ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR PIRACETAM IN PHARMACEUTICAL FORMULATION

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Abstract. A simple, rapid and validated HPLC method was developed for determination of Piracetam in film coated tablets. A Lichrosorb® (RP-18 column with a mobile phase consisting of acetonitrile – phosphate buffer (5:95v/v)) was used. Quantitative evaluation was performed at 205 nm. The HPLC method is selective, precise and accurate and can be used for routine analysis of preparations in pharmaceutical industry quality control laboratories.

Key Words: liquid chromatography, piracetam, tablets, validation

Introduction

Piracetam (2-oxo-pyrrolidin-1-yl)-acetamide is a nootropic psychopharmacological agent [1-3] having a variety of physiological effects that may result from the restoration of cell membrane fluidity. It is used for treatment of patients suffering from pathological, neurosensitive [4], and cognitive deficits [5], brain-organic psychosyndromes (e.g., primary degenerative dementia), vertigo, and myoclonus of cortical origin.

Piracetam is a white powder and is freely soluble in water, soluble in alcohol, and slightly soluble in methylene chloride [6]. The chemical structure of the molecule is presented in Figure 1. Literature survey revealed HPLC methods were developed for the estimation of piracetam in biological fluids [7-10]. Capillary electrophoresis [11], thin layer densitometric determination [12], micellar electrokinetic chromatography [13] methods were also developed for the estimation of piracetam in biological fluids. Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve. Derivative spectrophotometry is now a reasonably prized standard feature of modern micro computerized UV spectrophotometry [14-15]. The aim of the present study was to develop a simple, economical and accurate analytical method for the estimation of Piracetam in pharmaceutical dosage forms.

Figure 1. Chemical structure of Piracetam

Experimental Section

Materials and Methods

Reagents and Chemicals

All chemicals and reagents used were HPLC grade. Piracetam standard was obtained from Sigma Aldrich. Tablet formulation containing 400 mg Piracetam was obtained commercially. HPLC grade Acetonitrile was procured from Merck Ltd. All other chemical reagents were of analytical grade.

Instrumentation and Chromatographic Conditions

A Shimadzu HPLC system was utilized consisting of the following components: quaternary pump
LC – 20 AD, vacuum degasser unit DGU – 20 A5 and a UV/VIS variable detector SPD – 20 A. Separation was carried out on a LiChrosorb C 18 column (250 x 4.6 mm, particle size 5 µm) under reversed phase partition chromatographic conditions. The mobile phase consisted of an aqueous solution containing Acetonitrile – 1 g/l solution of Dipotassium hydrogen phosphate (5:95) with pH 6.0 adjusted with ortho-phosphoric acid. The mobile phase was filtered through 0.45 µm membrane filter and degassed by using sonicater for about 10 min before use. The sample solutions were also filtered using 0.45 µm membrane filters. The mobile phase was delivered isocratically at a flow rate 1 ml/min. The column was maintained at 25ºC temperature. The injection volume was a 20 µl and the total run time was 5 minutes. The detection was carried out at 205 nm.

**Preparation of the Standard Solution**

About 50 mg of Piracetam were accurately weighed and transferred into 100 mL volumetric flask and dissolved in mixture of acetonitrile – water (10 : 90 v/v). The final drug concentration of 50 µg/mL was obtained by dissolving the appropriate amount from this standard stock solution in the above said mixture. Calibration standards of Piracetam were prepared by making serial dilutions of the stock solution at concentrations of 12.5, 25.0, 50.0, 75.0, 100.0 µg/mL.

**Sample Preparation**

Twenty tablets were accurately weighed (to obtain the average mass of one tablet) then finely powdered. Weight equivalent to 200 mg of Piracetam (half a tablet) was weighed, transferred into a 100 ml volumetric flask and dissolved with about 50 mL mixture of acetonitrile – water (10 : 90 v/v). The contents were sonicated for 5 minutes. The mixture was made up to 100 ml with the same mixture. The solution was filtered through a membrane syringe filter (pore size 0.45 µm). The final drug concentration of 50 µg/mL was obtained by dissolving the appropriate amount from the filtrate solution. The sample solution was injected and the peak area was measured for determination of Piracetam in a tablet formulation.

**Results and Discussion**

After equilibration of column with the mobile phase indicated by a stable baseline, aliquots of sample (20 µL) were injected. The typical chromatogram is shown in Figure 2. The amount of Piracetam present in the tablets was calculated using single point analysis method and results are shown in Table 1.

![Figure 2. Chromatogram of Piracetam](image)

**Table 1. Results of Assay of Piracetam tablet**

<table>
<thead>
<tr>
<th>Drug #</th>
<th>Label claim (mg/1 tab)</th>
<th>Mean amount found (mg/1 tab) (n=6)</th>
<th>Mean % Assay RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piracetam</td>
<td>400</td>
<td>401.7</td>
<td>100.4±0.552</td>
</tr>
</tbody>
</table>

The method was developed and validated by using the ICH guideline [16]. The selectivity, limits of detection and quantification, linearity, precision, and accuracy were determined. Determination was carried out using tablet formulation. The presented RP-HPLC method has been proved to be rapid and was successfully used for determination of Paracetam.

**Linearity**

The linearity for Piracetam was determined by plotting a calibration graph of ratio of peak area of drug to concentration. The linearity of this method was found to be in the concentration range 12.5 - 100 µg/mL for Piracetam. Y=5.341E7x +1217.2 which is linear regression equation with correlation coefficients of 0.999 was determined from linearity curve (Table 2).

**Limit of detection and limit of quantification**

In order to estimate the limit of detection and limit of quantification, mobile phase was injected six times, and the noise level was determined. The limit of detection was calculated to be three times the noise value and ten times the noise, which gave limit of quantification, and was also cross-checked by formulas given below (Table 2).

LOD=3.3σ/S and LOQ=10σ/S where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve.
**Table 2. Linearity Results, Limit of Detection (LOD) and Limit of Quantification (LOQ)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>r²</th>
<th>Calibration equation</th>
<th>LOQ ng</th>
<th>LOD ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piracetam</td>
<td>0.999</td>
<td>( Y = 5.341 \times 10^7 x + 1217.2 )</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

**Accuracy/Recovery**

Accuracy of the developed method was confirmed by performing a recovery study as per ICH norms at three different concentration levels (50%, 100%, 150%) by replicate analysis (n = 3). The results obtained (Table 3) indicate that recovery is good, not less than 98% and percentage relative standard deviation is less than 2%.

**Precision**

The precision of the method was determined by repeatability, intermediate precision (intra-day, inter-day) and was expressed as % relative standard deviation (% RSD). Intra-day precision was determined by performing analysis of triplicate injections of two different concentrations of Piracetam on the same day at different time intervals and on two different days for inter-day precision. The % RSD of the study was found to be less than 2% as shown in Table 4.

**Conclusion**

The aim of the present research work was to achieve highest precision in quantitative estimation of Piracetam in tablet dosage form. The method was validated in terms of linearity, precision, accuracy, limit of detection and limit of quantification. The developed method has a simple procedure for the preparation of the samples, shorter run time for chromatographic analysis (less than 5 min) and a low percent of organic solvent (acetonitrile 5%) in the composition of the mobile phase. Hence the proposed RP-HPLC method can be considered as simple, rapid, suitable and easy to apply for routine analysis of Piracetam in pharmaceutical dosage form.

**Table 3. Recovery studies of Piracetam**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration added (μg/mL)</th>
<th>Concentration recovered (μg/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piracetam</td>
<td>25.00</td>
<td>25.05, 25.15, 24.69</td>
<td>100.2, 100.6, 98.76</td>
</tr>
<tr>
<td></td>
<td>50.00</td>
<td>49.85, 50.15, 49.93</td>
<td>99.70, 100.3, 99.86</td>
</tr>
<tr>
<td></td>
<td>75.00</td>
<td>75.22, 75.01, 75.15</td>
<td>100.3, 100.0, 100.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean</th>
<th>SD</th>
<th>% RSD</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>99.99</td>
<td>0.532</td>
<td>0.532</td>
<td>±0.409</td>
</tr>
</tbody>
</table>

**References**


16. **ICH, Q2 (R1) Validation of analytical procedure, Test and methodology, International Conference of harmonization, Geneva, 2005.**

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