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SIMULTANEOUS METHOD DEVELOPMENT AND VALIDATION OF CITICOLINE AND PIRACETAM IN BULK AND ITS TABLET DOSAGE FORM

Y. Samatha*[1], A. Sri Vidya A. Ajitha and V. Uma maheswararao

Department of Pharmaceutical Analysis, CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad. 501401.

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***Correspondence for Author**
Y. Samatha
Department of Pharmaceutical Analysis, CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad. 501401.
ysamatha3@gmail.com

ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Citicoline and piracetam, in its pure form as well as in tablet dosage form. Chromatography was carried out on a XBridge C18 (4.6 x 250mm, 5µm) column using a mixture of Methanol and Phosphate buffer pH3 (85:15 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 229nm. The retention time of the Citicoline & Piracetam was 1.933, 3.396 min respectively. The method produce linear responses in the concentration range of Citicoline- 12.5-62.5, and 5-25mg/ml of piracetam. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Citicoline & piracetam, RP-HPLC, validation. ; Tablet Dosage forms.

INTRODUCTION

Citicoline The drug is chemically 5O-[hydroxy({hydroxy[2-(trimethylammonio)ethoxy]phosphoryl}oxy)phosphroyl]cytidine **Figure 1**. It is an amorphous, somewhat hygroscopic powder having molecular weight 489.332 g/mol and pKa value of 4.4. It is soluble readily in water to form acidic solution, practically insoluble in most organic solvents. It is a psychotherapeutic agent used as psychostimulant, nootropics and neurotonics. It exerts its action by activating the biosynthesis of structural phospholipids in the neuronal membrane, increases cerebral metabolism, and increases the levels of various neurotransmitters, including acetylcholine and dopamine. Citicoline sodium is primarily

indicated in conditions like cardiac stroke, head trauma, ischemic heart disease, and paralysis of lower extremities and can also be given in adjunctive therapy as an alternative drug of choice in Parkinson's disease. It is generally prescribed as an oral tablet containing 500 mg drug.

Mechanism of action: Citicoline increases blood flow and O2 consumption in the brain. It is also involved in the biosynthesis of lecithin. Citicoline enhance the synthesis of phosphatidyl-choline in brain but could inhibit destructive process (activation of phospholipases).

Piracetam: (sold under many brand names) is a nootropic drug in the racetams group, with chemical name *2-oxo-1-pyrrolidine acetamide*. It shares the same 2-oxo-pyrrolidone base structure with pyroglutamic acid. Piracetam is a cyclic derivative of GABA.The empirical formula is $C_{25}H_{38}O_5$. The chemical structures of Citicoline and piracetam are shown in Figure2.

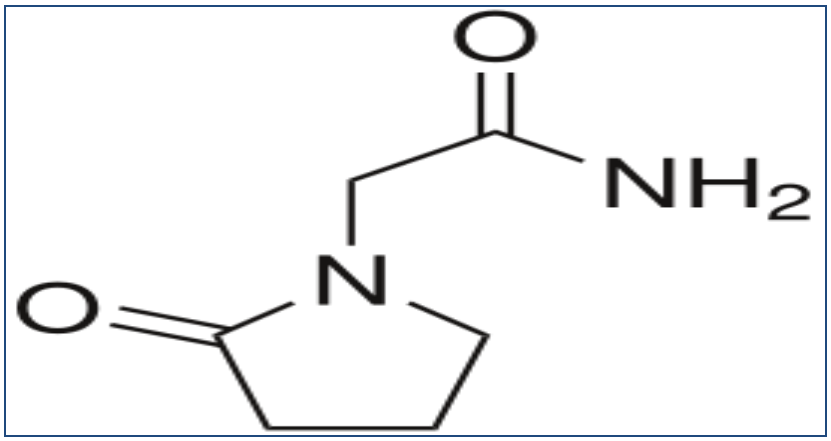

Figure 1: Chemical structure of Piracetam

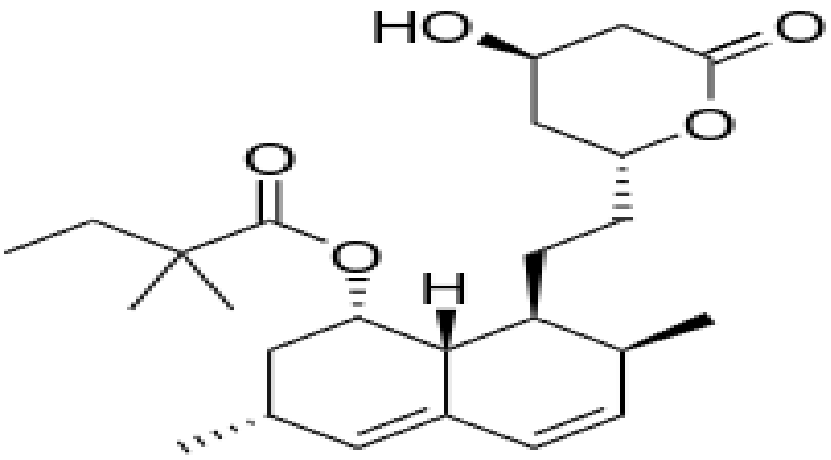

Figure 2: Chemical structure of Citicoline

MATERIALS AND METHODS

Reagents and chemicals

Methanol HPLC grade was procured from E.Merck Ltd, Mumbai. Methanol, Acetonitrile. Fine chemicals, Hyderabad. Water HPLC grade was prepared using Millipore purification system. Citicoline and piracetam reference standards procured from Sura Labs pharma Pvt,Ltd Hyderabad.

Instrumentation

The HPLC system consists of water Empower -2695 having photodiode array detector system, which was connected with the help of Empower 2 software for data integration and processing. XBridge (250 X 4.6 mm) 5µ column was used for the analysis.

HPLC conditions

The contents of the mobile phase were ACN: Triethylamine buffer pH4 (85:15 v/v . These were filtered through 0.45µ membrane filter and degassed by sonication before use. The flow rate of mobile phase was optimized to 1.0 ml / min. The 6min and column temperature was maintained at ambient. The volume of injection was 10µl, and the eluent was detected at 229 nm. Each of standard and test preparations was injected into the column and the responses recorded (Fig.05 and Fig.06.).

Table 1.Optimized method parameters

PARAMETERS	CHROMATOGRAPHIC CONDITIONS
Mobile phase ratio	ACN: Triethylamine buffer pH4 (85:15 v/v)
Column	XBridge C18 (4.6×250mm) 5µ
Detector	PDA Detector
Column temperature	40ºC
Wavelength	229 nm
Flow rate	1 ml/min
Injection volume	10 µl
Run time	6 minutes

METHOD VALIDATION

The RP-HPLC Method of Citicoline and piracetam were achieved by isocratic elution technique with PDA Detector. Citicoline and Piracetam were determined at 229nm respectively with the concentration range of 0.20-0.8µg/ml for both Citicoline and piracetam respectively.fig.03 &04. For analysis of tablet formulation the tablet powder equivalent to 25 mg was taken, dissolved in 25 ml volumetric flask and made up to 25ml with Methanol. The solution was sonicated for 15min, centrifuged at 100 rpm for 15 min and filtered through

Whatmann filter paper No.41. From clear solution, further dilutions were made to get 10 µg/ml of Citicoline and piracetam theoretically. Table 2.

System suitability parameters

Table: 2 Result of system suitability parameters

Parameter	Citicoline	Piracetam
Retention time	1.933	3.396
Theoretical plates	4242	6515
Tailing factor	1.15	1.78
Area	409905	392596

The system suitability parameters were found to be within specified limits for the proposed method

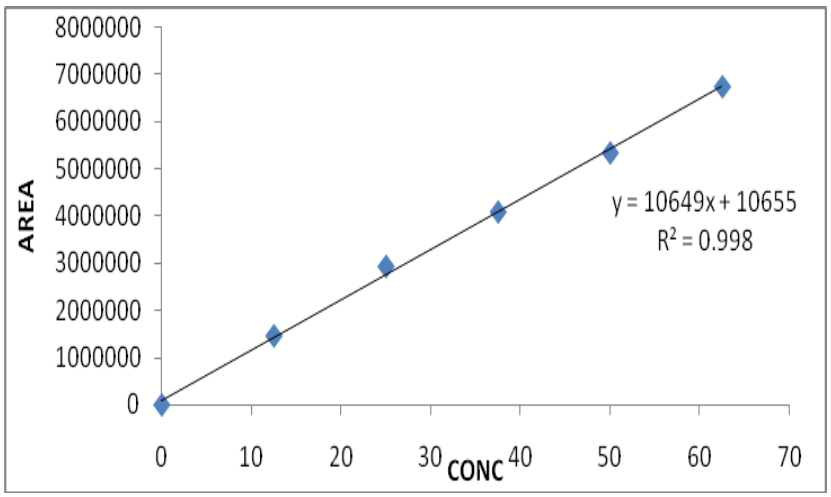

Fig. 6 :Calibration Curve for Citicoline

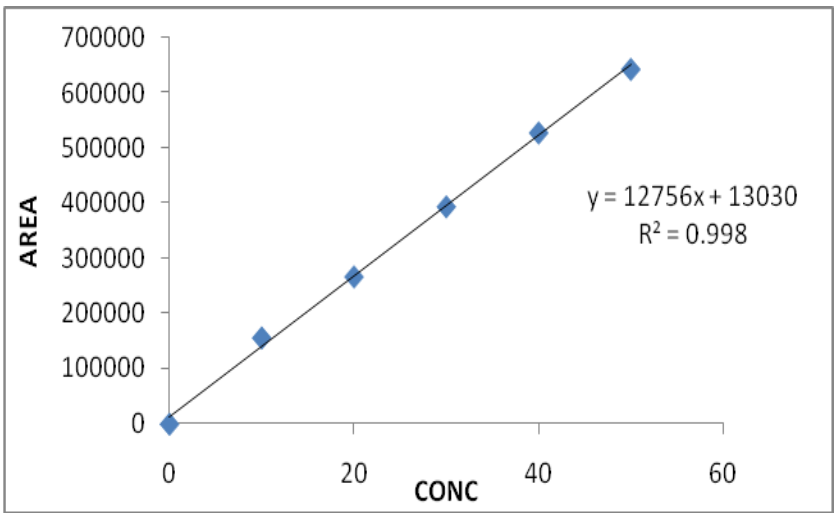

Fig. 7 Calibration Curve for Piracetam

Table: 7 Linearity Results of citicoline

S.no	Concentration	Area
1	12.5 ppm	1464449
2	25 ppm	2940463
3	37.5 ppm	4096252
4	50 ppm	5350193
5	62.5 ppm	6755619
	Correlation coefficient	0.998

Table: 8 Linearity Results of Piracetam

Linearity Level	Concentration	Area
1	10 ppm	156581
2	20 ppm	267461
3	30 ppm	394576
4	40 ppm	528761
5	50 ppm	644180
Correlation coefficient		0.998

The linearity range was found to be 12-62.5µg/ml for Citicoline and 10-50µg/ml for piracetam. calibration curve was plotted and correlation co-efficient for both the drugs found to be 0.998.Hence the results obtained were within the limits. the linearity curves were shown in The linearity chromatograms recorded were shown. The linearity results were reported in **Table.**

Limit of detection :LOD was calculated by using standard deviation and slope values obtained from calibration curve.

LOD= 3.3 × σ / s

Table 9: LOD results of the Method

Drug	Amount
Citicoline	27.9
Piracetam	1.76

Limit of quantitation: LOQ was calculated by using standard deviation and slope values obtained from calibration curve.

LOQ=10×σ/S

Table 10: LOQ results of the Method

Drug	Amount
Citicoline	84.5
Piracetam	5.33

From the above the LOD values of Citicoline and Piracetam were found to be 27.9 and 1.76µg/ml respectively. The LOQ values of Citicoline and Piracetam were found to be 84.5 and 5.33µg/ml respectively. Thus the method developed was found to be sensitive.

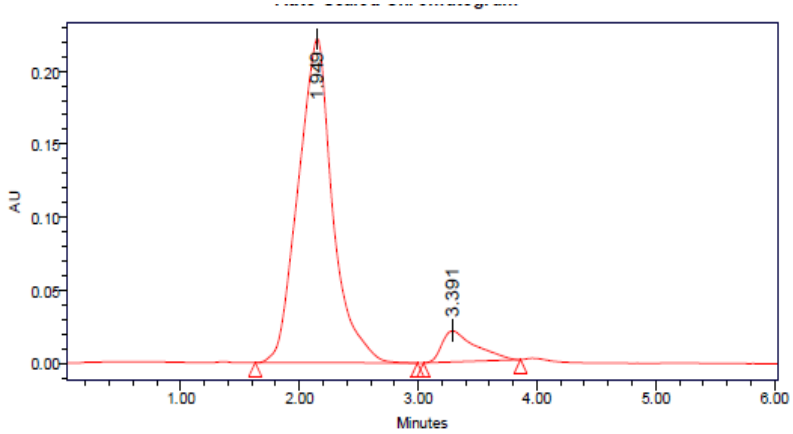

Figure 8: Typical chromatograms for recovery studies

For recovery studies, to the reanalyzed formulation, solutions of raw material containing different concentrations were added and the amount of drug recovered was calculated. The procedure was repeated as per the analysis of formulation. The amount of drug recovered was calculated by using slope and intercept values from the calibration graph. Finally the method was validated as per ICH guide lines for precision, accuracy, specificity, linearity, reproducibility, limit of detection and limit of quantification.

Table: 11 Accuracy Results of Citicoline

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	212026.7	18.75	18.9	100.8	100.2%
100%	406885.7	37.5	37.2	99.2	
150%	614558	56.25	56.7	100.8	

Table: 12: Accuracy Results of Piracetam

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	202430	15	14.8	98.9	99.9%
100%	394993.7	30	29.9	99.8	
150%	593559	45	45.5	101.1	

The accuracy studies were shown as % recovery for Citicoline and Piracetam at 50%,100%,150% ,the limits of recovery should be in range of 98-102% the limits obtained for Citicoline and Piracetam were found to be within the limits. Hence the method was found to be accurate. The accuracy studies shows % recovery of the Citicoline 100% and Piracetam. the limits of % recovery of drugs were 98-102% and from the above results its indicates that the method was accurate and also revealed that the commonly used exciepients present in the pharmaceutical information do not interfere in the proposed method. the chromatograms of shown in **Fig 8.** and results were shown **Tables:11& 12.**

Precision: In the precision study,% RSD was found to be less than 2 % for citicoline 0.2% and piracetam 0.8 which indicates the system has a good reproducibility for precision studies 5 replicate studies of Citicoline and piracetam formulation(method precision) was performed.% RSD was determined for peak areas of citicoline and piracetam. the acceptance limits should be NMT 2% and the results were found to be within the acceptance limits .results were reported in **Table:13**

Table 13 Results of System precision

INJECTION	CITI AREA	PIRA AREA
Injection1	409349	327876
Injection2	409980	320133
Injection3	407839	323930
Injection4	409739	324517
Injection5	408042	323107
Average	408989.8	323912.6
Standard deviation	986.596	2784.2
% RSD	0.2	0.8

Table 14. Results of Intermediate system precision

INJECTION	CITI AREA	PIRA AREA
Injection1	409600	323199
Injection2	409792	324588
Injection3	408131	326955
Injection4	409710	321726
Injection5	409596	323546
Injection6	409932	327755
Average	409460.2	324628.2
Standard deviation	663.3016	2114.593
% RSD	0.161994	0.713561

In the ID precision study, %RSD was found to be 2%.for citicoline 0.1 and piracetam 0.7 which indicates that the system has good reproducibility.

For intermediate precision studies 5 replicate injections of CITI and PIRA formulation(method id precision) was performed.% RSD was determined for peak ares of citicoline and piracetam. the acceptance limit were should be NMT 2% and the results were obtained for sample(method id precision) were found to be wth in the acceptance limits. the id preciion chromatograms were showed in **Fig.9.**

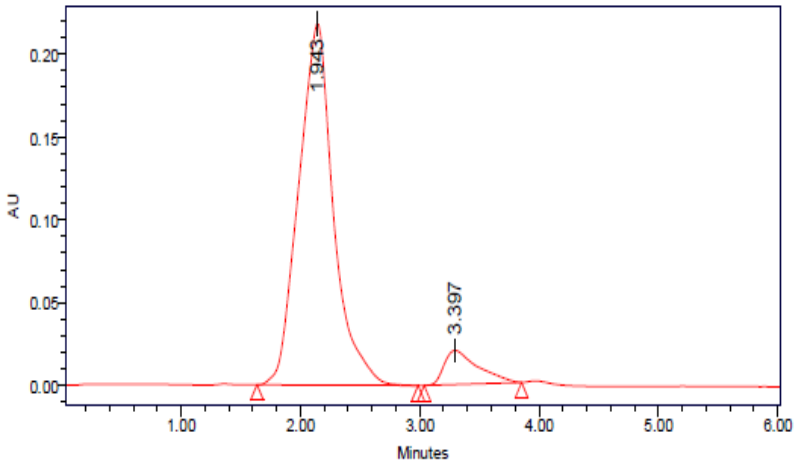

Figure 9: Typical chromatogram of Citicoline and piracetam

RESULTS AND DISCUSSION

A simple, selective, rapid and precise validated RP-HPLC Method for Simultaneous Estimation of Citicoline and piracetam in bulk material and in pharmaceutical formulation has been developed and validated. The correlation coefficient was found to be 0.9997&0.9998 for Citicoline and piracetam respectively. In this method the % purity of Citicoline and piracetam were found to be 101.25 ± 1.074 and 100.19 ± 1.031 respectively. The recovery studies range is.99.98-100.01% and 99.94 – 100.03 % for Citicoline and piracetam, respectively. The Intraday and Inter day analysis carried out for precision. The ruggedness study was performed. In First order Derivative method the % purity were found to be 100.25 ± 1.0054 and 101.49 ± 1.9305 for Citicoline and piracetam, respectively. The recovery studies range is 99.98-100.01% and 99.94 – 100.03 %. Table 1.The Intraday and Inter day analysis carried out for precision. The ruggedness study was performed. The method was validated for statistical analysis.

CONCLUSION

The developed RP-HPLC method was validated and the statistical validation was performed with the simplicity and ease of operation ensures that the validated method can successfully used for routine Analysis of Citicoline and piracetam in bulk and tablet dosage formulation.

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