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UV-VISIBLE SPECTROPHOTOMETER METHOD DEVELOPMENT AND VALIDATION OF CITICOLINE IN SYRUP FORMULATION

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ABSTRACT

An accurate, simple and specific UV-Vis spectrophotometer method has been developed for the quantitative estimation of citicoline in bulk syrup. The wavelength was set to 280 nm. The correlation coefficient of citicoline (0.9990) and the regression equation (y = 1.0302×-2.2688) were shown by a linear calibration curve ranging from 16 µg/ml to 24 µg/ml. The %RSD result is less than 2%. The method has been validated against USP and ICH guidelines to verify method validation parameters such as linearity, accuracy, recovery and robustness. Therefore, a validated method can be used to quantify citicoline in the form of bulk syrup.

KEYWORDS: Calibration curve, Analytical Method, Citicoline, UV spectroscopy.

INTRODUCTION

Citicoline sodium is a complex organic particle, also known as cytidine 5' (dihydrotriphosphate) P'[2-trimethylamino]ethyl]ester internal salt. Citicoline is an intermediate that produces brain phosphatidylcholine from choline. It acts on the level of neurotransmitters and behaves like a presynaptic cholinergic agent.[1],[2] The action of citicoline can also be explained by decreasing phospholipase A2 activity.[3] Citicoline increases the synthesis of phosphatidylcholine.[4],[5],[6]

Citicoline is used as a psychostimulant, a stimulant and a dietary supplement.[7],[8]

It is mainly manifested in cognitive disorders, nerve regeneration, heart stroke, cerebral ischemia, brain and spinal cord injury, and diseases of the nervous system. Citicoline has low toxicity drug characteristics, so it can also be used as an alternative drug. Alzheimer's disease, Parkinson's disease, Huntington's disease in 6 cases, dementia in 7 cases, dyslexia in 8 cases, epilepsy and cerebral hemorrhage.[9,10]

Chemical and reagents: The working standard for citicoline was from Global Pharmaceutical. All chemical reagents and instruments was of analytical grade. Hydrochloric acid and diethylether were purchased from E. Merck Ltd of Pakistan.

Instrumentation and UV spectral conditions: Digital electronic balance, Hitachy 5300 UV spectrometer and 1 cm quartz cell, measurement characteristic wavelength range (200-800) nm.

Standard stock solution preparation: Accurately weighed citicoline sodium working standard equivalent of 100 mg citicoline and is transferred to a 100 ml volumetric flask. It was dissolved in 0.1 N HCl and made up volume up to mark with the same solvent.

Sample Preparation: A sample containing 100 mg of citicoline was transferred in a separator funnel, and 20 ml of 0.1 N HCl was added to the sample and shaked vigorously. It was extracted with 30-40 ml of diethyl ether.

This process was repeated three times.

The upper ether layer was discarded and the lower aqueous layer was collected in a 100 ml volumetric flask and the volume was adjusted to the mark with 0.1 N HCl.

Absorbance spectrum of Citicoline: 2 ml of the standard stock solutions and sample were dispensed into 0.1 N HCl in a 100 ml volumetric flask separately, and diluted to the mark with 0.1 N HCl. The 20 µg/ml solution measured in the range (200-400 nm) using 0.1 N HCl as a blank showed an absorbance spectrum and λmax at 280 nm.

Method development: From standard stock solution of citicoline (1000 µg/ml), series of solutions were prepared by diluting different volumes having different concentrations of citicoline (16-24 µg/ml) and the calibration curve was determined by plotting the absorbance versus citicoline concentration (µg/ml).

METHOD VALIDATION PARAMETERS

ICH guideline was followed for validation of developed method.

Specificity: The specificity of the method for determining citicoline is determined by comparing the spectrum of the sample solution with the spectrum of the standard solution. An aliquot of the stock solution was further diluted to obtain a solution of 20 µg/ml concentration with and without excipients, respectively. The resulting solution was measured at 280 nm. (Figure 1 a&b).

Linearity: The linearity of the analytical program is its ability to obtain test results that are directly proportional to the concentration of the analyte in the sample. Six different concentrations corresponding to 16-24 µg/ml citicoline were prepared from working standard solutions (1000 µg/ml). A calibration curve was drawn on the citicoline concentration range (16-24 µg/ml) at 280 nm. Regression analysis of the line equation found is y = 1.0302 × - 2.2688 and reported the correlation coefficient (R2 = 0.9990). (Table 1) (Figure 2).

Precision

Repeatability: The aliquots of the stock solution were further diluted with 0.1N HCl to obtain six solutions (20 µg/ml) of the same concentration. The resulting solution was measured at 280 nm using 0.1N HCl as a blank. (Table 2).

Intermediate Precision: Determined by evaluating it once in two consecutive days. From standard stock solution 2ml of sample were added to 100ml volumetric flask and volumed up by 0.1N HCl having concentration of 20µg/ml citicoline solution, and was measured at 280 nm every day for two consecutive days. (Table 3).

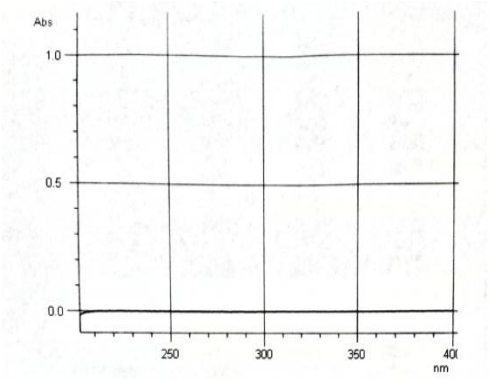
Robustness: In Robustness, the process variables are slightly changed (normality of HCl) during the validation study to observe the variation in results. (Table 4).

Accuracy: The accuracy of the method was determined by the recovery of the standard. A known amount of the pure drug having concentration of 16 µg/ml, 20 µg/ml, and 24 µg/ml citicoline was added to the solution and the sample was analyzed. Recovery studies were performed at three different concentration levels that were 80%, 100%, and 120%, respectively. (Table 5).

RESULTS AND DISCUSSION

Citicoline syrup is a mixture of different excipients and citicoline, and the mixture of citicoline and excipient must be separated. To do this, we used the extraction method. Extraction was carried out using diethyl ether and the procedure was modified three times. Based on the solubility, 0.1 N HCl was used. The drug followed the Beer-Lambert law at a concentration range of 16-24 µg/ml and showed a maximum absorbance at 280 nm with a correlation coefficient of 0.999.

The recovery rate of the proposed method is between 98 and 102. The accuracy of repeatability and intermediate precision %RSD is less than 2.



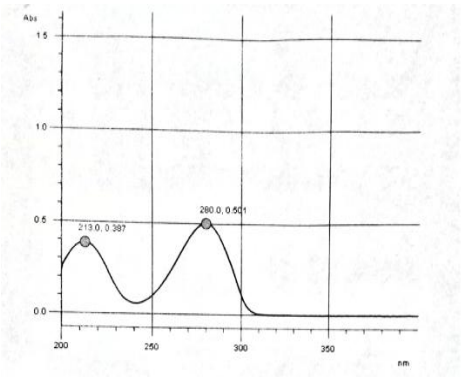


Figure 1: (a) Spectrum of Placebo. **(b) Chromatogram of Citicoline.**

Table 01: Linearity.

Concentration (µg/ml)		Absorbance at 280nm
16		**0.415**
18		**0.464**
20		**0.502**
22		**0.542**
24		**0.589**

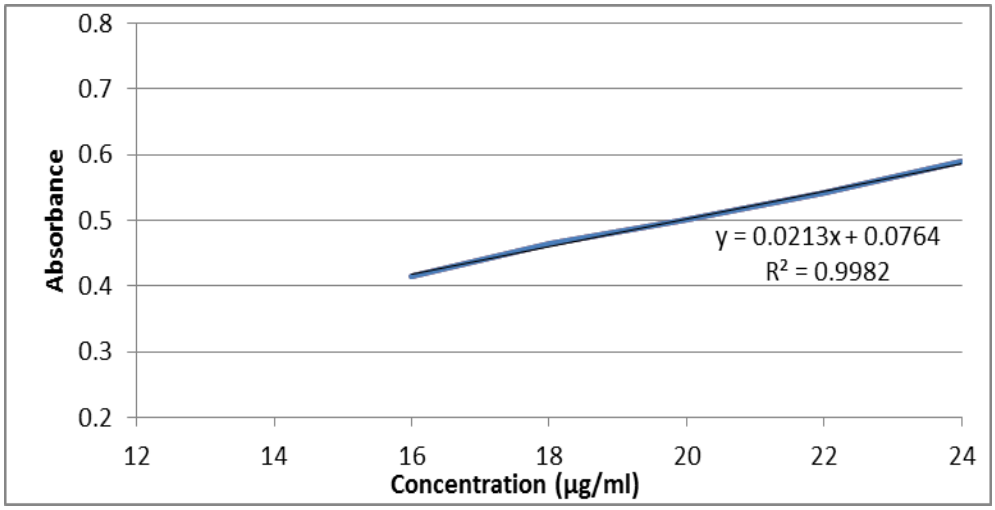


Figure 02: LINEARITY.

Table 02: Repeatability.

Level	Absorbance of Replicate	absorbance of Standard	Assay %	Mean	Standard Deviation	%RSD
100%	0.504	**0.503**	**100.15**	**100.38**	**0.52**	**0.5267**
	0.507		**100.74**			
	0.509		**101.14**			
	0.502		**99.75**			
	0.503		**99.94**			
	0.506		**100.54**			

Table 03: Intermediate precision.

	Day 1		Day 2
	Analyst I & Equipment I		**Analyst III & Equipment II**
(%age Assay)	**100.94**		**100.54**
	100.34		**100.34**
	100.34		**100.34**
Mean	100.54		100.41
RSD (Analyst to Analyst)	**0.3445%**		
RSD (Day to Day)	**0.1154%**		

Table No. 04: Robustness.

Factor	Variation Level	Assay (%)
Normality of HCl	0.09N	**100.75**
	0.1N	**100.34**
	0.11N	**99.75**
Evaluation:-	**The RSD of the assay is 0.502%.**	

Table No. 05: Recovery.

Recovery Level	Amount Citicoline(mg)		Average	Recovery (%age)	Standard Deviation	RSD (%)
	Spiked	Recovered				
80%	16	16.29	16.21	101.31	0.68	0.0680
	16	16.05				
	16	16.29				
100%	20	20.02	20.00	100.03		
	20	20.02				
	20	19.98				
120%	24	24.12	24.06	100.25		
	24	24.08				
	24	24.00				

CONCLUSION

On behalf of the above experimental results, the method was verified according to the ICH guidelines. The method developed is simple, inexpensive, accurate, fast, and validated in terms of linearity, accuracy, precision and recovery. Therefore, the developed spectroscopy method was used to routinely estimate citicoline in syrup formulation.

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