Exploring Novel Isocratic HPLC Method for Quantitative Determination of Cinnarizine and Piracetam in Their Capsule Preparations

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Abstract
A novel isocratic HPLC method had been developed for rapid simultaneous separation and determination of cinnarizine and piracetam in pure form or in pharmaceutical formulations within less than 10 minutes. Separation was carried out on a Hypersil gold C_18 (10μm, 100x4.6mm) column. Effect of pH and composition of mobile phase in addition to flow rate was studied. Calibration was obeyed in the range of (10-80) μg/ml for cinnarizine or (160-960) μg/ml for Piracetam. The method was applied for the simultaneous determination of these drugs in both bulk and pharmaceutical forms and this is the first published method that uses isocratic HPLC method for cinnarizine and piracetam determination in combined formulation and the method were validated according to ICH parameters.

Keywords: Cinnarizine; Piracetam; CinaretamR; HPLC

Introduction
Cinnarizine is antihistaminic, ca-channel blocker and sedative drug [1]. It is described for the treatment of motion sickness, nausea and vertigo. The main problem with cinnarizine administration is drowsiness [2]. British pharmacopoeia states a non-aqueous titration method for cinnarizine assay. Cinnarizine have been determined by Several other reported methods, including spectrophotometric methods [3-7] and high-performance liquid chromatography (HPLC) [8-10].

Piracetam is a nootropic and psychopharmacological drug [11]. It is a synthetic cyclic derivative of GABA. It is described as a myoclonus and neuroprotective agent. The mechanism of action depends on eliminating of calcium chloride those results in a decrease of the rhythm and an increase of the contraction amplitude [12]. Manufacturing occur by condensing ethyl chloroacetate with 2-pyrrolidinone in the presence of a metal hydride and converting the ester into an amide with ammonia [13]. British pharmacopoeia states a chromatographic method for piracetam determination. Several methods have been developed for piracetam determination, including spectrophotometric methods [14-17], high-performance liquid chromatography (HPLC) [18-22] and electrochemical method [23].

Review of literature revealed that only limited reports have been published on the chromatographic assay of cinnarizine and piracetam in the combined formulation [24,25]. However, there is no report on the isocratic RP-HPLC method for assay these two drugs. The main objective of the present work is to describe a specific, linear, accurate, precise and a new reversed phase HPLC method using isocratic elution for the simultaneous determination of cinnarizine and piracetam in the combined formulation. The optimized mobile phase was determined as a mixture of acetonitrile:water (50:50 v/v), detection at 229 nm, pH 2.7 adjusted by orthophosphoric acid and a flow rate of 0.5 ml/min. Under these conditions, piracetam and cinnarizine eluted at 3.8, and 8.1 minutes respectively (Figures 1 and 2).

Experimental
Instrumentation
The HPLC system used was a computer based Agilent 1200 series instrument comprising of a quaternary pump, a UV detector (Thermo. Scientific Co. USA) and Auto sampler (injector). The system equipped by Agilent chemistation PC program.

Materials and reagents
All reagents used were of analytical grade or HPLC grade. Orthophosphoric acid was supplied by (Germany), Acetonitrile HPLC grade and Water. Cinnarizine and piracetam standard working powders were kindly supplied by Egyptian sigma company (Egypt), and were used without further purification.

Figure 1: Cinnarizine structure IUPAC name: (E)-(Diphenylmethyl) -4-(3-phenylprop-2-enyl)piperazine.

Figure 2: Piracetam structure IUPAC name: 2-(2-oxopyrrolidin-1-yl) acetamide.

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Chromatographic procedure

20 μl from the sample solutions of the drugs were taken and detected at lambda 229 nm. Liquid chromatography was performed on a Hypersil gold C18 (10μm, 100x4.6mm) column. The used mobile phase was (acetonitrile: water 50:50 v/v) and pH at 2.7 using orthophosphoric acid. Mobile phase pumped at a flow rate equals to 0.5 ml/min at ambient temperature. Before all, the mobile phase was filtered by a 0.45 μl Nylon membrane filter (USA,MA) under vacuum and degassed by ultra-sonication (USA, Vernon Hills).

Pharmaceutical preparation

Cinaretam capsules labeled to contain in each capsule 25 mg cinnarizine and 400 mg of piracetam and batch No. 805072.

Preparation of stock standard solutions

25 and 800 mg of cinnarizine and piracetam respectively weighted and dissolved in the least amount of methanol in 10 ml volumetric flask. Sonicating for 5 m and then completing the final volume with mobile phase. The resulted concentrations of solutions were of 5, 80 mg/ml of cinnarizine and piracetam respectively.

The solutions of calibration plot (working standard solutions)

To construct calibration plots, the working standard solutions have been prepared by dilution the stock standard solutions with mobile phase and preparing working solutions with concentration ranges (10-80 and 160-960 μg/ml) for cinnarizine and piracetam respectively. Each solution (n=5) was injected in triplicate and chromatographed under the mentioned conditions above. Linear relationships were obtained when average drug (n=5) was injected in triplicate and chromatographed under the mentioned conditions above. Linear relationships were obtained when average drug concentrations of solutions were plotted against the corresponding concentrations and its toxicological impact on healthy Sprague Dawley Rats. J App Pharm 8: 225. doi: 10.21065/1920-4159.1000225

The developed methods were validated according to international conference on harmonization guidelines [26]. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include degradants, impurities, matrix, etc. A Bulk of Cinaretam capsules (solution contains excipients only) had been prepared by mixing its excipients like StarCap 1500, silicon dioxide, and calcium phosphate di or tri basic disodium edetate. Known concentration of studied excipients was added to the bulk

Results and Discussion

Optimization of Chromatographic Conditions: All chromatographic conditions were illustrated in Table 1.

The chromatographic detection was performed at 229 nm after screening of absorption at UV spectrum using Surveyor photodiode array detector (PAD) (Thermo Scientific Co. USA) where we found that 229 is the best one that give more sensitivity to the method and also linearity and validation to the method. Detection at 190 nm was more sensitive but it was not valid as it did not give linear relationship between the concentration and the peak area. The method was performed on a Hypersil gold C18 (10μm, 100x4.6mm) column (Thermo Scientific Co. USA). Chromatographic conditions were optimized by changing the mobile phase composition, pH of the mobile phase, the flow rate and also studying the detection at different wave length. Different experiments were performed to optimize the mobile phase but adequate separation of drugs could be achieved by altering the composition of mobile phase from (methanol 60: water 40 v/v) [where both drugs appear at the same retention time] to (acetonitrile 60: water 40: v/v) [where cinnarizine peak is too broad and piracetam peak appear with tailing] at pH 2.7 by orthophosphoric acid, flow rate at 0.5 ml/min and detection at 229 nm as shown in Figure 3.

The optimized mobile phase was determined as a mixture of acetonitrile:water (50:50, v/v) at a flow rate of 0.5 ml/min, pH 2.7 using orthophosphoric acid and detection at 229 nm as shown in Figure 4.

Method Validation

The developed methods were validated according to international conference on harmonization guidelines [26]. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include degradants, impurities, matrix, etc. A Bulk of Cinaretam capsules (solution contains excipients only) had been prepared by mixing its excipients like StarCap 1500, silicon dioxide, and calcium phosphate di or tri basic disodium edetate. Known concentration of studied excipients was added to the bulk

**Figure 3:** HPLC Chromatograms of authentic mixture (800 μg. ml⁻¹ piracetam and 60 μg ml⁻¹ of cinnarizine at different Mobile phase (A, B) at 229 nm, pH 2.7 and flow rate 0.5 ml/min.

(A) (acetonitrile 60: water 39: orthophosphoric acid 1 v/v) [where cinnarizine peak is too broad and piracetam peak appear with tailing].

(B) (methanol 60: water 39: orthophosphoric acid 1 v/v) [where both drugs appear at the same retention time].
then was injected under the illustrated chromatographic conditions. Recovery results showed that the bulk has negligible effect Which Means That the bulk did not interfere with developed method.

**Linearity and range**

When the results of analytical procedure are directly proportional to injected concentration (in certain range) of analyte this mean that the ability of the method to give linearity in the specific range. For the conformation of linearity, a minimum of 5 concentrations is recommended [27]. Five Concentrations were chosen in the ranges (10-80 and 160-960 μg/ml) for corresponding levels of 50-150% w/w of the nominal analytical concentration of cinnarizine and piracetam respectively. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis (Figures 5 and 6).

**Limits of detection and Limits of quantitation**

According to the ICH recommendations, determination of limits of detection and quantitation was based on the standard deviation of the y-intercepts of regression lines (n=3) and the slope of the calibration plots [26] as in Table 2.

**Precision**

The precision of the assay was investigated by measurement of both repeatability and Intermediate precision.

**Repeatability:** Repeatability was investigated by injecting a minimum of 6 determinations at 100% of the test concentration and percentage SD were calculated in Table 3.

**Intermediate precision:** In the inter-day studies, standard and sample solutions prepared as described above, were analyzed in triplicate on three consecutive days at 100% of the test concentration and percentage SD were calculated table 3.

**Accuracy**

Accuracy was assessed using 9 determinations over 3 concentration levels covering the specified range (80,100 and 120%). Accuracy was reported as percent recovery by the assay of known added amount of analyte in the sample Table 3.

**Robustness**

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small variations in method parameters and provides an indication of its reliability during normal usage [26]. Robustness was tested by studying the effect of changing mobile phase pH by ± 0.5, the percentage of organic solvent (acetonitrile) in the mobile phase by ± 5 %, temperature ± 5 °C, wavelengths ± 5 nm and flow rate ± 0.1 ml/min had no significant effect on the chromatographic resolution of the method.

**Stability of analytical solution**

As a part of confirmation of robustness, the stability of standard stock solutions was evaluated. Analysis of the standard stock solutions after preparation 1, 2 and 3 days at room temperature and notification the change in peak area response over 3 days which was (0.56 and 1.09% for cinnarizine and piracetam respectively. Their solutions were found to be stable for 3 days at room temperature at least.

**Applications on Pharmaceutical Preparation**

The Pharmaceutical formulation containing stated drugs have been successfully analyzed by the proposed method. Results obtained were compared to those obtained by applying reported reference methods [27,28] for cinnarizine and piracetam respectively where Student’s t-test and F-test were performed for comparison. Results are shown in table 4 where the calculated t and F values were less than

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**Table 1:** Chromatographic Conditions for the proposed method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Hypersil gold C18 (10um, 100x4.6mm) column</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Isocratic binary mobile phase of acetonitrile:water (50:50, v/v), filtered</td>
</tr>
<tr>
<td>Wave length nm</td>
<td>229</td>
</tr>
<tr>
<td>Flow rate, ml/min</td>
<td>0.5</td>
</tr>
<tr>
<td>Injected volume, µl</td>
<td>10</td>
</tr>
<tr>
<td>Pressure, psig</td>
<td>11</td>
</tr>
<tr>
<td>Temperature</td>
<td>Ambient (25 ± 5°C)</td>
</tr>
<tr>
<td>pH</td>
<td>2.7 adjusted by orthophosphoric acid</td>
</tr>
</tbody>
</table>

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**Figure 4:** Typical HPLC chromatograms obtained from 20 µl injections of piracetam at (3.888 min) and cinnarizine at (8.103 m) respectively under optimized chromatographic conditions.

**Figure 5:** The linear regression equations of piracetam was Y = 15328 X–2000000, and regression coefficient (r²)=0.9999, Where Y is the peak area of standard solution and X is the drug concentration.

**Figure 6:** The linear regression equations of cinnarizine was Y=651557 X–2000000, and regression coefficient (r²)=0.9999, Where Y is the peak area of standard solution and X is the drug concentration.
again shows the strength of the method. Very sensitive, rapid, accurate, selective, and reproducible. The method validated. Linearity was observed over a concentration range of 10 to 80 µg ml−1 for cinnarizine and in the range of 160 to 960 µg. ml−1 for piracetam. The results obtained indicate that the proposed method is reported method (T-student test) and (F-test for variance) by reported method (T-student test) and (F-test for variance) tabulated values for the both drugs which in turn indicate that there is no significant difference between proposed method and reference methods relative to accuracy and precision.

Table 2: Results of the analysis for the proposed method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cinnarizine</th>
<th>Piracetam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>100.4</td>
<td>100.16</td>
</tr>
<tr>
<td>± SD</td>
<td>0.88</td>
<td>0.78</td>
</tr>
<tr>
<td>± RSD</td>
<td>0.87</td>
<td>0.78</td>
</tr>
<tr>
<td>± SE</td>
<td>0.39</td>
<td>0.35</td>
</tr>
<tr>
<td>Variance</td>
<td>0.77</td>
<td>0.62</td>
</tr>
<tr>
<td>Slope</td>
<td>651557</td>
<td>15328</td>
</tr>
<tr>
<td>L.D.</td>
<td>1.04</td>
<td>16</td>
</tr>
<tr>
<td>L.Q.</td>
<td>3.4</td>
<td>48</td>
</tr>
</tbody>
</table>

Table 2: Results of the analysis for the proposed method.

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Cinnarizine</th>
<th>Piracetam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AV ± SD mg/ ml</td>
<td>AV ± SD %</td>
</tr>
<tr>
<td>Repeatability</td>
<td>24.96 ± 0.01</td>
<td>99.86 ± 0.11</td>
</tr>
<tr>
<td>Intermediate precision</td>
<td>24.23 ± 0.03</td>
<td>99.97 ± 0.08</td>
</tr>
<tr>
<td>Accuracy &amp; Recovery%</td>
<td>80%</td>
<td>19.96 ± 0.04</td>
</tr>
<tr>
<td>100%</td>
<td>25.11 ± 0.02</td>
<td>100.18 ± 0.98</td>
</tr>
<tr>
<td>120%</td>
<td>30.51 ± 0.06</td>
<td>99.7 ± 1.30</td>
</tr>
</tbody>
</table>

Table 3: Repeatability and Intermediate precision and Accuracy (Recovery %) of cinnarizine and piracetam respectively.

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Recovery ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proposed method</td>
</tr>
<tr>
<td>Cinnarizine [27]</td>
<td>99.74 ± 0.78</td>
</tr>
<tr>
<td>Piracetam [28]</td>
<td>101.34 ± 0.79</td>
</tr>
</tbody>
</table>

Table 4: Statistical comparison of the proposed and published methods for determination of Cinnarizine and piracetam in the form of Cinaretam® capsules by reported method (T-student test) and (F-test for variance)

Conclusion

A novel RP-HPLC method for rapid simultaneous estimation of cinnarizine and piracetam within less than 10 minutes was developed and validated. Linearity was observed over a concentration range of 10 to 80 µg ml−1 for cinnarizine and in the range of 160 to 960 µg. ml−1 for piracetam. The results obtained indicate that the proposed method is very sensitive, rapid, accurate, selective, and reproducible. The method has been successfully applied for the analysis of marketed formulations. It can be used for the routine analysis of formulations containing any one of the above drugs or their combinations without any alteration in the assay. The most we can talk about, HPLC method is also being used in other drug formulation recently such as nanomedicine [29] which again shows the strength of the method.

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References


