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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CITICOLINE IN BULK AND DOSAGE FORM BY UV SPECTROSCOPY

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ABSTRACT

An accurate, precise, specific, and economic method for estimation of citicoline in bulk is developed. The calibration curve method showed a wavelength for citicoline at 270 nm with distilled water. The calibration curve shows linearity in the concentration range of 10-60 µg/ml with good regression equation y = 0.016x + 0.012 and correlation coefficient 0.999 for citicoline. The precision results are not more than 2%. The results of analysis have been validated in order to verify linearity, precision, and accuracy for the goal intended and further implementation for the analysis in the pharmaceutical dosage form.

KEY WORDS

Calibration curve, Validation, Analytical Method, Citicoline, CDP-choline, UV spectroscopy.

INTRODUCTION:

Citicoline monosodium is a complex organic particle, also known as cytidine 5' (trihydrogen diphosphate) P'[2-trimethylammonio) ethyl] ester inner salt. Citicoline is a white or off-white crystalline powder, freely soluble in water. It is insoluble in ethanol, acetone, and chloroform.[1, 2] Citicoline is an intermediate in the generation of cerebral phosphotidylcholine from choline. It acts upon the levels of neurotransmitters and behaves like a presynaptic cholinergic agent. [3, 4]

Citicoline used as psychostimulant, nootropic agent, and dietary supplement.[1,2] It is primarily indicated in conditions like cognitive impairment, nerve regeneration, cardiac stroke, cerebral ischemia, cerebral and spinal injury, and neurological disease.[5] Citicoline has a low toxicity drug profile, thus it also can be given as an alternative drug of choice in Alzheimer's disease, Parkinson's disease,[6] Huntington's disease,[7] dementia,[8] dyslexia, epilepsy, and Intracerebral hemorrhage.[9, 10]

Literature survey revealed that much work has been published. The survey described drug physic-chemical properties, pharmacokinetics, pharmacological, toxicity, bioavailability, neuronal injury, and neurodegenerative disease. The extensive survey revealed several analytical methods cited for determination of citicoline individually or in combination with another drug by UV-Vis spectroscopy[11-17] HPLC[18-31] Gas Chromatography[32] and UPLC[33] in biological fluids and in pharmaceutical dosage forms.

EXPERIMENTAL:

MATERIALS:

Chemical and reagents: Citicoline used as a standard drug. Distilled water as a solvent used and all other chemical reagents and apparatus are of analytical grade.

Instrumentation and UV spectroscopy condition: Digital electric balance, and Agilent Carry 60 UV Spectroscopy and 1 cm Quartz cells with a fixed slit width (2nm); the measurement properties wavelength

range (200-800) nm, scan speed: 100nm/min, medium sampling interval: 2.0, scan mode: single, measuring mode: absorbance.

PROCEDURE:

Standard solutions preparation: Standard stock solution of citicoline (1000µg/ml) was prepared by weighing 50 mg of Citicoline, dissolving in distilled water in 50 ml volumetric flask, then diluting with distilled water up to the mark. Then further working standard solution (100µg/ml) of citicoline was prepared with distilled water and again dilutions were made as such that solutions prepared to contain 10-60 µg/ml citicoline.

Determination of Absorbance spectrum of Citicoline: Aliquot 1 ml of standard stock solution into distilled water in 10 ml volumetric flask and dilute up to the mark with distilled water. The resulted 10µg/ml solution was measured at range (200- 400nm) using distilled water as blank, show the absorbance spectrum and λ_{max} at 270 nm.

Method development: A series of solutions were prepared by diluting different volumes of a standard solution of citicoline 100 µg/ml, in 10ml volumetric flasks and dilute with distilled water up to the mark. The method was determined at different concentration levels ranging (10-60 µg/ml) for Citicoline, the calibration curve was determined by plotting absorbance versus concentration of Citicoline (µg/ml) (Fig. 2).

METHOD VALIDATION PARAMETERS:

Validation of the developed method according to ICH guideline [34, 35]

Linearity and Range: The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of an analyte in the sample. Six different concentrations corresponding to 10-60 µg/ml of citicoline were prepared from the working standard solution (100µg/ml) using distilled water. A calibration curve was plotted over a concentration range (10-60 µg/ml) of citicoline at 270 nm. The regression analysis was performed for the line equation was found to be y = 0.016x + 0.012 and correlation coefficient (R^2=0.999) was reported. (Table 1) (Fig. 2)

Precision:

Repeatability- Aliquot stock solutions to further diluted with distilled water to get the six solutions of the same concentration (15µg/ml). Resultant solutions were measured at 270 nm using distilled water as the blank. (Table 2)

Intra-day Precision- It is determined by analyzing the corresponding responses 3 times on the same day. Intraday precision was determined by evaluating 10, 30 and 50 µg/ml concentration solution of citicoline for three times in the same day at 270 nm using distilled water as a blank. (Table 3)

Inter-day Precision- It is determined by evaluating once on three consecutive days. The aliquot working standard solution in 10 ml volumetric flasks to further diluted with distilled water to get the 10, 30 and 50 µg/ml solution of citicoline. Resultant solutions of citicoline were measured daily once for three consecutive days at 270nm using distilled water as a blank. (Table 4)

Accuracy: The accuracy of the method was determined in terms of % recovery of standard, with no detectable impurities at an appropriate concentration. A known amount of citicoline of the pure drug was added to pre-analyzed tablet powder and the sample was then analyzed. The recovery studies were carried out at three different concentration levels; 80%, 100% and 120% by standard addition method. (Table 5)

Specificity: The specificity of the method for determination of citicoline in tablet dosage form was determined by comparing the spectrum of tablet solution with that of standard solution. Aliquots of stock solution were further diluted with distilled water to get the solution of 10µg/ml concentration with and without excipients, separately. The resultant solutions were measured at 270 nm using distilled water as the blank. (Table 6)

Estimation of Citicoline in Pharmaceutical dosage form: Weigh 20 tablets and the average weight determined and crushed into fine powder. Weigh accurately the quantity of powder equivalent to 500 mg of citicoline and transfer into 50 ml volumetric flask. Add 35 ml distilled water and sonicate for 15 minutes. Make up to the volume up the mark with distilled water, then filtered. 1ml of the resultant solution was further diluted with distilled water to get the final concentration of citicoline 10 µg/ml. The absorbance of the resulting solution of the tablet solution measured at 270 nm. (Table 7)

RESULTS AND DISCUSSION:

The solubility of citicoline studied and distilled water is selected as a solvent. For the calibration curve method citicoline showed wavelength maxima at 270 nm. The drug follows Beer-Lambert's law over the concentration range of 5-60 µg/ml with a correlation coefficient of 0.998. The present study of proposed method showed precision in terms of the repeatability and, reproducibility is found to be not more than 2%. The recovery results are in the range of 100 to 104%. Hence, the results of the analysis are validated as per ICH guidelines.

Quantitative determination of citicoline in tablet dosage form by employed the method, the assay values found 95.02± 0.626.

Concentration (µg/ml)	Absorbance at 270nm
10	0.1726
20	0.3329
30	0.5004
40	0.6578
50	0.8167
60	0.9781

Tablet No. 1: Standard solutions of Citicoline (Concentration and Absorbance)

Nominal Conc. (µg/ml).	Absorbance	Mean ± SD	%RSD
	0.2479		
	0.2446		
15	0.2504	0.24707 ± 0.00197	0.797
	0.2459		
	0.2468		
	0.2468		

Table No. 2: Results of repeatability

Nominal Conc. (µg/ml)	Absorbance			Mean ± SD	%RSD
	0 hr	3 hr	6 hr.		
10	0.1664	0.1617	0.1619	0.16333 ±0.00266	1.63
30	0.4926	0.4847	0.4844	0.48723 ± 0.00465	0.954
50	0.7662	0.7646	0.7635	0.76477 ± 0.00136	0.177
Mean					0.92033

Table No. 3: Results of intra-day Precision

Nominal Conc. (µg/ml)	Absorbance			Mean±SD	%RSD
	0 hr	24 hr	48 hr		
10	0.1738	0.1741	0.1754	0.17443 ± 0.00085	0.48757
30	0.492	0.4989	0.5024	0.49777 ±0.00529	1.06311
50	0.7754	0.7885	0.7914	0.7851 ±0.00852	1.08581
Mean					0.87883

Table No. 4: Results of inter-day Precision

Recovery at (%)	Nominal Conc. (µg/ml)	Absorbance	Observed Conc. (µg/ml)	% Recovery
80	18= 10+8	0.2996	18.23	101.25
80	18= 10+8	0.2956	17.98	99.86
80	18= 10+8	0.2973	18.08	100.45
100	20= 10+10	0.3345	20.41	102.03

100	20= 10+10	0.3398	20.74	103.69
100	20= 10+10	0.3382	20.64	103.19
120	22= 10+12	0.3687	22.54	102.48
120	22= 10+12	0.3722	22.76	103.47
120	22= 10+12	0.3709	22.68	103.09
Mean ± SD				102.167 ±1.37

Table No. 5: Results of accuracy

Nominal Conc. (µg/ml)	Without Excipients		With Excipients		% Interference
	Absorbance	Observed Conc. (µg/ml)	Absorbance	Observed Conc. (µg/ml)	
10	0.1636	9.72	0.1706	10.16	0.44
10	0.1612	9.57	0.1809	10.81	1.23
10	0.1692	10.07	0.1795	10.72	0.64
10	0.1637	9.73	0.1833	10.95	1.22
10	0.1639	9.74	0.1765	10.53	0.79
10	0.1557	9.23	0.1766	10.54	1.31
Mean					0.94

Table No. 6: Results of specificity

Absorbance	Conc. (µg/ml)	Content (mg)	Weight Taken (mg)	%Assay
0.1893	11.33	476.05	500.00	95.21
0.1876	11.23	471.59	500.00	94.32
0.1899	11.37	477.63	500.00	95.52
Mean±SD				95.02± 0.626

Table No. 7: Analysis of Citicoline tablets

S. No.	Parameter	Results
1.	Wavelength	270 nm
2.	Beer's law limit (µg/ml)	10-60 µg/ml
3.	Regression equation	y = 0.016x + 0.012
4.	Correlation coefficient	0.999
5.	Precision	
a)	Repeatability	0.797
b)	Intra-day precision	0. 92
c)	Inter-day precision	0. 878
6.	Accuracy	102.167 ±1.37
7.	Specificity	0.94

Table No. 8: Results of validation parameters of Citicoline

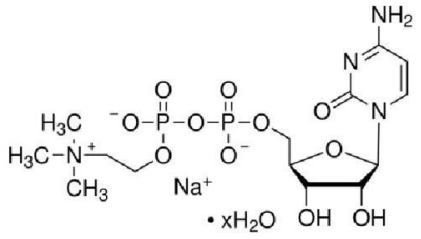

Figure 1: Chemical structure of Citicoline

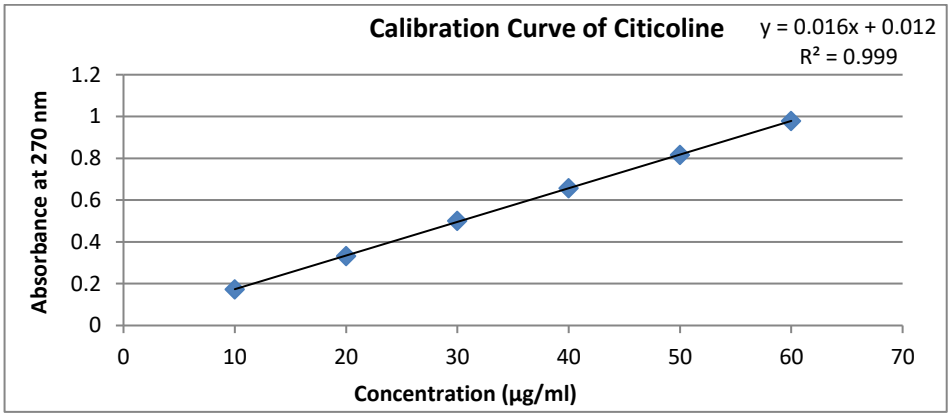

Figure 2: Calibration curve of Citicoline at 270nm

CONCLUSION:

The newly developed method of simple, precise, rapid, and validate in terms of linearity, precision, accuracy, and reproducibility. Therefore, the developed spectroscopic method used for routine estimation of citicoline in tablet dosage form.

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