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Development and Validation of Dissolution Method for Citicoline Controlled Release Tablets by Reverse Phase High Performance Liquid Chromatographic Method

Y. Sreenivasa Reddy[1*], A .Dinakar[1]

1. Sun Institute of Pharmaceutical Education and Research Nellore -524346, A.P, India

ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method has been developed for determination (drug release) of Citicoline in controlled release tablet dosage form by reverse phase separation was carried out on a columns containing different stationary phases, the final choice giving satisfactory theoretical plates and tailing with good reproducibility and run time, with dimension 250 mm × 4.6 mm internal diameter, 5-µm particle; Zorbax C18 reversed-phase column. The mobile phase consisting of buffer: methanol (95:5, pH 6.0) at a flow rate of 0.8mL/min. The UV detection wavelength was set at 270nm. The retention time for Citicoline was found to be 6.4 min. and recoveries from controlled release tablet dosage form were between 99.7% and 104.4%. The method was validated for specificity, linearity, accuracy, precision and robustness. The proposed method was optimized and validated as per the ICH guidelines.

Key words: Citicoline monosodium, Reverse Phase -High performance liquid chromatography, Dissolution, Validation.

*Corresponding Author Email: srinuyeruva@gmail.com

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INTRODUCTION

Citicoline sodium is a chemically designate as Cytidine 5'-(trihydrogendiphosphate) P'-[2-(trimethylammonio) ethyl] ester monosodium salt, its molecular formula is $C_{14}H_{25}N_4NaO_{11}P_2$ and molecular weight 510.31(salt) and 488.32 (base). It is a white crystalline, hygroscopic powder and readily soluble in water but practically insoluble in alcohol. Its dissociation constant (pKa) was 4.4[1].Citicoline belongs to the therapeutic use of a Neuroprotectant or as a cerebroprotectant,in Particular citicoline is useful the victims of ischemic stroke, head trauma and neurodegenerative disease.Citicoline is also used to treat unconsciousness resulting from cerebral thrombosis, hemorrhages, cranial trauma and cerebropathies due to atherosclerosis.[2]

Citicoline serve as a donor of choline in the metabolic pathways for biosynthesis of acetyl choline and neuronal membrane phospholipids.

Citicoline is a complex organic molecule that functions as an intermediate in the biosynthesis of cell membrane phospholipids.Citicoline also called as CDP-choline and cytidine diphosphate choline. Citicoline is degraded into uridine and choline during intestinal absorption.[3] These two compounds then pass through the blood brain barrier to reconstitute as citicoline in the brain[4].

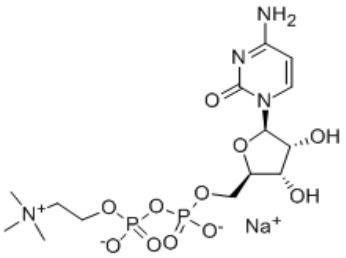

Figure 1: Structure of Citicoline mono sodium

Drug absorption from a dosage form after oral administration depends upon the release of the drug substance from the pharmaceutical dosage form. The Dissolution test is a very important tool in product development and quality control[5] .At present there was no official monograph for the citicoline sodium and its controlled release tablet. A liquid chromatography method for the determination of citicoline sodium and its injection was reported in the literature[6]. A rapid and sensitive high performance liquid chromatography assay method for citicoline in formulation dosage form was also reported in literature[7]. As per our knowledge none of the reported analytical procedures to describe a method for the determination of drug release (dissolution method) in controlled release tablet formulation.In the present study to develop a simple, accurate dissolution test method for the determination of citicoline from its controlled release tablets.

MATERIALS AND METHODS

Chemicals/Reagents

Citicoline monosodium was procured from Micro labs, Bangalore, India, as a gift sample. Potassium di-hydrogen orthophosphate (AR Grade), Sodium hydroxide (AR Grade), Tetra butyl ammonium hydrogen sulphate (AR Grade), Triethylamine (AR Grade), Acetic acid (AR Grade) and Methanol (HPLC Grade) were purchased from Merck (India) Ltd. Mumbai, India.

Instrumentation

Analysis was performed on a chromatographic system Waters Alliance e2695 series separation module equipped with an auto injector, UV detector, and Quaternary pump. Data was made with Empower software.

Determination of Solubility of Citicoline Monosodium

The solubility of Citicoline monosodium was determined in the various dissolution media as follows. Citicoline monosodium (1000mg) were transferred into separate 100 mL volumetric flask. Dissolution medium (60mL) was added to each flask. These flasks were sonicated for 30 min. at 37°C with intermittent shaking, make up to the mark. Further diluted 5 ml of this solution into 100mL of volumetric flask with dissolution media. Again further diluted 5ml of this solution into 50mL of volumetric flask with dissolution media. The solutions were each filtered, through a 0.45µ membrane filter. Each filtrate was analyzed separately by UV-Vis Spectrophotometer at 270 nm to determine the solubility of the drug in the particular dissolution medium.

Evaluation of Stirring Rate, Dissolution Medium and Volume

Dissolution experiments were performed using (Distek 2100B dissolution apparatus) standard USP dissolution apparatus 1(Basket) and apparatus 2 (Paddle). Stirring rates of 75 rpm, 100 rpm for basket and 75 rpm, 100 rpm for paddle were evaluated in 900mL dissolution media. All the dissolution media used were de-aerated by sonication followed by filtration using membrane filters under vacuum.

Table 2: Dissolution profile of Citicoline controlled release tablets in 0.1N HCl at different RPM.

Time (hrs.)	Basket 75 RPM	Basket 100 RPM	Paddle 75 RPM	Paddle 100 RPM
1	21.70	30.10	20.10	28.60
3	44.60	49.10	39.60	46.60
6	55.90	70.60	46.80	57.10
9	65.80	81.20	58.40	69.50
12	81.50	90.40	71.50	87.90

Table 3: Dissolution profile of Citicoline controlled release tablets in Acetate Buffer pH 4.5 at different RPM.

Time (hrs.)	Basket 75 RPM	Basket 100 RPM	Paddle 75 RPM	Paddle 100 RPM
1	23.20	28.20	19.50	27.60
3	45.20	52.50	40.20	51.20
6	57.70	62.20	48.90	59.60
9	66.30	75.10	57.40	65.90
12	80.10	91.30	68.70	80.90

Table 4: Dissolution profile of Citicoline controlled release tablets in Phosphate Buffer pH 6.8 at different RPM.

Time (hrs.)	Basket 75 RPM	Basket 100 RPM	Paddle 75 RPM	Paddle 100 RPM
1	22.2	30.2	20.1	30.1
3	42.5	47.6	40.8	55.6
6	55.5	58.2	53.5	62.9
9	64.2	80.3	61.2	70.8
12	72.3	99.7	70.1	85.7

Finally with Phosphate buffer pH 6.8 as a dissolution media, we have got good consistency results, so we have selected the Phosphate buffer pH 6.8 as suitable dissolution media for this formulation.

Development and Optimization of chromatographic conditions

Different columns, mobile phases, flow and column temperatures were tested in the development of the analytical method. C-8 and C-18 columns of the same length, different lengths and diameters were also tested and pH of buffer variations from 4.0 to 6.5 were also tested by keeping all parameters and conditions were constant (0.8 mL/min.,injection volume of 20µL, temperature at 25°C). Then the mobile phases with different buffer concentrations and organic content were also tested by keeping the all parameters and conditions were constant. Finally we got the good chromatographic peak with more than 5000 theoretical plates, tailing factor of less than 2.0 and Relative standard deviation of less than 2.0% for six replicate standard injections.(Flow: 0.8 mL/min., Column: C-18, 250mm×4.6mm, 5µ,Wave length at 270nm, injection volume of 20µL and temperature at 25°C).

Chromatographic Conditions

Column :C18, 250 mm X4.6mm, 5µ or equivalent column (Zorbax)

Flow rate :0.8mL/min

Detection :270nm

Injection volume : 20µL

Column temperature : 25°C

Run time : 10min

Preparation of Buffer

Dissolve 1.697 gm of Tetra butyl ammonium hydrogen sulphate in 1000 mL of water, add 2mL of Triethylamine and adjust the pH to 6.0 with diluted acetic acid.

Preparation of Mobile Phase

Prepare a degassed mixture of buffer and Methanol (95:5).

Dissolution Parameters

Apparatus :USP- I (Basket)

Medium :Phosphate Buffer pH 6.8

Volume :900mL

RPM :100

Temperature :37° C ± 0.5° C

Specified Time (hours) :1, 3, 6,9 and 12.

Preparation Of Phosphate Buffer Ph 6.8: (Dissolution Media)

Weigh accurately and transfer about 40.83g of Potassium di-hydrogen orthophosphate and add 3000mL of water and dissolve, add to this 5.4gm of Sodium hydroxide and dilute with water to 6000mL. and adjust the pH to 6.8 ± 0.05.

Standard Preparation

1. Weigh accurately and transfer about 46.0mg of Citicoline monosodium (equivalent to 44mg of Citicloine) and dissolve and makeup to 100mL with Dissolution Media. (Phosphate Buffer pH6.8)

2. Dilute 5mL of this solution to 50mL with Phosphate buffer pH 6.8 and mix.

Sample Preparation

Set the parameters of dissolution apparatus as mentioned above. Place one tablet in to each of the six dissolution jars and operate the apparatus. At the end of the specified time point, withdraw 10mL of the sample solution from each dissolution vessel. Replace the aliquots withdrawn with equal volumes of dissolution medium maintained at 37°C ± 0.5°C. Filter the solution through 0.45µm membrane filter. Dilute 2mL of withdrawn sample to 50mL with Phosphate buffer pH 6.8 and mix.

Procedure

Separately inject 20µL of Blank solution, standard solution(six injections) and each sample solution into the Chromatographic system. Record the chromatograms and measure the peak

responses.

System Suitability Parameters

From standard solution:

1. The theoretical plates (N) of Citicoline peak should be not less than 2000.
2. Tailing factor (T) for Citicoline peak should be not more than 2.0.
3. The Relative Standard deviation for peak area counts of Citicoline from six replicate injections of Standard solution should be not more than 2.0%.

METHOD VALIDATION[8]

The HPLC method was validated for accuracy, precision, linearity, specificity and robustness according to ICH guidelines.

Accuracy

The accuracy of the method in the range of 10 % to 120 % of test concentration of Citicoline controlled release tablets (44 mg/mL of Citicoline) was satisfactory. Recovery was between 99.7% to 104.4% for Citicoline controlled release tablets.

Precision

The intra-day and inter-day precision, compared in terms of %RSD, were close to each other indicating satisfactory level of precision of the method. %RSD for intra-day precision was 2.7 and 1.9 for Citicoline controlled release tablets on first and second day of analysis, respectively while %RSD for inter-day precision was 1.0 for Citicoline controlled release tablets.

Linearity

The analytical method was linear for Citicoline controlled release tablets in the concentration range of 10 % – 120 % of test concentration of the drug. The test concentration of Citicoline controlled release tablets was 44 µg/mL. The regression analysis of the linearity data yielded correlation coefficient value 0.999.

Specificity

The chromatograms obtained from standard solutions were identical to those obtained from tablet solutions containing an equivalent concentration of Citicoline. The representative chromatograms show no other peaks in retention time of Citicoline. In addition, when the solution prepared from the blank tablet was injected into the HPLC system, no co-eluting peaks were obtained at the retention time of Citicoline.

Robustness

The robustness of the method was evaluated by the varying chromatographic conditions of flow rate, pH and composition of mobile phase of the analytical method. As per the standard test

method, flow rate 0.8mL/min, pH of mobile phase 6.0 and composition of mobile phase 95:5buffer: methanol. To evaluate the robustness of the method, the chromatograms were recorded with flow rate of 0.6 mL/minute and 1.0 mL/minute, mobile phase pH of 5.8 and 6.2, and mobile phase composition of 97:3 v/v and 93:7 v/v of buffer: methanol. The results of the chromatographic test under varied conditions indicated that the accuracy and precision are within the specified limits of 95.0 % - 105.0 % for accuracy and %RSD is 5.0 for precision. No significant change was observed in system suitability parameters such as tailing factor, number of theoretical plates and %RSD for replicate injections of the standard.

RESULTS AND DISCUSSION

The method was validated, in accordance with ICH guidelines for accuracy, precision, linearity, specificity, and robustness. For the RP-HPLC, chromatographic conditions were optimized to get best peak shape. The selection of mobile phase was based on peak parameters; (theoretical plates, tailing factor) ease of preparation and cost. The optimum wave length for detection and quantification was 270 nm, at which good detector response was obtained with symmetrical peaks.

The sink conditions tested showed that Citicoline was soluble in 0.1N HCL pH 1.2, Acetate buffer pH4.5 and Phosphate buffer pH6.8. Then, dissolution tests for Citicoline controlled release tablets were performed using three dissolution medium at the stirring speed of 75 rpm and100 rpm, to investigate the drug release in each medium. The results show that Phosphate buffer pH6.8 with 100 rpm was the best dissolution medium, since it provides good and consistency results with highest drug release percent.

1. The specificity test by HPLC demonstrated that the excipients from tablets do not interfere in the drug peak. Thus, the HPLC method is useful to quantify Citicoline in pharmaceutical formulation from the dissolution tests.
2. To assess the linearity, standard curves for Citicoline were constructed, plotting percentage concentrations (µg/mL) versus absolute area (mV/s) and showed good linearity on the 10% to 120%. The representative linear equation was $y = 11614\ x + 6181.7$, where x is concentration and y is the peak absolute area. The correlation coefficient was0.999, indicating good linearity (Table-5). The data were validated by means of the analysis of variance, which demonstrated significative linear regression and no significant linearity deviation.

Table 5: Linearity

S.No.	Linearity Solution (%)	Area Response
1	10	120652
2	20	238203
3	40	468324
4	60	706371
5	100	1177778
6	120	1390719
Slope		11614
Intercept		6181.7
Correlation Co-efficient		0.999

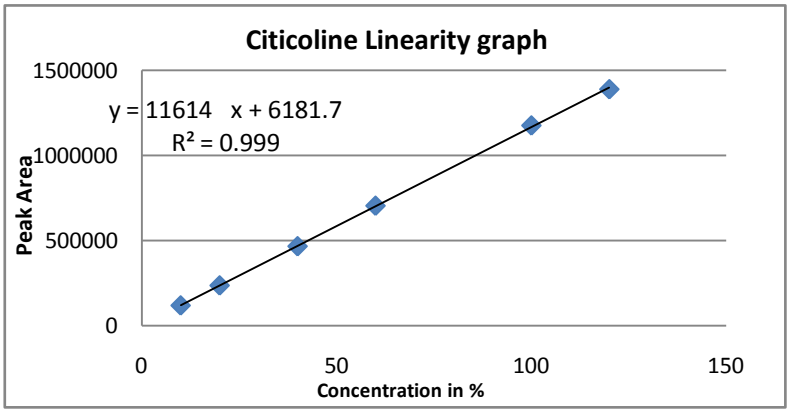

Figure 2: Linearity curve of Citicoline controlled release tablets

3. The Precision and Intermediate Precision of the dissolution test was verified through the comparison of the results of the percentage drug release. The drug release percent for method precision 96.3%, for Intermediate Precision 97.0%. The overall %RSD was 1.9% only. (Table-6)

Table 6: Method Precision and Intermediate Precision Results

S.No.	Method precision Test area	% drug release for 12hr	Intermediate precision Test area (other day)	% drug release for 12hr
1	1028341	94.2	1065361	95.7
2	1036316	94.9	1086594	97.6
3	1090135	99.8	1068913	96.1
4	1050645	96.2	1077589	96.8
5	1024651	93.8	1083658	97.4
6	1082436	99.1	1092164	98.1
Avg.		**96.3**	**Avg.**	**97.0**
Min		**93.8**	**Min**	**95.7**
Max		**99.8**	**Max**	**98.1**
SD		**2.57**	**SD**	**0.94**
% RSD		**2.7**	**%RSD**	**1.0**
Overall % RSD		**1.9**		

4. The accuracy expresses the agreement between the accepted value and the value found. The mean recovery was found to be 102.1% for the Citicoline controlled release tablets (Table-7). This value shows the good accuracy of the proposed method.

Table 7: Accuracy/ Recovery of spiked Citicoline.

Level	Area	% recovery
10%	115141	101.9
40%	465866	102.8
80%	952784	104.4
100%	1181814	99.7
120%	1372804	101.9
Mean		**102.1**
SD		**1.7**
%RSD		**1.7**

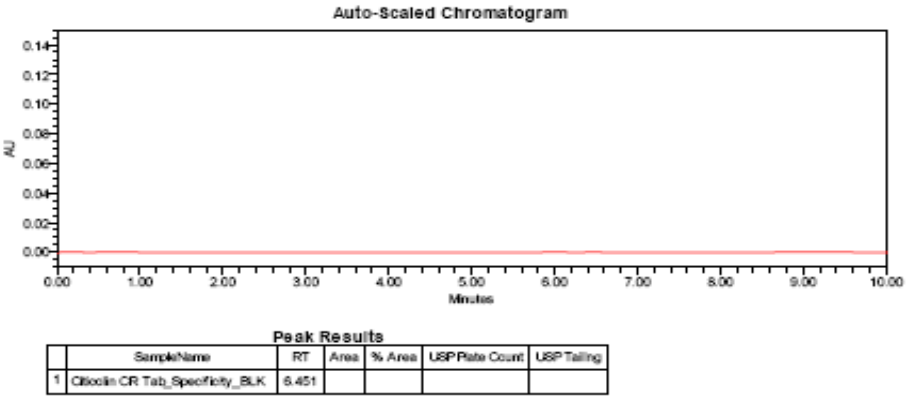

Figure 3: Typical chromatogram of Blank

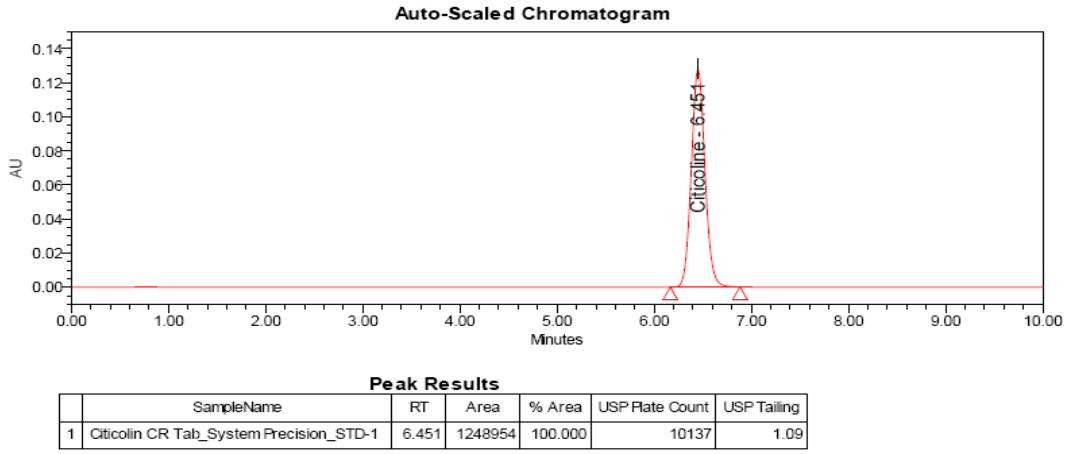

Figure 4: Typical chromatogram of Standard

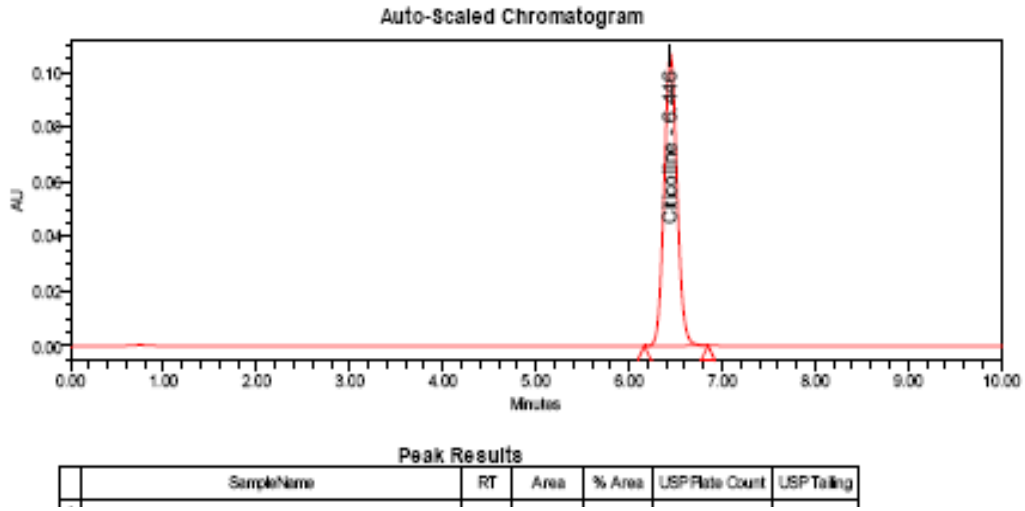

Figure 5: Typical chromatogram of Test

CONCLUSION

It is a well-known that validation procedure is an integral part of the analytical method development. Therefore, the developed method was validated according to the ICH guidelines. Based on the results, it can be concluded that there is no other co-eluting peak with the main peak and that the method is specific for estimation of Citicoline. The proposed method has linear response in the stated range and is accurate and precise. To our knowledge, the developed HPLC method is the first reported method for determination of Citicoline (Drug release) in controlled release tablet dosage form. Taken together, these results clearly showed that this method can be used for dissolution of Citicoline in their drug product.

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