Stability indicating and simultaneous determination of cinnarizine and piracetam from capsule dosage form by reversed phase high performance liquid chromatography

G Navaneethan¹, K Karunakaran¹, * & K P Elango²

¹Department of Chemistry, Sona College of Technology, Salem 636 005, India
²Department of Chemistry, Gandhigram Rural Institute, Gandhigram 624 302, India

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A stability indicating reversed phase HPLC assay method has been used for the simultaneous assay of piracetam and cinnarizine drugs in hard gelatin capsule formulation. The separation is achieved by gradient elution using Hypersil BDS C8 column (250 mm × 4.6 mm, i.d. 5 µm particle size) with mobile phase (A) consisting of 0.015 M dipottassium hydrogen phosphate and 2 mL of triethyl amine adjusted at pH 6.0 with orthophosphoric acid and acetonitrile (990:10 v/v); and mobile phase (B) consisting of 2 mL of orthophosphoric acid in 1000 mL of acetonitrile and with a flow rate of 0.6 mL min⁻¹.

The retention time is found to be approximately 11 min for PR and 51 min for CN. The analysis is conducted at ambient temperature and monitored using a diode array UV-Vis detector set at wavelength 205 nm. The proposed method is found to be specific, linear, accurate and precise for the simultaneous determination of both the drugs from capsule.

Keywords: Cinnarizine, Liquid chromatography, Piracetam, Peak purity

A combined preparation of piracetam (PR) and cinnarizine (CN) exhibits pronounced antihypoxic effect. The effects of both these components are mutually enhanced with respect to the antihypoxic and cerebrovascular resistance reducing effects. The preparation also increases the cerebral blood flow. Cinnarizine could also be viewed as a nootropic drug because of its vasorelaxating abilities due to calcium channel blockage, which happen mostly in brain. It is also effectively combined with other nootropics, primarily piracetam; in such combination each drug potentiate the other in boosting brain oxygen supply¹,³.

Review of literature revealed that only limited reports have been published on the chromatographic assay of PR and CN in the combined formulation¹,⁵ though many methods have described the determination of individual PR, CN and combination with other drug⁶-¹³. However, so far there is no report on the stability indicating chromatographic method for these two drugs. The main objective of the present endeavor is therefore, to describe a specific, linear, precise and accurate reversed-phase HPLC method for the simultaneous determination of CN and PR in the combination formulation. Also, the gradient LC method without using ion-pair aqueous mobile phase and getting unadulterated peak of PR and CN became the target of our work. The important aspects of the HPLC method validation have been reported in many publications and according to the ICH guidelines¹⁴ the validation of analytical procedure has to include linearity, specificity, accuracy and precision.

Experimental Procedure

Materials and reagents

Pure PR (2-oxo-1-pyrrolidineacetamide) and CN (1-(Diphenylmethyl)-4-(3-phenyl-2-propenyl)-piperazine) reference standards were purchased from European Pharmacopoeia and their chemical structures are given in Fig. 1. Bulk materials of PR and CN were obtained as gift sample from M/S Sun Lab, Baddi. PR and CN combination capsule contains

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*Corresponding author.
E-mail: drkk@sonatech.ac.in

Fig. 1— Chemical structure of piracetam and cinnarizine
25 mg of CN and 400 mg of PR and these were prepared in house. Acetonitrile used was of chromatographic grade from Merck, India. Buffer material and all other chemicals used were of high purity analytical reagents grade. High purity water was prepared using a Millipore Milli-Q plus purification system.

**HPLC instrumentation**

Chromatographic separation was performed using Waters Alliance HPLC system equipped with a 2695 separation module and 2996 photo diode array detector (Waters corporation, Milford, USA). Empower software build-2154 workstation was employed for data collection and processing. Chromatographic separation was performed using a Hypersil BDS C8 (250 mm × 4.6 mm, i.d. 5 µm particle size) column. The gradient mobile phase (A) consisting of a 0.015 M dipotassium hydrogen phosphate and 2 mL of triethyl amine adjusted at pH 6.0 with orthophosphoric acid and acetonitrile (990:10, v/v) was used. The mobile phase (B) consisting of 2 mL of orthophosphoric acid in 1000 mL of acetonitrile was used. The mobile phase (A) and (B) was delivered initially in the ratio of 100:0 (v/v) for 10 min then changed to 50:50 (v/v) for the next 30 min and finally equilibrated back to 100:0 (v/v) from 60 min to 70 min. The mobile phase was delivered at a flow rate of 0.6 mL min⁻¹, separation was performed at an ambient temperature and the detection was made at 205 nm. The injection volume was 20 µL.

**Preparation of standard solutions**

Both the working standards were prepared by standardization of bulk drug material against EP reference standard. 25 mg of CN and 400 mg of PR working standards were weighed into a 100 mL volumetric flask. Then 20 mL of methanol was added and sonicated for 5 min and made up to 100 mL with water. 5 mL of above solution was diluted to 100 mL with water. This gave a concentration of 12.5 µg mL⁻¹ of CN and 200 µg mL⁻¹ of PR.

**Sample preparation**

Powdered equivalent to 25 mg CN and 400 mg PR was weighed in a 100 mL volumetric flask, methanol (20 mL) was added and sonicated for 30 min, the solution was cooled and made up to the mark with water. 5 mL of the solution was diluted to 100 mL with water. The resulting solution was used as the sample solution for chromatographic analysis.

**Results and Discussion**

**Development and optimization of HPLC method**

Gradient method was employed to optimize the separation of PR and CN from their combined formulation. In the reversed phase LC, using a buffer in the range of 2.0 - 4.0, PR peak is observed on column void. To rectify this, several phosphate, acetate and citrate buffers have been tried and that is achieved by using dipotassium phosphate (pH 6.0) buffer. The PR peak is obtained at around 12 min. Further, addition of small quantity of triethyl amine improves peak sharpness and shape. A Hypersil BDS C8 (250 mm × 4.6 mm, i.d. 5 µm particle size) column is selected due to its high efficiency and suitability for polar molecules compared with commercially available C8 silanised silica gel packing material.

The flow rate of mobile phase through the column is kept at 0.6 mL min⁻¹ throughout the analysis. Detection is carried out using a PDA detector at 205 nm. Under the described experimental conditions the peaks are well defined and free from tailing. Specificity of the described method is determined by analyzing forcibly degraded powder samples. Forced degradation studies are performed to provide indication of the stability indicating property of the proposed method. Forced degradation is achieved by exposing both the formulation product and bulk material to stress condition of acid (0.5 N HCl), base (0.5 N NaOH), peroxide oxidation (3% H₂O₂), UV light (254 nm) and heat (80°C). For heat and light studies, study period is 2 days, whereas for the other studies it is 8 h. Figure 2 shows that most of the degradation products are well resolved from the active components and the proposed method displays satisfactory selectivity to CN and PR and their degradation products. The corresponding peak purity and degradation data are given in Table 1. The ability of the method to separate the drugs from their degradation products and the non-interference from the matrix indicates the good selectivity and specificity of the developed method.

**Method validation**

The nominal concentrations of standard and test solutions for CN and PR are 12.5 and 200 µg mL⁻¹ respectively. The system suitability results of five standard solution injections are shown in Table 2.
Fig. 2 — HPLC chromatograms of (a) unstressed sample (b) acid degradation (c) base degradation (d) peroxide oxidation (e) UV light and (f) heat. Peaks 1, 2 and 3 are for degraded components.

Table 1 — Results of forced degradation study

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Unstressed sample</th>
<th>UV light (254nm)</th>
<th>HCL (0.5N)</th>
<th>NaOH (0.5N)</th>
<th>H2O2 (3%)</th>
<th>Heat (80°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>For CN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purity angle</td>
<td>0.520</td>
<td>0.321</td>
<td>0.275</td>
<td>0.571</td>
<td>0.675</td>
<td>0.362</td>
</tr>
<tr>
<td>Purity threshold</td>
<td>0.701</td>
<td>0.652</td>
<td>0.527</td>
<td>0.812</td>
<td>0.852</td>
<td>0.452</td>
</tr>
<tr>
<td>% Assay</td>
<td>99.1</td>
<td>99.3</td>
<td>95.6</td>
<td>96.8</td>
<td>97.1</td>
<td>99.0</td>
</tr>
<tr>
<td>For PR</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Purity angle</td>
<td>0.321</td>
<td>0.357</td>
<td>0.389</td>
<td>0.352</td>
<td>0.327</td>
<td>0.318</td>
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<tr>
<td>Purity threshold</td>
<td>0.578</td>
<td>0.589</td>
<td>0.552</td>
<td>0.529</td>
<td>0.302</td>
<td>0.498</td>
</tr>
<tr>
<td>% Assay</td>
<td>99.6</td>
<td>99.4</td>
<td>97.5</td>
<td>97.2</td>
<td>96.7</td>
<td>99.2</td>
</tr>
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</table>

Table 2 — Results of system suitability study

<table>
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<tr>
<th>Parameter</th>
<th>CN</th>
<th>PR</th>
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</thead>
<tbody>
<tr>
<td>% RSD (n=5)</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>11.8</td>
<td>49.5</td>
</tr>
<tr>
<td>USP tailing factor</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td>USP theoretical plates</td>
<td>2300</td>
<td>3600</td>
</tr>
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</table>

The % RSD of both CN and PR are found to be less than 2, indicating the precise nature of the developed method. Linear function is determined by preparing standard solution at five different concentrations ranging from 6.25 μg mL\(^{-1}\) to 18.75 μg mL\(^{-1}\) for CN and from 100 μg mL\(^{-1}\) to 300 μg mL\(^{-1}\) for PR. The statistical parameters of the linear regression equation...
for both the components are presented in Table 3. The high correlation coefficients of the calibration curves (r>0.999) confirm good linearity. Known amounts of CN and PR samples are taken and spiked with known amounts of CN and PR working standards at three different levels (110, 120 and 130%) in triplicate. The percentage recoveries at each level (n = 3), and mean percentage recovery (n = 9) are determined and the results are given in Table 4. The mean recovery was found to be 99.7% and 99.9% for CN and PR respectively. A batch of capsules is analyzed by two different analysts on different days using different columns to establish the precision of the method and intermediate precision of the method (Table 5). The significant difference between method precision and intermediate precision mean values at 95% confidence level considering the T value of 1.812 at 10º of freedom are found to be 0.5881 for PR and 0.5911 for CN. The results indicate that there is no statistically significant difference. Thus, based on the peak purity results, obtained from the analysis of forced degraded samples using the described method, it can be concluded that the proposed method is specific for the estimation of CN and PR in presence of degradants. Also the method has linear response in the stated range and is accurate and precise. Hence, the described method can be used for the assay of CN and PR in their combination products.

Conclusion

Simple and convenient reversed phase HPLC method for the simultaneous determination of CN and PR in the combination formulation has been described. The result of peak purity study has proven their stability indicating nature and hence this method is found to be superior over the earlier published methods. The proposed method is linear, accurate and precise for the determination of CN and PR from capsule. Hence, it can be employed for routine quality control work containing these two drugs.

Acknowledgement

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References