Original Article

Estimation of citicoline sodium in tablets by difference spectrophotometric method

Abstract

Aim: The present work deals with development and validation of a novel, precise, and accurate spectrophotometric method for the estimation of citicoline sodium (CTS) in tablets. This spectrophotometric method is based on the principle that CTS shows two different forms that differs in the absorption spectra in basic and acidic medium. **Materials and Methods:** The present work was being carried out on Shimadzu 1800 Double Beam UV-visible spectrophotometer. Difference spectra were generated using 10 mm quartz cells over the range of 200-400 nm. Solvents used were 0.1 M NaOH and 0.1 M HCl. **Results:** The maxima and minima in the difference spectra of CTS were found to be 239 nm and 283 nm, respectively. Amplitude was calculated from the maxima and minima of spectrum. The drug follows linearity in the range of 1-50 μg/ml (R^2=0.999). The average % recovery from the tablet formulation was found to be 98.47%. The method was validated as per International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology guidelines. **Conclusion:** This method is simple and inexpensive. Hence it can be applied for determination of the drug in pharmaceutical dosage forms.

Key words:
Citicoline sodium, difference spectroscopy, International Conference on Harmonization, validation

Introduction

Citicoline sodium (CTS) is believed to increase blood flow and oxygen consumption in brain and has been given in treatment of cerebrovascular disorders, parkinsonism, and brain injury.[1] Chemically, it is cytidine 5'-{sodium P'-(2-[trimethylammonio]-ethyl) hydrogen diphosphate}, inner salt.[1] The structure of CTS is shown in Figure 1. Literature survey revealed that so far several spectrophotometric[2-4] and High Performance Liquid Chromatographic (HPLC)[5-12] methods have been reported for estimation of CTS in different sample matrix.

Difference spectrophotometry is an analytical methodology which provides assay results with increased selectivity and accuracy.[13] This method can reduce the interferences in absorbance caused by the impurities present in the analyte. It generates a difference in absorbance (ΔA) between the equimolar solutions of sample showing different spectral properties in two different mediums. Several methods are reported for estimation of various drugs in pharmaceutical dosage forms by difference spectrophotometry.[14-16] So far no difference spectrophotometric method has been reported for estimation of CTS in marketed tablet formulations. So a successful attempt was made to develop and validate a new difference spectrophotometric method for determination of concentration of CTS in tablet formulation.

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Materials and Methods

Experimental

Reagents and standards

Pure standard drug of CTS (purity >99%) was procured as gift samples from Intas Pharmaceuticals Ltd., India. Sodium hydroxide and hydrochloric acid was purchased from S.D. Fine Chem. Pvt. Ltd., Mumbai, India. Purified water obtained

Sagar Suman Panda, Ganeswar Mohanta, Bera Venkata Varaha Ravi Kumar

Department of Pharmaceutical Analysis and Quality Assurance, Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, India

Address for correspondence:
Prof. Sagar Suman Panda,
Department of Pharmaceutical Analysis and Quality Assurance, Roland Institute of Pharmaceutical Sciences, Berhampur - 760 010, Odisha, India.
E-mail: sagarguddu2002@gmail.com

by TKA Water Purification System, Germany was used for preparing drug and reagent solutions. Tablet formulation containing CTS (500 mg) was purchased from the local market.

Instrumentation

A Shimadzu 1800 UV Visible Double beam spectrophotometer (Shimadzu Corporation, Kyoto, Japan) with 10 mm matched quartz cuvettes were used for spectral measurements. A high precision analytical balance was used for weighing the reagents. Ultrasonicator (Enertech, Mumbai, India) was used to affect dissolution of the marketed formulation.

Preparation of standard stock solution

Standard stock solution of the drug was prepared by dissolving 10 mg of the CTS in purified water and volume was made up to 10 ml. This gave a stock solution of 1000 µg/ml. From this 2.5 ml of the solution was taken in two separate 25 ml volumetric flasks and were diluted upto the mark with 0.1 M HCl and 0.1 M NaOH to produce working standard solutions of concentration 100 µg/ml.

Procedure

Preparation of calibration curve

Different aliquots were taken from the respective working standard solutions in separate 10 ml volumetric flasks and finally diluted with 0.1 M HCl and 0.1 M NaOH solutions to prepare a series of concentrations ranging from 1 to 50 µg/ml. The difference spectrum for CTS was recorded by placing the drug in 0.1 M HCl in reference cell and drug in 0.1 M NaOH in sample cell. The amplitude was calculated from the absorbance of CTS at 239 nm (maxima) and 283 nm (minima). Calibration curve was plotted by taking concentration of drug (µg/ml) on X-axis and amplitude on Y-axis. The overlying difference UV absorption spectrum of CTS at concentrations of 5 and 10 µg/ml is shown in Figure 2.

Analysis of commercial dosage forms

Twenty tablets were weighed accurately and powdered finely. A quantity of powder equivalent to 10 mg of CTS was transferred into a 10 ml volumetric flask containing 5 ml of purified water and the content was ultrasonicated for 20 min. The volume was made up with purified water and mixed well. The solution was further filtered using Whattmann filter paper to remove particulate matter, if any. The filtered solution was further appropriately diluted with 0.1 M HCl and 0.1 M NaOH separately for analysis as already described. The amount of drug present in the sample solution was determined using the calibration curve of standard drug.

Method validation

The developed method was validated statistically for linearity, accuracy, and precision (intra-day and inter-day). Linearity of the developed method was determined by scanning the standard solutions of CTS in a seven-point concentration range. Statistical analysis of the regression equation of the calibration

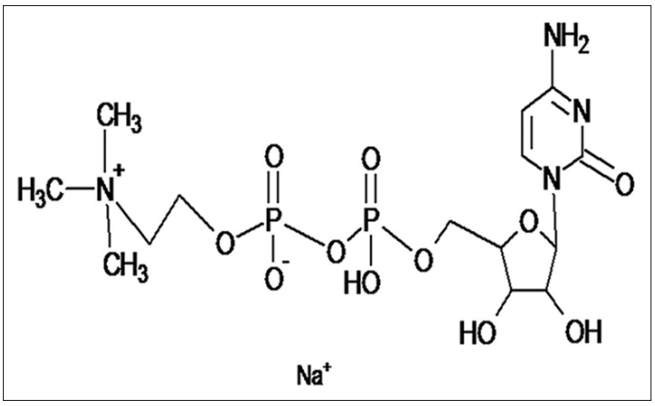

Figure 1: Chemical structure of citicoline sodium

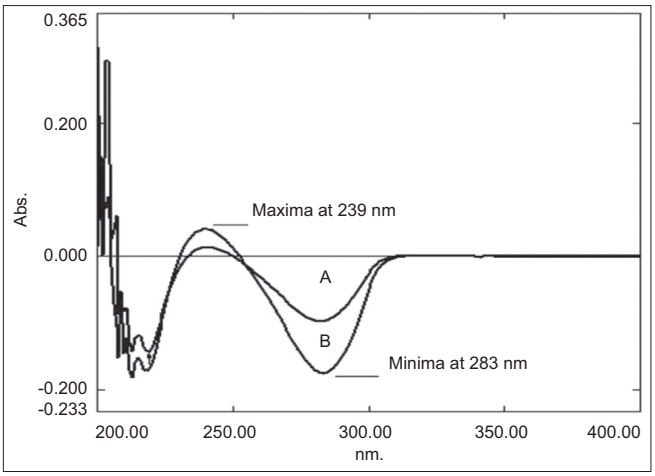

Figure 2: Overlaid UV absorption spectrum of citicoline sodium (a) 5 µg/ml and (b) 10 µg/ml

curve was carried out. To check the accuracy of the proposed method, recovery studies were carried out at 80%, 100%, and 120% of the test concentration as per ICH guidelines.[17] The recovery study was performed three times at each level. The intra-day and inter-day precision was ascertained by actual determination of six replicates of fixed concentration within the Beer's range and the amplitude was found out by the developed method. The percent relative standard deviation was calculated. The molar extinction coefficient and Sandell's sensitivity were also calculated to evaluate the optical characteristics for CTS by the developed method.

Results and Discussion

A new difference UV-spectrophotometric method has been developed to determine the amount of CTS present in tablet formulation. In this methodology, the responses are expressed as amplitude, i.e., the difference between two equimolar solutions of the analyte in two different chemical forms exhibiting different spectral properties. The difference spectrum of CTS in 0.1 M NaOH was recorded by dissolving the drug in 0.1 M HCl solution as reference. The typical difference spectrum shows characteristic maxima at

239 nm and minima at 283 nm. A critical evaluation of the method was performed. The calibration curve was found to be linear over a concentration range of 1-50 μg/ml. The linear regression equation was $y = 0.022x - 0.001$ with a correlation coefficient of 0.999. The optical characteristics are shown in Table 1. The percentage recovery from commercial formulation was found to be 98.47% as shown in Table 2. Accuracy of the developed method was evaluated by recovery studies of the drug. The average recovery ranged from 101.03% to 104.83%. The percent relative standard deviation (RSD) was also <2%, for both intra-day and inter-day determinations showing high degree of precision of the proposed method. The results of validation studies for the method lie within the prescribed limit, indicating that the current method is free from interference from excipients.

Conclusion

A validated difference UV-spectrophotometric method was developed for determination of CTS in pharmaceutical dosage form. The developed method is novel, simple, accurate, and precise. The method is economical when compared with other sophisticated instrumental analytical techniques. The method is suitable for the determination of the CTS in tablet formulation without interference from commonly used excipients. Hence it can be used for routine analysis of CTS in bulk and commercially available formulations.

Table 1: Optical characteristics and validation parameters

Parameters	Obtained values
Wavelength (nm)	239 (maxima), 283 (minima)
Linearity range (μg/ml)	1-50
Sandell's sensitivity (μg/cm^2/0.001AU)	0.046
Molar extinction coefficient (L/mol.cm)	1.107×10^4
Regression equation ($y=ax+b$)*	$0.022x-0.001$
Precision (% RSD)	
Intra-day	0.19
Inter-day	0.28
Accuracy (% Recovery† ± SD)	
80	104.83±0.89
100	101.03±0.71
120	102.88±0.58
% Range of error	
0.05 confidence limits	±0.0320
0.01 confidence limits	±0.0421

RSD – Relative standard deviation; SD – Standard deviation; AU – Absorbance units; *Is $y=ax+b$; where y is absorbance; a is the slope; b is the intercept and x is the concentration; †Is average of three determinations at each level

Table 2: Analysis of commercial formulation

Formulation	Labelclaim (mg)	% Recovery‡ ± SD
Tablet	500	98.47±0.86

SD – Standard deviation; ‡Is average of three determinations

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