International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.3,pp1311-1313, July-Sept 2011

# Method development and Validation of RP-HPLC for Simultaneous estimation of Citicoline and Piracetam in Tablet dosage form

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**Abstract:** A simple, rapid, sensitive, validated reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Citicoline and Piracetam in pharmaceutical dosage form. The chromatographic conditions used for the separation was Phenomenex Luna C18 (4.6 x 250 mm x 5 µm) with mobile phase comprised of dibasic potassium phosphate: acetonitrile (40: 60). The flow rate was set at 1.0 ml/min with detection at 272 nm. The retention time of Citicoline and Piracetam was found to be 2.562 and 6.310. The method was developed in terms of accuracy, precision, linearity, specificity, system suitability, and stability studies, limit of detection, limit of quantification, ruggedness and robustness. The linearity of Citicoline and Piracetam was in the range of 10 - 60 µg/ml and 20 - 120 µg/ml respectively. All the proposed parameters were with in the specification limits. The proposed methods are suitable for the simultaneous determination of Citicoline and Piracetam in pharmaceutical dosage form.
***Keywords*:** Citicoline, Piracetam, RP-HPLC.

## 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[1]. Piracetam is chemically 2-oxo -1- pyrrolidine acetamide. It is a fine white crystalline powder having molecular weight 142.16g/mol. 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 bio-synthesis 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[2-7]. 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.

## EXPERIMENTAL

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 acetonitrile was obtained from Qualigens Fine Chemicals Ltd., Mumbai and Water obtained from Thomas Baker Chemicals Ltd., Mumbai. The reagents Dibasic potassium phosphate and 1-Hexane sulphonic acid were obtained from Spectrochem, Mumbai, India.

### Preparation of Solutions

### Preparation of Mobile phase

First the phosphate buffer was prepared by dissolving 6.8 g of dibasic potassium phosphate in 100 mL of water. The buffer solution was mixed with acetonitrile in the ratio 40: 60 and 1 g of 1-hexane sulphonic acid sodium salt was added and then pH of the mobile phase solution was adjusted to 3.4 with ortho phosphoric acid.

### 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 milliQ water.

### Sample preparation

Samples were powdered, and from that 1.5 g of sample was drawn and it was dissolved in 100 ml of milliQ water. The samples were filtered through a 0.22 micron filter prior to run in HPLC.

### 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 obtained from the PDA detector the wavelength of 272 nm was selected, where Citicoline and Piracetam gives the maximum absorption in the UV region.

**Table 1 Summary of validation parameters of citicoline and piracetam**

| S. No | PARAMETERS | CITICOLINE | PIRACETAM |
|---|---|---|---|
| 1. | System Suitability | | |
| | a) Resolution | 7.213 | |
| | b) Tailing Factor | 0.9375 | 1.13 |
| | c) No. of Theoretical Plates | 2179 | 5518 |
| 2. | Linearity ($R_2$) | 0.999 | 0.999 |
| 3. | Range | 10-20 µg/ml | 20-120 µg/ml |
| 4. | Accuracy (% Recovery) | 99.79 | 100.16 |
| 5. | System Precision (% RSD) | 0.0571 | 0.02408 |
| 6. | Method Precision (% RSD) | 0.0613 | 0.0314 |
| 7. | LOD | 1.131 µg/ml | 1.88 µg/ml |
| 8. | LOQ | 3.427 µg/ml | 5.70 µg/ml |
| 9. | Robustness | Robustted | Robustted |
| 10. | Ruggedness<br>a)Analyst 1 (% RSD)<br>b)Analyst 2 (% RSD) | <br>0.00148%<br>0.00621% | <br>0.373%<br>0.359% |

## RESULTS AND DISCUSSION

A new reverse-phase, isocratic, liquid chromatographic method with UV detection at 210 nm and 272 nm was developed for the quantitative determination of Citicoline and Piracetam in Pharmaceutical dosage forms. The chromatographic method was performed on Phenomenex–Luna C18 (250 × 4.6 mm) column with an isocratic mobile phase of dibasic potassium phosphate: acetonitrile (40: 60) with a flow rate of 1.0 ml/min was used. The resulting chromatogram exhibited a retention time of 2.562 and 6.310 min. The above method was optimized with a view to develop an assay method for Citicoline and Piracetam.

A linear range of 10-50 µg/ml was established for Citicoline and 20-120 µg/ml was established for Piracetam drugs. The accuracy is reproducibility which is evident from the data as results are close to 100%. This serves a good index of accuracy and reproducibility of the proposed method. Estimation was carried out for both the drugs Citicoline and Piracetam. All the values shown are with in the acceptance criteria.

All the developed methods are simple, economical, rapid, precise and accurate. Hence these methods can be used for routine analysis of Citicoline and Piracetam in combined tablet dosage form. The results obtained for tablets and recovery studies are summarized in Table 1.

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