International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.3, pp 1136-1141, July-Sept 2012

A Reverse Phase Liquid Chromatography Analysis of Citicoline Sodium in Pharmaceutical Dosage Form using Internal Standard Method

Sanjay Surani[1], Prachi Kabra[1], Ritu Kimbahune*[2], Sunil K[1], L.V.G. Nargund[1]

[1]Department of Quality Assurance, Nargund College of Pharmacy, Dattatreya Nagar, II Main,100 Ft Ring Road, BSK III Stage, Bangalore- 560 085,India.
[2]Department of Quality Assurance, Al-Ameen College of Pharmacy, Hosur Road, Opp. Lalbagh Main Gate, Bangalore-560 027,India.

Corres.author: rituvivekk@gmail.com
Phone number: +91 97403 30413

Abstract: In the present study, a reverse phase high performance liquid chromatographic method was developed and validated for the determination of Citicoline sodium in pharmaceutical dosage form. Chromatographic separation was carried out on a RP-18 column using a mobile phase consisting of acetonitrile: water (20:80, v/v) adjusted at pH 3.0 using 1% orthophosphoric acid. Flow rate was maintained at 0.7ml min^{-1} and UV detection was carried at 260 nm. Caffeine was used as an internal standard. The calibration curve was found to be linear over the range 1–500µgml^{-1}. The results of accuracy study were ranged between 99.32% and 101.90% with a R.S.D. less than 1%. LOD and LOQ were 1.4µgml^{-1} and 4.3µgml^{-1}, respectively. The method was found to be simple, rapid, and easy to apply, making it very suitable for routine analysis of Citicoline sodium in pharmaceutical dosage form.
Keywords: Citicoline Sodium, Caffeine, RPHPLC.

1. INTRODUCTION

Citicoline Sodium (CT) is white crystalline, somewhat hygroscopic powder. Chemically it is Cytidine 5‘-{trihydrogendiphosphate} p‘-[2-{trimethylammonio} ethyl] ester inner salt [1]. It is freely soluble in water but insoluble in ethanol, acetone and chloroform [2]. CT is derivative of choline and cytidine involved in biosynthesis of lecithin**.** It is claimed to increase blood flow and oxygen consumption in the brain and has been given by injection in the treatment of cerebrovascular disorders. It is primarily used in pharmacotherapy of brain insufficiency and other related neurological disorders viz., as stroke, brain trauma and parkinsonism’s disease [3].

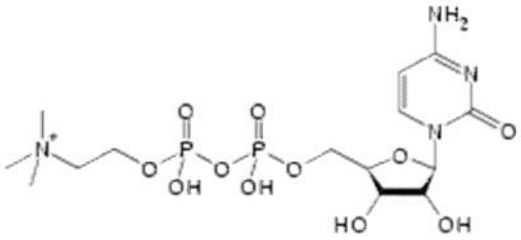

Fig 1. Chemical Structure of Citicoline Sodium.

A liquid chromatography method for the determination of CT in injection, oral drops and tablet dosage form were reported in the literature [4, 5]. Spectrophotometric methods like colorimetric by complexation and UV-visible spectrophotometric method using standard absorptivity value for determination of CT in pharmaceutical dosage form were also reported [6,7]. Since none of RP-HPLC method reported for the determination of CT using an internal standard (IS). An aim of the present work was to develop a rapid, precise, accurate and comparatively economical RP-HPLC method with UV detection for quantitative estimation of CT in tablet dosage form to avoid uncertainties introduced by sample injection in quantitative chromatography.

2. EXPERIMENTAL

2.1. Reagents and chemicals

All the reagents like ortho phosphoric acid, acetonitrile (Qualigens fine chemicals, Mumbai) and water used were of HPLC grade. The gift samples of CT and CAF were obtained from Strides Arco Laboratories (Bangalore, India), Juggat Pharma (Bangalore, India) respectively. The marketed formulation (Citistar, CT 500mg) was purchased from the local pharmacy.

2.2. Instrumentation

The HPLC system used was Shimadzu LC-20AT pump, Rheodyne injector (20µl), SPD-20A UV detection and the system was controlled through Spinchrome software. Analytical column used for this method was Gracesmart RP18 (250 X 4.6mm, 5µm). Sartorius digital Balance was used for weighing.

2.3. Chromatographic conditions

The composition of the mobile phase was acetonitrile: water (20:80, v/v) (adjusted to pH 3.0 with 1% orthophosphoric acid). The mobile phase was vacuum-filtered through 0.2 µm Supor200 membrane and degassed by ultrasonication for 10min before use. The mobile phase flow rate was set at 0.7 ml min^{-1}. All standard and assay samples were filtered through Supor200 membrane before injection. After equilibration of column with the mobile phase indicated by a stable baseline, aliquots of sample (20 µl) were injected and the total run time was kept 20 min. The absorbance of the eluent was monitored at 260nm with a detection sensitivity of 0.1000 aufs. CAF (5µgml−1) was used as an internal standard.

2.4. Standards and sample solutions preparation

Standard stock solutions of CT (1000µgml−1) and CAF (1000µgml−1) were prepared in HPLC grade water. These solutions were stored under refrigeration (4.0±0.5 ◦C). Working standard solutions were freshly prepared daily by appropriate dilution of the stock solutions with mobile phase.

Sample solution was prepared by weighing 20 tablets accurately and finely powdered. The powder equivalent to 100 mg of CT was taken in a 100 ml volumetric flask, about 60 ml of HPLC grade water was added and kept in Ultrasonic bath for 10 minutes then made up to volume. The resulting solution was filtered through whatman filter paper no. 41. From the above solution 1 ml was transferred in to 10ml volumetric flask along with 1ml of CAF solution (50 µgml−1) made up to volume with mobile phase (100µgml−1 CT and 5µgml−1 CAF) and results are as given in Table 1.

Table 1: Assay of Citicoline sodium Tablets (CT : Label claim 500mg**)**

Sr.No.	Amount found(mg)	Amount found (%)
1	510.2	102.0
2	520.0	104.0
3	525	105.0
4	520	104.0
5	515	103.0
6	530	106.0
Mean	520.0	104.0
%RSD	1.3	1.3

2.5. Method validation

Method validation was carried out under the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures[8]. The assay was validated with respect to linearity, precision, accuracy, sensitivity and robustness.

2.5.1. Linearity

Calibration curves were obtained from injecting the six sets of ten serial different drug concentrations (1, 5, 10, 20, 30, 40, 50, 100, 250 and 500µgml−1 of CT). The curves were generated by plotting the peak area ratios between CT and CAF against CT concentration. Linearity was evaluated by linear regression equation.

2.5.2. Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as relative standard deviation (R.S.D.). Repeatability was determined by performing nine determinations from triplicate injections of three different concentrations of CT (10, 50 and 100µgml^{-1}) on the same day at different time intervals and on three different days for inter-day precision.

2.5.3. Accuracy/recovery

In this study, accuracy was determined based on the recovery (percentage) of known amounts of standard CT added in the assay samples. This was performed by analyzing CT at three concentration levels (50, 100 and 150%), with a constant concentration of 5µgml−1 of internal standard. Samples were prepared in triplicate. The accuracy of the assay was determined by comparing the found concentration with the added concentration.

2.5.4. Sensitivity

Sensitivity of the method was determined by means of the detection limit (LOD) and quantification limit (LOQ). The LOD and LOQ were measured based on the method described by the International Conference on Harmonization. Calculations for LOD and LOQ were based on the standard deviation of the calibration curve (σ) and the slope of curve (*S*), using the equation LOD= 3.3×σ/*S* and the equation LOQ= 10×σ/*S*.

2.5.5. Robustness

Robustness of the method was evaluated by the analysis of CT solution under different experimental conditions such as pH of the mobile phase and flow rate. The flow rate was varied ±0.02 ml min^{-1} and pH of the mobile phase was changed ±0.1 units. Their effects on the retention time (*t*R), tailing factor (*T*), resolution of the peaks (*R*), recovery and repeatability were studied.

3. RESULTS AND DISCUSSION

3.1. Optimization of the chromatographic method

The chromatographic conditions were adjusted to provide the best performance of the assay. For system optimization the important parameters such as type and concentration of organic solvents, pH and mobile phase flow rate were investigated.

3.1.1. Effect of pH

Different pH values of the mobile phase were checked to establish the optimum separation and highest analytical sensitivity for CT and CAF. The pH values tested were 3.0, 4.5 and 7.0. Finally, the best results were obtained at pH 3.0±0.2 by using 1% orthophosphoric acid. The choice of this pH for the mobile phase is justified by the excellent symmetry of the peaks and the adequate retention times of CT and CAF.

3.1.2. Effect of mobile phase composition

Different mobile phase composition were tried to achieve better separation and resolution(R) between CT and CAF. It was observed that the water–acetonitrile system gave a better resolution and peak symmetry than the water–methanol system.

Different proportions of water–acetonitrile (50:50, 30:70, 70:30, 20:80, 80:20, and 25:75 v/v) were tested and evaluated before the final chromatographic conditions were selected. As a result, modification of CT retention times occurred from 3.8 min to 5.5min as the percentage of acetonitrile increased. Finally, water–acetonitrile (80 : 20, v/v) (adjusted to pH 3.0±0.2with1% orthophosphoric acid) was chosen as mobile phase. As a result, the standard solutions of CT and CAF showed symmetric and well-defined peaks, with an average retention time for CT of 3.8 min and 7.0 min for the CAF. Resolution between peaks was 14.13. Tailing factor was 1.39 for CT and 1.34 for CAF.

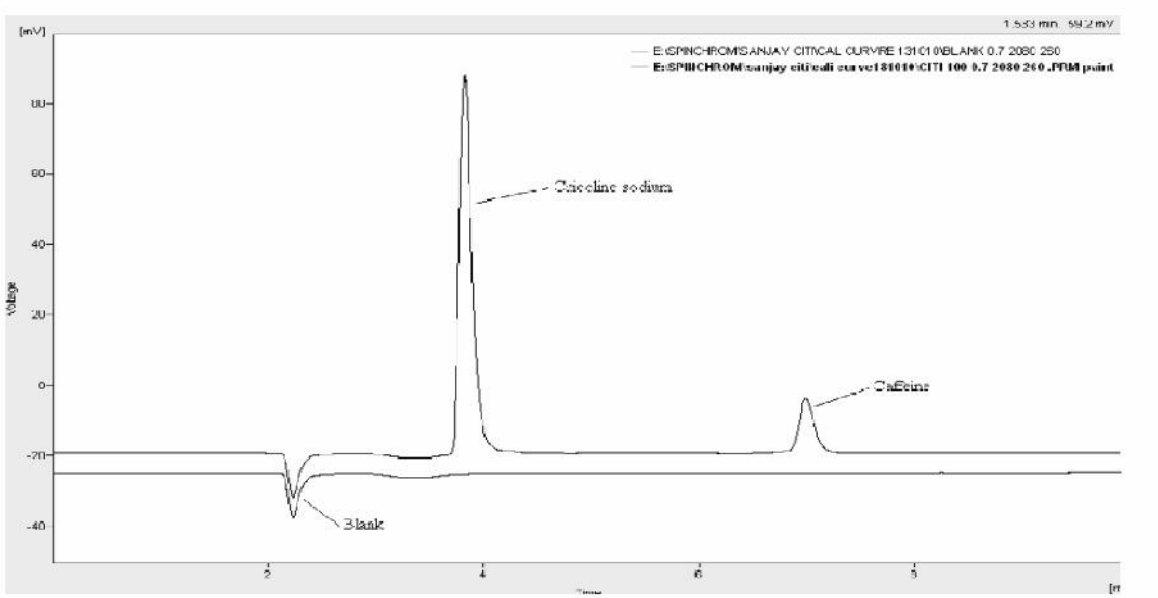

Fig2. Overlay chromatogram of blank and CT (tR 3.8min) with CAF(tR 7.0min)

3.1.3. Effect of flow rate

Different mobile phase flow rates (0.7, 1.0 and 1.2mlmin^{-1}) were investigated. The optimum flow rate for which the column plate number (N) was maximum, with the best resolution between all components and with a short run time (<20min) was found to be 0.7 mlmin$^{-1.}$

3.1.4. Internal standard

Different compounds were tested as IS for the chromatographic procedure. Among them, CAF eluted before 10 min of the analysis and has a better symmetry and resolution with respect to CT. Therefore, CAF has been chosen as an IS.

3.2. Method validation

3.2.1. System suitability

System suitability was performed to confirm that the equipment was adequate for the analysis to be performed. The test was carried out by making six replicate injections of a standard solution containing 10.0µgml−1 and 5.0µgml−1 of CT and CAF (IS), respectively, and analyzing each solute for their peak area, theoretical plates (*N*), resolution and, tailing factor. The results of system suitability in comparison with the required limits are shown in table 2. The proposed method fulfils these requirements within the accepted limits.

3.2.2. Linearity

The standard calibration curve was linear over the concentration range 1–500µgml^{-1}. The correlation coefficient obtained after linear regression analysis was 0.9998. The equation of the calibration curve based on the peak ratio of CT/IS with respect to CT concentration is y= 0.061780x-0.050093 with the standard error 0.038.

Table 2: System suitability results of the proposed method.

Analyte	R	N	T	RSD	
				tR	peak area
CT	14.70	5877	1.39	0.43	2.3
IS		13365	1.34	0.59	2.8
Required limits	R >2	N > 2000	T < 1.5	R.S.D. < 5%	

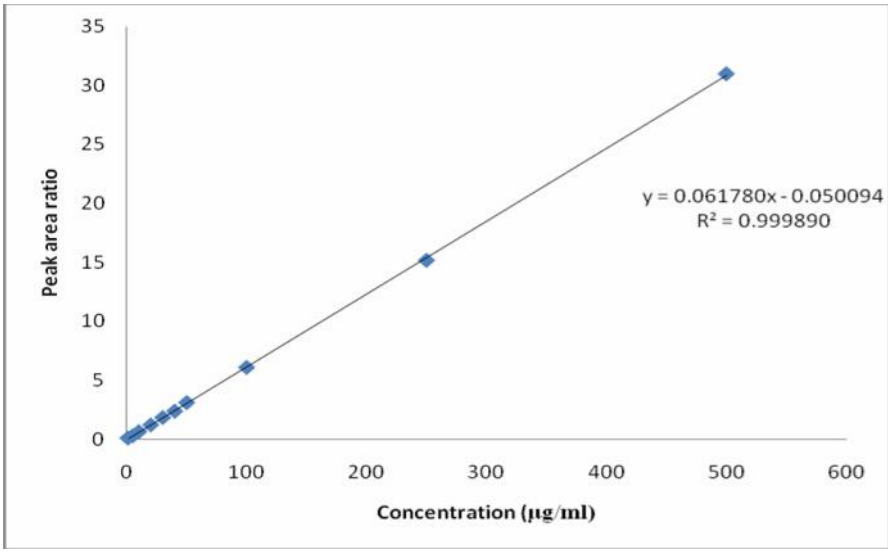

Fig3. Calibration curve of Citicoline sodium

3.2.3. Precision

The R.S.D. of repeatability (intra-day) and intermediate precision (inter-day) ranged between 2.8% and 3.5%.These values show a low variability between the values obtained for each concentration. These values are shown in Table 3.

3.2.4. Accuracy

The results of the accuracy studies are shown in Table 4. Recovery ranged between 99.32% and 101.9% with R.S.D. less than 1%. The values obtained show a suitable accuracy for the analytical method.

Table 3: Summary of precision determined during method validation

Concentration (µgml−1)	R.S.D. (%), intra-day	R.S.D. (%),inter-day
10	5.5	4.0
50	2.8	4.2
100	2.8	3.5

Table 4: Accuracy of the method determined according to ICH Q2 guidelines.

Concentration (µgml−1)		Recovery (%)[a]	R.S.D. (%) (*n*=3)
Added	Recovered		
50	50.59	101.18	
50	50.95	101.90	0.46
50	50.50	101.00	
100	99.63	99.63	
100	99.32	99.32	0.16
100	99.60	99.60	
150	150.64	100.43	
150	148.39	98.92	0.84
150	150.50	100.33	

[a] (Found concentration/added concentration) ×100.

Table 5: Robustness of the method.

Parameter	Value	R	T	*t*R (min)	%Recovery	R.S.D. (%)
pH	2.9	15.514	1.55	3.803	99.73	4.4
	3.0	14.435	1.39	3.813	100.00	2.9
	3.1	15.457	1.57	3.830	100.44	4.5
Flow rate	0.68	13.925	1.53	3.933	103.1	0.9
($mlmin^{-1}$)	0.70	14.435	1.39	3.813	100.0	2.9
	0.72	13.707	1.46	3.727	97.74	3.9

3.2.5. Sensitivity

LOD and LOQ were found to be 1.4µgml−1 and 4.3µgml−1, respectively. These values are adequate for the detection and quantification of CT.

3.2.6. Robustness

During the robustness study, peak symmetry (T) was maintained and the retention times were not significantly changed as shown in Table 5. These facts suggest that the method did not change with time and experimental conditions. However, it could be noted that organic composition of the mobile phase can influence the method performance.

4. CONCLUSIONS

In the present research work to achieve highest precision in quantitative chromatography of CT in pharmaceutical dosage form, a reverse phase liquid chromatography method for CT using IS was developed and validated. The method was validated in terms of linearity, precision, accuracy, detection limit, quantification limit and robustness. It involves a simple procedure for the preparation of the samples and shorter run times for analytical procedure (less than 20 min). A low percent of organic solvent (acetonitrile 20%) was used in the composition of the mobile phase. Hence the present HPLC method can be considered simple, rapid, suitable and easy to apply for routine analysis of Citicoline sodium in pharmaceutical dosage form.

5. ACKNOWLEDGEMENTS

The authors shall remain grateful to M/s Strides Arcolab limited, Bangalore, India and Juggat Pharma, Bangalore, India for providing Citicoline sodium and Caffeine in the form of 'Gift Samples' respectively and Nargund College of Pharmacy, Bangalore for providing lab and research facilities to complete this project.

6. REFERENCES

1. O'Neil, M.J. The Merck Index an Encyclopaedia of Chemical, *Drugs and Biologicals*, 14th ed., MERCK and Co. Inc. USA; 2006; p 2319.
2. Sweetman, S.C. Martindale the Complete Drug Reference, 35th ed., Pharmaceutical press:London UK; 2007; p 2072.
3. Secades J.J., Lorenzo J.L., Citicoline Pharmacological and Clinical Review. Methods Exp Clin Pharmacol 2006; 28: 1-56.
4. Mirakor, V.A., Vaidya, V.V., Baing, M.M., Joshi, S.S., Rapid and sensitive high performance liquid chromatography method for Citicoline in formulation dosage form. *Indian Drugs*. 2007, 44(9), 693.
5. Ganduri, R.B., Peddareddigari, J.R., Dasari, N.R., Stability indicating LC method for the determination of citicoline sodium in injection formulation. *Int J Pharma Tech Res*. 2010, 2(1), 427.
6. Malipatil, S.M.; Patil, S.K.; Deepthi, M.; Kishwar, J. *J Pharma Res*. 2010, 3(4), 785.
7. Surani, S.; Kimbahune, R.; Kabra, P.; Urmila, G. Der Pharmacia Lettre. 2010, 2(5), 353.
8. International Conference on Harmonization (ICH), Harmonized Tripartite Guidelines on 'Validation of Analytical Procedures: Methodology', Operational from June 1997, The European Agency for the Evaluation of Medicinal products, Human Medicines Evaluation Unit.

*****