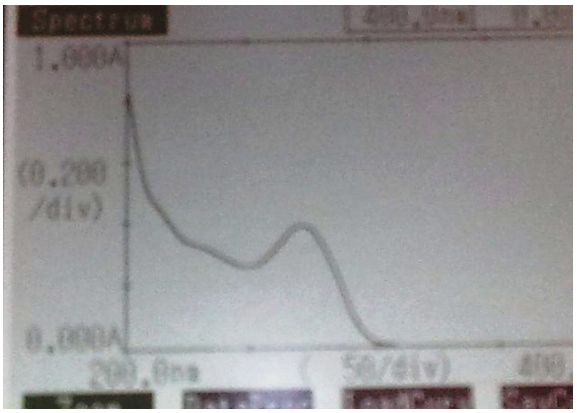

Fig. 2 UV Spectrum of Citicoline in distilled water.

Estimation of citicoline in tablet (recovery study)

Twenty tablets were accurately weighed and powdered. A quantity of powder sample equivalent to 10mg of citicoline was transferred to 100ml volumetric flask. The drug content was dissolved in 50ml of distilled water and was kept in ultrasonicator for 20 min. Finally, the volume was adjusted with distilled water. The solution was filtered through Whatman filter paper No. 41. The above solution (1ml) was transferred to 10 ml volumetric flask and diluted to mark with distilled water to obtain final solution with 10µg/ml of citicoline. Recovery studies were carried out at 80%, 100% and 120% level of label claim. The tablet analysis obtained by proposed method was validated by statistical evaluation (table 1).

Table 1 Results of % Recovery data (Accuracy) for tablet.

Levels	Amount of drug (Tablet) taken (µg/ml)	Amount of Pure drug added (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery
80%	10	8	18.29	101.6
100%	10	10	19.78	98.9
120%	10	12	22.02	100.1
			Mean % recovery	100.2
			SD	1.35

Method validation

The optimized spectrophotometric method was completely validated according to the procedures described in ICH guidelines Q2 (R1) for the validation of analytical methods (ICH, 2005).

Linearity

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. For linearity studies (calibration curve), six different concentrations corresponding to 5, 10, 20, 30, 40 and 50µg/ml of citicoline were prepared from standard stock solutions (200µg/ml) using distilled water. Calibration curve with concentration versus absorbance was plotted (figure 3) and the linear regression equation of citicoline with correlation coefficient value was reported in table 2.

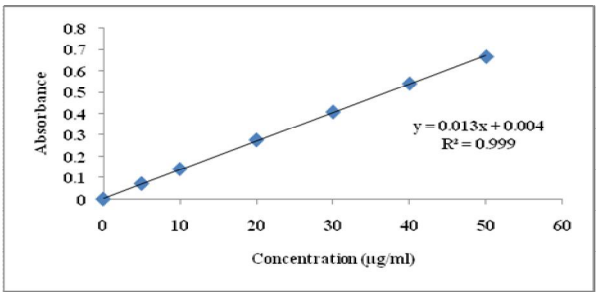

Fig. 3 Calibration curve for Citicoline.

Table 2 Overview of linearity data obtained for citicoline

Regression Parameters	Citicoline
λ_{max} (nm)	272nm
Concentration range (µg/ml)	5-50 µg/ml
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	7.036×10^3
Regression Coefficient, R^2	0.9998
Slope	0.0134
Intercept	0.0049

Table 3 Validation parameters of evaluated method for citicoline

Intra-day Precision* (%RSD)	Inter-day Precision* (%RSD)	LOD* (µg/ml)	LOQ* (µg/ml)
0.48	1.07	0.49	1.49

**n =6*

Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation (% RSD) for a statistically significant number of samples. Intermediate precision was done to evaluate within laboratory variation, on different days at three different concentrations (low, medium and high concentrations) and %RSD was found to be less than 2% (table 3).

Accuracy

For the accuracy of proposed method, recovery studies were performed by standard addition method at three different levels (80%, 100% and 120% of final concentration). A known amount of citicoline pure drug was added to pre-analyzed tablet powder and the sample was then analyzed by proposed method (Wankhede et al., 2008). Results of recovery studies were found to be satisfactory and are reported in table 1.

Limit of detection

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantitated. It is a limit test that specifies whether, or not an analyte is above or below a certain value is shown in table 3.

Limit of quantitation

The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operation conditions of the method is shown in table 3.

RESULTS AND DISCUSSION

The present study describes a highly sensitive, economic, accurate, precise and reproducible method for determination of citicoline. Percent recovery results were found in the range of 98 to 102%. The precision of the proposed method was checked in terms of the inter-day and intra-day time periods and percent RSD was found to be <2%. LOD and LOQ were found to be 0.49µg/ml, 1.49µg/ml respectively. Hence the results of the analysis were validated and recovery studies were carried out as per ICH guidelines. Therefore the newly developed method was successfully applied in tablet dosage form.

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