DEVELOPMENT AND VALIDATION OF DIFFERENCE SPECTROSCOPIC METHOD FOR CITICOLINE SODIUM IN TABLET DOSAGE FORMS BY ULTRAVIOLET SPECTROSCOPY

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Abstract-*Simple, sensitive and specific spectrophotometric method was developed and validated for quantification of Citicoline Sodium by difference spectroscopy method. Citicoline sodium exhibits a substantial difference in absorbance in the two solvents that is in 0.01 N HCL and 0.01 N NaOH at 283 nm. Beer's law was obeyed in the concentration range of 5 to 50 µg/ml. Results of tablet analysis showed assay value ranging 98 % to 100 % with standard deviation of 0.85 for Citicoline Sodium which indicates repeatability of the method. The results indicated excellent recoveries ranging from 96 % to 103.9 % for Citicoline Sodium with mean of 100 %. Method was validated according for all method parameters for assay as per ICH guidelines Q2R1. Developed method was found to be accurate, linear, precise, sensitive, robust and rugged as per ICH Q2R1 guidelines.*

Keywords-**Citicoline sodium, difference spectroscopy method, ICH Q2R1**

I. INTRODUCTION

Citicoline sodium 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.

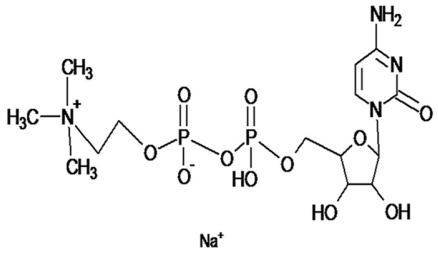

Fig. 1 Chemical structure of Citicoline Sodium

Literature survey revealed that so far several spectrophotometric [2-4] and High Performance Liquid Chromatographic (HPLC) methods have been reported for estimation of Citicoline sodium in different sample matrix. [5-12]

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] UV spectrophotometry method can detect the Citicolione Sodium specifically. Citicoline Sodium is present in different formulations in combination with other drugs. Citicoline Sodium shows specific absorbance in UV spectrophotometry by using 0.01 N HCL and 0.01 N NaOH as solvent. So far no difference spectrophotometric method has been reported for estimation of Citicoline sodium in marketed tablet formulations. So a successful attempt was made to develop and validate a new difference spectrophotometric method for determination of concentration of Citicoline sodium in tablet formula.

II. MATERIALS AND METHODS

A. *Materials*

Citicoline sodium was procured as a gift samples from SRS Pharmaceuticals, Mumbai. All chemicals and reagents used were of UV spectrophotometric grade. UV spectrophotometric method was developed using double beam UV- visible spectrophotometer (Shimadzu, Model No. 1800) having tow matched quartz cells with 1 cm path length..

B. *Selection of Working Solvent*

The selection of working solvent was made after using different acids and bases and their different normalities.

C. *Preparation of Standard Drug Solution*

Standard stock solution containing Citicoline Sodium was prepared by dissolving 10 mg of Citicoline Sodium separately in 50 ml of 0.01 N HCl and 0.01 N NaOH, sonicated for 5 minutes and then final volume of both the solutions was made up to 100 ml with same solvents to get stock solution containing 100 µg/ml of Citicoline Sodium in 0.01 N HCl and 0.01 N NaOH in two different 100 ml volumetric flasks.

D. *Selection of sampling wavelength for Analysis*

By appropriate dilution of two standard drug solutions with 0.01 N HCl and 0.01 N NaOH solutions containing 10 µg/ml of Citicoline Sodium were scanned separately in the range of 200-350 nm to determine the wavelength of maximum absorption for the drug. The difference spectrophotometric method developed for analysis of Citicoline Sodium and one wavelength was selected for estimation of Citicoline Sodium from the overlain spectra as shown as Fig. 1.

E. *Construction of Calibration Curve*

From standard stock solution of drug, six working standard solutions prepared and scanned in the wavelength range of 200-400 nm. The appropriate aliquots of drug were pipetted out from standard stock solution of the drug in 0.01 N HCl and 0.01 N NaOH into series of 10 ml volumetric flask. The volume was made up to get solution of concentration 5, 6, 8, 10, 12, 14, 15, 16 µg/ml of Citicoline Sodium in both 0.01 N HCl and 0.01 N NaOH separately. Calibration curve was constructed at wavelengths 283nm by recording absorbance of concentrations 5-20 µg/ml in each 0.01 N HCl and 0.01 N NaOH and plotting data of difference in absorbance at 283nm vs. related concentration. By using quantitative modes slope, intercept and correlation coefficient values for calibration curve was obtained.

F. *Method Validation*

The method was validated according to ICH Q2B and Q2R1 guidelines for validation of analytical procedure in order to determine linearity, range, accuracy, precision, LOD, LOQ, ruggedness and robustness.

1. *Linearity and Range*

Linearity of the method was studied over calibration curve and range was demonstrated for 50-150 % of assay concentration over linearity.

2. *Accuracy (recovery studies)*

To determine the accuracy of proposed method, recovery studies were carried out at 50%, 100%, 150% of assay concentration from standard bulk sample of Citicoline Sodium which were within the linearity range and percentage recovery values are calculated. Each concentration was prepared in triplets at each level and assayed.

3. *Precision*

The precision is determined by two methods as Intra-day precision and Inter-day precision by preparing three different concentrations of the Citicoline Sodium.

Intra-day precision

The Intra-day precision was determined by analyzing Citicoline sodium at three different time points of the same day. The absorbance, standard deviation, and % RSD were calculated.

Inter-day precision

The Inter-day precision was determined by analyzing Citicoline sodium at three different time points on different days. The absorbance, standard deviation, and % RSD were calculated.

4. ***Ruggedness***

The ruggedness of the proposed method was evaluated by applying the developed procedures by assay of 10 µg/ml of Citicoline sodium by using the same instrument by two different analysts under the same optimized conditions at different days.

5. ***Robustness***

The robustness of method was determined by introducing small change in UV parameters, such as changing the wavelength.

E. Analysis of tablet formulation

Marketed tablet formulations containing 20 mg of Citicoline sodium were analyzed by this method. From the triturate of 20 tablets, an amount equivalent to 10 mg of Citicoline sodium was weighed and transferred to 100 ml volumetric flask. The contents of the flask were dissolved in the 50 ml of 0.01 N HCL and 0.01 N NaOH separately with the help of ultrasonication for 10 minutes. The solution was filtered through Whatman Filter paper No. 41 and then final volume of the solution was made a to 100 ml with the same solvent to get a stock solution containing 100 µg/ml of Citicoline sodium in 0.01 N HCL and 0.01 N NaOH. After appropriate dilutions the absorbance were measured and the concentration of analyte was determined with the equation obtained calibration curve. The tablets analysis done in replicate determinations (n=6).

III. RESULTS AND DISCUSSION

The goal of the study is to develop and validate difference spectroscopic method for quantitation of Citicoline Sodium from tablet dosage. Method was carried out under optimized conditions. It was validated according to ICH Q2B and Q2R1 guidelines. The result of the validation parameters were within acceptable limits.

A. Selection of Common Solvent

0.01 N HCL and 0.01 N NaOH was selected as a common solvent for developing spectral characteristics of drug.

B. Selection of sampling wavelength for Analysis

The difference absorption maximum of pure Citicoline Sodium was found to be 0.153 at wavelength 283 nm as obtained from overlain spectrum Citicoline Sodium in 0.01 N NaOH and 0.01 N HCl. Wavelength selected for the estimation of Citicoline Sodium was 283 nm. This is shown in Fig. 2.

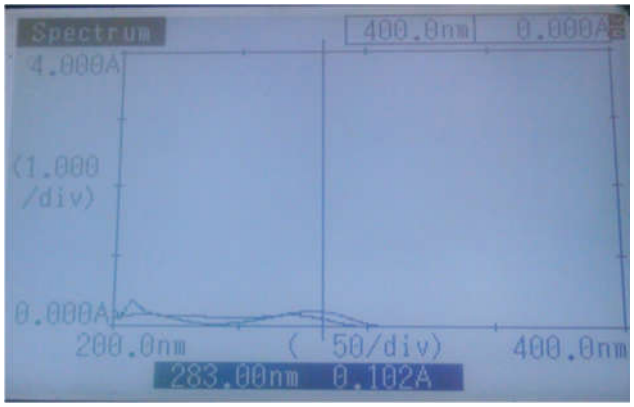

Fig. 2: Overlain spectrum Citicoline Sodium in 0.01 N NaOH and 0.01 N HCl

C. Calibration curve (Regression data)

Calibration curve of Citicoline Sodium was found to be in linear in the range of 4-16 µg/ml in 0.01 N NaOH and 0.01 N HCL with correlation coefficient 0.998. Difference in absorbance at 283nm Vs. related concentration data. Results are shown in Fig. 3 and 4 and summarized in Table 1 and 2.

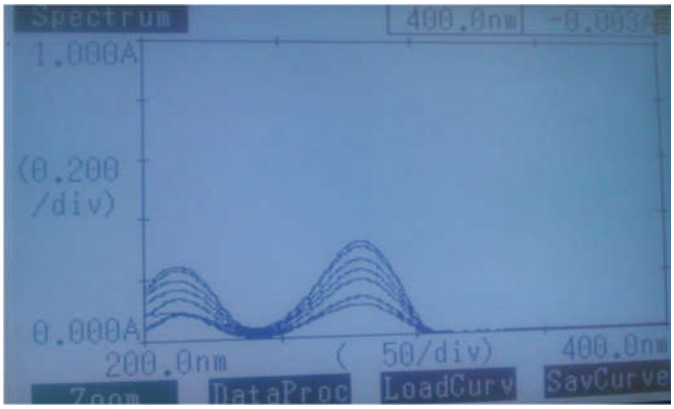

Fig.3: Overlain spectrum of citicoline sodium in 0.01 N HCl

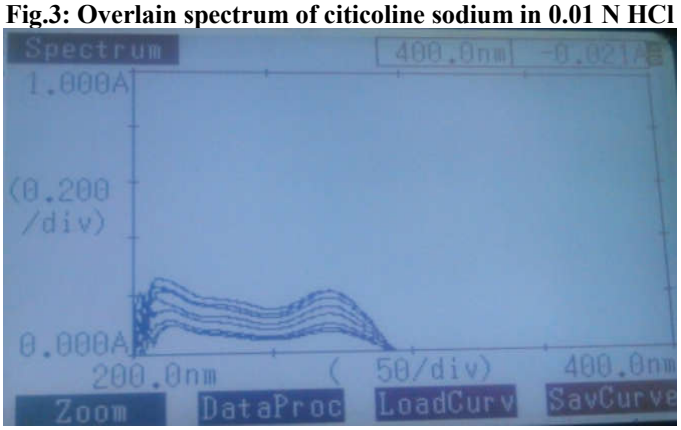

Fig.4: Overlain spectrum of citicoline sodium in 0.01 N NaOH

Table 1: Data for calibration curve

Concentration (µg/ml)	Difference in Absorbance
4	0.091
5	0.102
6	0.109
8	0.131
10	0.153
12	0.167
14	0.197
15	0.211
16	0.219

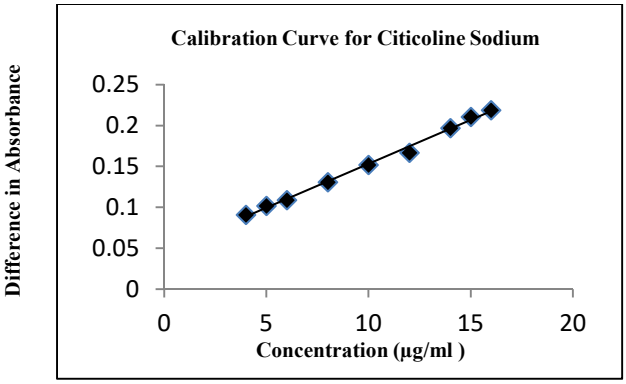

Fig. 5: Calibration Curve for Citicoline Sodium

Table 2: Calibration curve statistics

Sr. No.	Parameter	Result

1	Working Wavelength	283nm
2	Slope	0.011
3	Intercept	0.046
4	Regression Coefficient	0998

D. Method Validation

1. Linearity and Range

Citicoline Sodium follows linearity within the concentration range 5–15 µg/ml. The observed linearity range fitted well Beer-Lambert's law and corresponding regression coefficient (r=0.998) is an indicating of a high degree of method sensitivity. Linearity and Range of method was demonstrated throughout calibration data.

2. Accuracy (recovery studies)

Accuracy of proposed method was demonstrated by recovery studies at 50%, 100%, and 150% of assay concentration from standard bulk sample of Citicoline Sodium which were within the linearity range. For all nine determinations percent recoveries were within the limits of 96%-103.9% and % RSD was less than 1. The results are given in Table 3.

Table 3: Accuracy by recovery studies

Sr. No.	Theoretical Concentration (µg/ml)	Difference in Absorbance	Practical Concentration (µg/ml)	% purity (X)	Mean $\overline{(X)}$	S.D.	% RSD
1	5	0.102	5.17	103.4	103.6	0.85	0.820
2	5	0.100	5.19	103.9			
3	5	0.101	5.18	103.8			
4	10	0.152	9.81	98.1	97.2	0.81	0.833
5	10	0.150	9.63	96.3			
6	10	0.151	9.72	97.2			
7	15	0.211	15.18	101.2	100.37	0.91	0.908
8	15	0.210	15.09	100.6			
9	15	0.208	14.90	99.33			

3. Precision

The precision is determined by two methods as Intra-day precision and Inter-day precision by preparing three different concentrations of the Citicoline Sodium.

Intra-day precision

% RSD for Intra-day precision was found to be less than 2 when determined at different points of the day. The results are shown in Table 4.

Table 4: Results for Intra-day precision

Sr. No.	Concentration µg/ml	Absorbance	SD	% RSD
1	10	0.160		
2	10	0.162	0.17	0.164
3	10	0.164		

Inter-day precision

% RSD for Inter-day precision was found to be less than 2 when determined at different days. The results are shown in Table 5.

Table 5: Results for Inter-day precision

Sr. No.	Concentration µg/ml	Absorbance	SD	% RSD
1	10	0.166		
2	10	0.169	0.10	0.927
3	10	0.170		

4. Ruggedness

The obtained results for method ruggedness were reproducible without significant difference when operated by different validated analysts. Thus the proposed method is rugged. The results are shown in Table 6.

Table 6: Results for ruggedness of method

Sr. No.	Analysts	Concentration (µg/ml)	Absorbance	SD	% RSD

1	Analysts-1	10	0.152	0.22	0.224
		10	0.153		
		10	0.153		
2	Analysts-2	10	0.149	0.35	0.370
		10	0.148		
		10	0.148		

5. Robustness

After deliberately changing method wavelength absorbance was found to be unaffected. The results are shown in Table 7.

Table 7: Results for robustness of method

Sr. No.	Wavelength (nm)	Absorbance
1	282	0.131
2	283	0.147
3	284	0.135

6. Analysis of tablet formulation

A fully validated assay method was applied to marketed tablet formulation of Citicoline Sodium to estimate percent assay value. The result of tablets analysis after replicate determinations (n=6) is shown in Table 8.

Table 8: Results for analysis of tablet formulation

Formulation	Labeled amount (mg)	Amount prepared (µg)	Absorbance	% assayed	Mean	SD	% RSD
Nurocot	500	10	0.153	99.0	98.56	0.85	0.870
Nurocot	500	10	0.152	98.1			
Nurocot	500	10	0.151	97.2			
Nurocot	500	10	0.154	100			
Nurocot	500	10	0.153	99.0			
Nurocot	500	10	0.152	98.1			

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