

Method Development and Validation of RP-HPLC for Simultaneous Estimation of Citicoline and Piracetam in Tablet Dosage Form

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ABSTRACT

Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method have been developed and validated for the estimation of Citicoline and Piracetam in Bulk drug and Pharmaceutical dosage form. The developed method is rapid, accurate, precise, simple and economical than the previous methods. The separation was carried out using ACE C₈, column having 250mm X 4.6mm, 5 μ m particle size, in isocratic mode, with mobile phase containing Methanol: Water [60: 40, v/v]. The flow rate is 0.6 ml / min and effluents are monitored at 220 nm. Chromatogram showed peak at a retention time of 3.096 \pm 0.008 min for Citicoline and 4.110 \pm 0.008 min for Piracetam. The method is validated for system suitability, linearity, precision, accuracy specificity, ruggedness, robustness, LOD and LOQ. Recovery of Citicoline and Piracetam is found to be in the range of 99.22 - 100.11 %. The LOD and LOQ for estimation of Citicoline and Piracetam are found to be 0.2 μ g / ml, 0.76 μ g / ml, and 0.1664 μ g / ml, 0.5632 μ g / ml respectively. Proposed method can be successfully applied for the quantitative determination of Citicoline and Piracetam in Bulk drug and Pharmaceutical dosage form.

Key words: Citicoline, Piracetam, RP-HPLC, Method Development, Validation and ACE.

1. INTRODUCTION

Citicoline is an intermediate in the generation of phosphatidyl choline from choline. It is chemically 5'-O [hydroxyl ((hydroxyl [2(trimethylammonio) ethoxy] phosphoryl)oxy) phosphoryl] cytidine. Citicoline is a white or off-white amorphous, hygroscopic powder having molecular weight 488.3g/mol. It helps to improve focus and mental energy and may possibly be useful in the treatment of attention deficit disorder [1-2].

Piracetam is a cyclic derivative of GABA. It is one of the group of racetams. It is chemically 2-oxo -1- pyrrolidine acetamide, it shares the same 2-oxo-pyrrolidone base structure with 2-oxo-pyrrolidine carboxylic acid(pyroglutamate). It is a fine white crystalline powder having molecular weight 142.16g/mol. It may enhance, elevate, and improve cognitive functions and abilities linked and associated to the central nervous system, memory development and memory processes. Many people across the world use the nootropic, piracetam, to effectively retain knowledge and improve memory. Piracetam appears to be effective in treating cognitive impairment in alcoholism [3-8]. Piracetam improves the

functioning of the (ACh) transmitters and receptors.

Both drugs are psychotherapeutic agents, used as psycho stimulant, nontropic and neurotonics. Both drugs are freely soluble in water. These drugs will increase cerebral metabolism and increase level of various neurotransmitters, including acetylcholine and dopamine, exerting its action by activating the biosynthesis of structural phospholipids in neuronal membrane. This drug will increase the blood flow and oxygen consumption in brain. The review of literature regarding quantitative analysis of Citicoline and Piracetam revealed that the attempts were made to develop analytical methods for Citicoline and Piracetam in serum. Some spectrometric methods and LC methods have been reported for the estimation of the individual drugs [9-13] and one RP-HPLC method in an expensive and time taking way. The focus of the present study was to develop and validate a rapid, stable, specific, and economic RP-HPLC method for the estimation of Citicoline and Piracetam in tablet dosage form.

2. MATERIALS AND METHODOLOGY

Citicoline and Piracetam were obtained as gift samples from Sun pharmaceuticals. Tablets were also obtained from Sun pharmaceuticals. Tablets contain 800 mg of Piracetam and 500 mg of Citicoline. The HPLC grade methanol was obtained from Qualigens Fine Chemicals Ltd., Mumbai and Water obtained from Thomas Baker Chemicals Ltd., Mumbai.

2.1. Preparation of Solutions

2.1.1. Preparation of Mobile phase

The HPLC grade methanol was mixed with milli Q water in the ratio 60: 40 and was subjected to vacuum filtration.

2.1.2. Preparation of standard solution

Standard stock solution was prepared by dissolving 10 mg of drug in sufficient amount of water in a 100 ml volumetric flask and diluted up to the mark. From that 1 to 5 ml of standard solutions were pipette out in to a clean and dry HPLC vials and it was made up to 10 ml using mobile phase.

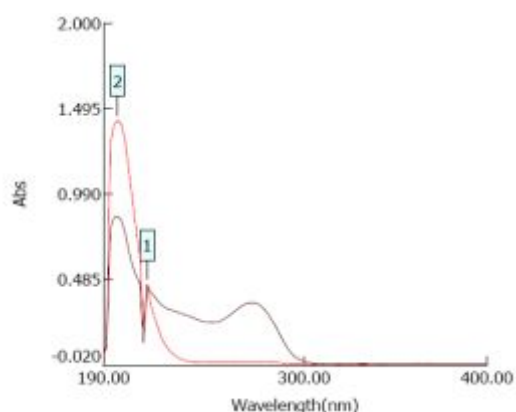
2.1.3. Sample preparation

Tablets were powdered, and from that 0.19 g of powder were accurately weighed and taken in 100 ml volumetric flask, sufficient mobile phase was used for dissolving the drug. Then volume is made up to the mark with mobile phase. The samples were filtered through a 0.22 micron filter prior to run in HPLC.

2.1.4. Selection of wavelength

Spectrum of diluted solutions was scanned in the spectrum mode between 200 nm to 400 nm with a bandwidth of 1 nm. From the overlain spectra of Citicoline and Piracetam as shown in fig. 1, obtained from the PDA Detector the wavelength of 220 nm was selected, where Citicoline and Piracetam gives the maximum absorption in the UV region.

Fig -1: Overlain spectra of Citicoline and Piracetam



3. RESULTS AND DISCUSSION

A new reverse-phase, isocratic, liquid chromatographic method with UV detection at 220 nm and 224 nm was developed for the quantitative determination of Citicoline and Piracetam in Pharmaceutical dosage forms. The chromatographic separation was achieved on ACE C₈ (250 × 4.6 mm) column with mobile phase containing methanol: water (60: 40 % v/v) with a flow rate of 0.6 ml/min was used. The resulting chromatogram (Fig 2 and 3) exhibited a retention time of 3.096 and 4.110 min. The above method was optimized with a view to develop an assay method for Citicoline and Piracetam.

3.1. Validation of the method

The accuracy of a method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analysed sample formulation as 50%, 100% and 150% levels. The recovery studies were carried out 3 times of each level and the percentage recovery and mean of the percentage recovery were calculated and given in Table 3. From the data obtained, it was observed that the recoveries of standard drugs were found to be accurate and within the specified limits. The precision of the method was determined by repeatability and intermediate precision. The area of drug peaks and percentage relative standard deviation were calculated. The results revealed that the developed method was found to be reproducible in nature. The standard drug solutions in varying concentrations ranging from 60-160% of the targeted level of the assay concentration were examined by the assay procedure. Citicoline and Piracetam were found to be linear in the range of 60-100mg/ml and 96-160mg/ml respectively.

The slope, intercept and correlation coefficient values were also calculated. The correlation coefficient of piracetam and Citicoline were found to be 0.9997 and 0.9995 respectively. The calibration curves were plotted as peak area Vs concentration of the standard solutions. The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. The assay results are given in Table 2. These data demonstrates that the methods have adequate sensitivity of the concentration of the analytes.

Table -1: System Suitability Parameters

Parameters	Citicoline	Piracetam
Tailing factor	1.7	1.5
Theoretical Plate	2082.7	2561.8

Table -2: Assay Results

Drug	Percentage Assay	Amount Present (mg/tab)
Citicoline	98.7%	500mg
Piracetam	98.2%	800mg

Table -3: Validation Parameters

Parameters	Citicoline	Piracetam
Accuracy	%Recovery= 99.4%	% Recovery=99.7%
System precision	% RSD = 1.3	% RSD = 1.3
Method precision	% RSD = 0.46	% RSD = 0.45
Linearity	R ² = 0.9994	R ² = 0.9997
Range	60-100 mcg/ ml	96-160 mcg/ml
LOD	2.96 ng/ml	3 ng/ml
LOQ	9.96 mcg/ml	10 mcg/ml

. The range demonstrates that the method is linear outside the limits of expected use. The additional peaks The LOD and LOQ of the developed method were determined by analyzing progressively low concentration of the standard solutions using the develop methods. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3.3). LOD of Citicoline and Piracetam were found to be 2.96mg/ml and 3.00mg/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Citicoline and Piracetam were found to be 9.96 mg/ml and 10.0 mg/ml respectively. The system suitability studies were performed for the standard solutions and were presented in Table.1. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination. From the above experimental data results and parameters it was concluded that the developed RP-HPLC method is simple, economical, rapid, precise and accurate. Hence these methods can be used for routine analysis of Citicoline and Piracetam in combined tablet dosage form.

Fig- 2: Standard Chromatogram for Optimized Method

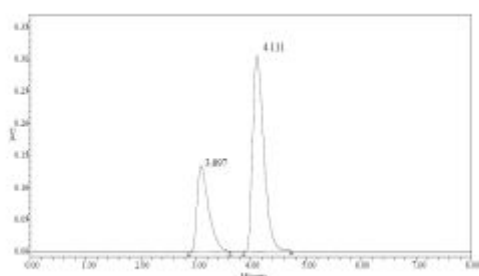
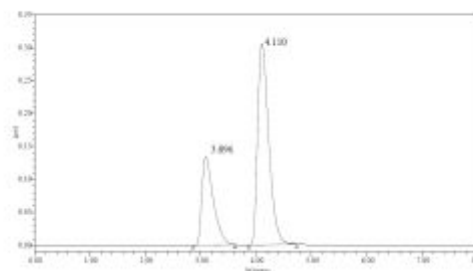


Fig -3: Sample Chromatogram for Optimized Method



6. CONCLUSION

The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness robustness, LOD and LOQ. As there was no interference due to excipients and mobile phase, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate and Mobile phase composition separately and analysis being performed by different analysts.

Good agreement was seen in the assay results of Pharmaceutical formulation by developed method. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Citicoline and Piracetam in Bulk drug and Pharmaceutical formulation.

7. REFERENCES

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