Reverse Phase Ultrafast Liquid Chromatography Method for Simultaneous Estimation of Citicoline Sodium and Piracetam in Tablets

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ABSTRACT
A simple, precise and accurate RP-UFLC method was developed for simultaneous determination of citicoline sodium and piracetam in tablets. Separation was carried on an Enable C18G column (250 mm × 4.6 mm i.d., 5 μm) using methanol: water (10 mM TEA) (75:25, v/v) as mobile phase at flow rate of 1 ml/min in isocratic mode. The PDA detection wavelength was 225 nm. The retention time of citicoline sodium and piracetam were 1.985 and 3.007 min, respectively. The method was validated for linearity, specificity, precision, accuracy and robustness as per ICH guidelines. The method was linear in the concentration range of 1.0 –250 µg/ml with correlation coefficients of 0.999 for both the drugs. The LOD and LOQ for citicoline sodium were 0.25 µg/ml and 0.81 µg/ml, respectively, and for piracetam 0.28 µg/ml and 0.93 µg/ml, respectively. The average recoveries for recovery study were found to be in the range of 100.24-101.09% and 100.08-101.05% for citicoline sodium and piracetam, respectively. The R.S.D. values for intraday, interday and system precision were found to be less than 2%. The method was applied successfully for simultaneous estimation of citicoline sodium and piracetam in combined tablet formulation.

KEYWORDS: Citicoline; piracetam; reverse phase-ultra fast liquid chromatography; triethylamine

Introduction
Citicoline sodium (CTS) is used in treatment of mild head injury (Aniruddha et al., 2009), glaucoma (Parisi et al., 1999) and cognitive decline (Garcia-Cobos et al., 2010). Chemically, it is cytidine-5’-{sodium P’-[2- (trimethylamino)-ethyl] hydrogen diphosphate}, inner salt (Sweetman, 2009). Piracetam (PRM) is a nootropic agent (Gouliaev and Senning, 1994) used in treatment of epilepsy (Chaudhry et al., 1992), dyslexia (Helfgott et al., 1986) and cognitive impairment (Flicker and Grimley, 2001). Chemically, it is 2-(2-oxopyrroolidin-1-yl) acetamide (Sweetman, 2009). Many methods have been reported so far for determination of both the drugs by various analytical techniques. The reported methods for CTS includes several spectrophotometric (Malipatil et al., 2010; Surani et al., 2010; Sachan et al., 2011) and HPLC (Zhao-Hua et al., 2003; Ganduri et al., 2010; Raveendra Babu et al., 2010; Chen et al., 2011; Patil et al., 2011; Bindaiya et al., 2011; Uttawar et al., 2011). Several reported methods for PRM includes spectrophotometric (Metwally et al., 2005; El-Saharty, 2008; Bhomick et al., 2010), FTIR (Karamancheva et al., 2000), HPLC (Metwally et al., 2005; Lestari at al., 2005; Cuticapean and Imre, 2007; El-Saharty, 2008; Arayane et al., 2010) and LC-MS/MS (Wang et al., 2010).

The combined tablet formulations containing CTS and PRM are used in the management of cognitive disorders. Only one spectrophotometric (Prajapati et al., 2012) and one HPLC (Babu et al., 2011) methods are reported till the date for simultaneous estimation of CTS and PRM in pharmaceutical dosage form. However, the reported liquid chromatographic method is having one or the other drawback like lack of higher sensitivity, use of complex reagents and narrow linear range. In this study, we made an attempt to develop a simple, accurate and sensitive RP-UFLC method for simultaneous estimation of CTS and PRM in combination tablet dosage form.

Materials and Methods

Chemicals and Reagents
All the chemicals and reagents used were of analytical reagent grade. The standard drugs CTS and PRM (purity >99 %) were received from Intas Pharmaceuticals Ltd., India. HPLC grade methanol and triethylamine were procured from Merck Ltd., Mumbai, India. The water for HPLC was obtained by using TKA Water Purification System, Germany. The combined tablet formulation (Somazina Plus® Tablets, Elder Pharmaceuticals Ltd., India) containing 500 mg of CTS and 800mg of PRM was purchased from the local market.
Instrumentation and Chromatographic conditions

Quantitative UFLC was performed on a binary gradient UFLC with two Shimadzu LC-20AD pumps, with a 20μl sample injection loop (manual) and SPD M20A PDA detector. The output signal was monitored and integrated using Shimadzu LC Solution Software. An Enable C18G, (250 mm × 4.6 mm i.d., particle size 5 μm) was used for separation. Chromatographic analysis was carried out at the ambient temperatures on the column using the methanol: water (10mM TEA) (75:25, v/v) as mobile phase at a flow rate of 1.0 ml/min in isocratic mode. The mobile phase required no special preparation technique except filtration using 0.45μm filter paper. Afterwards, both the methanol and water were ultrasonicated (Enertech, India) up to 20 min for degassing prior to use. The PDA detection was carried out at 225 nm. Analytical balance, Model-GR-202 (AND Instrument India Pvt. Ltd., Gurgaon, India) of sensitivity 0.1 mg was used to weigh the chemicals and reagents.

Preparation of Standard Solutions

Standard stock solutions for both the drugs were prepared separately by dissolving 25 mg of the drugs in mobile phase up to 25 ml. The volumetric flasks having 10 ml of mobile phase along with the drugs were ultrasonicated for 5 min. Finally, the volumes were made up to the 25 ml mark, which gave 1000 μg/ml solutions. From this a mixed standard stock solution was so prepared that the drugs CTS and PRM were in the ratio (1:1). Working standard solutions of CTS and PRM were prepared in the concentration ratio of 1:1.

Method Validation

The developed method was validated statistically as per the requirements of ICH for linearity, accuracy, precision, specificity, LOD, LOQ and robustness.

Results and Discussion

Optimization of the Chromatographic conditions

Optimization of the mobile phase was carried out basing on resolution, tailing factor and number of theoretical plates obtained for CTS and PRM. During the trial runs, both the drugs were tested with different mobile phase compositions like acetonitrile: methanol, acetonitrile: 0.01M tetra butyl ammonium hydrogen sulfate, methanol: water (10 mM TEA) and methanol: 0.01M tetra butyl ammonium hydrogen sulfate at various compositions (50:50, 60:40, 70:30, 75:25, v/v) and flow rates (0.8, 1.0 and 1.2 ml/min) for selection. The mobile phase consisting of methanol: water (10mM TEA) (75:25, v/v) at a flow rate of 1.0 ml/min was selected, which gave sharp, symmetric, well-resolved peaks for CTS and PRM. The retention times for standard CTS and PRM were 1.963 and 1.297 minutes, respectively. The tailing factor for CTS and PRM were 1.485 and 1.366, respectively. Response was measured at 225 nm using a PDA detector. The separation was carried out at room temperature. Fig. 2-5 represents the typical chromatograms of CTS, PRM, standard solution containing a combination of CTS and PRM, and a sample solution containing a combination of CTS and PRM, respectively. The present method is found to be superior from the reported LC method in literature for simultaneous estimation of CTS and PRM (Table 1).

Fig. 1 Chemical structure of (A) CTS and (B) PRM.

Fig. 2. Typical chromatogram of CTS.
Fig. 3. Typical chromatogram of PRM.

Fig. 4. Typical chromatogram of CTS and PRM in standard solution.

Fig. 5. Typical chromatogram of CTS and PRM in combined tablet formulation.
TABLE 1
Comparison of performance characteristics.

<table>
<thead>
<tr>
<th>Method</th>
<th>Linearity (µg/ml)</th>
<th>No. of Theoretical Plates</th>
<th>LOD (µg/ml)</th>
<th>LOQ (µg/ml)</th>
<th>Recovery(%) from Marketed Formulation</th>
<th>Mobile Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTS PRM</td>
<td>CTS PRM</td>
<td>CTS PRM</td>
<td>CTS PRM</td>
<td>CTS PRM</td>
<td></td>
</tr>
<tr>
<td>1.Reported (HPLC)</td>
<td>10-60</td>
<td>20-120</td>
<td>2179</td>
<td>5518</td>
<td>1.131</td>
<td>1.88</td>
</tr>
<tr>
<td>2.Current (UFLC)</td>
<td>1-250</td>
<td>1-250</td>
<td>2862</td>
<td>6325</td>
<td>0.25</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Remarks for the current method
- Wider linearity range
- Higher number of theoretical plates indicating better separation
- Higher sensitivity
- Higher percentage of recovery
- Simple to prepare and cost effective mobile phase, do not require additional reagent for pH maintenance

**Linearity**

Mixed standard solutions of CTS and PRM (1:1) were injected into the UFLC and peak areas for each drug were calculated. Two calibration curves were plotted for peak area Vs concentration of both the drugs. Both the calibration curves (10 point) were found to be linear over a concentration range of 1.0-250 µg/ml for CTS and PRM, respectively. The linear regression equations were \( y = 17185x - 11134 \) and \( y = 9900x - 29789 \) for CTS and PRM, respectively and \( r = 0.999 \) for both the curves.

**Precision**

The precision (intra-day, inter-day and system) of the method was ascertained separately from the response obtained by actual determination of six replicates of a fixed amount of drug. The percent RSD values for precision were calculated. The method was found to be precise as the result for % RSD values (Table 2) was well below 2%.

**Accuracy**

To check the accuracy of the proposed method, recovery studies were carried out in 80, 100 and 120% of the test concentration. The recovery study was performed three times at each level. The amounts of both the drugs present in the sample were calculated using the calibration curve. Accuracy of the method was determined by recoveries of CTS and PRM by standard addition methods. The values show high levels of accuracy of the method. The result of accuracy study is shown in Table 3.

### TABLE 2
Intraday, interday and system precision studies (n=6).

<table>
<thead>
<tr>
<th>Type of Study</th>
<th>Concentration Taken (µg/ml)</th>
<th>Peak Area±SD, RSD (%)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>CTS PRM</td>
<td>CTS PRM</td>
</tr>
<tr>
<td>Intraday precision</td>
<td>100 100</td>
<td>1780384 ± 6472.14,0.36</td>
</tr>
<tr>
<td>Interday precision</td>
<td>100 100</td>
<td>1788432 ± 7190.52,0.40</td>
</tr>
<tr>
<td>System precision</td>
<td>100 100</td>
<td>1743143 ± 5142.7,0.30</td>
</tr>
</tbody>
</table>

*average of six determinations

### TABLE 3
Accuracy of the method.

<table>
<thead>
<tr>
<th>Different concentration level comparing to sample concentration (%)</th>
<th>Amount Added Pure Drug (µg/ml)</th>
<th>Recovery(%) Pure Drug ± SD, RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTS PRM</td>
<td>CTS PRM</td>
</tr>
<tr>
<td>80</td>
<td>40</td>
<td>64</td>
</tr>
<tr>
<td>100</td>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td>120</td>
<td>60</td>
<td>96</td>
</tr>
</tbody>
</table>

*average of three determinations at each level
TABLE 4
System suitability results for robustness study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Retention Time, min.</th>
<th>Resolution</th>
<th>Tailing Factor</th>
<th>Theoretical Plates</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CTS PRM</td>
<td>CTS PRM</td>
<td>CTS PRM</td>
<td>CTS PRM</td>
</tr>
<tr>
<td>Flow rate (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>2.220 3.328</td>
<td>6.685</td>
<td>1.350 1.320</td>
<td>2927 6143</td>
</tr>
<tr>
<td>1.0</td>
<td>1.985 3.007</td>
<td>6.912</td>
<td>1.374 1.229</td>
<td>2862 6325</td>
</tr>
<tr>
<td>1.1</td>
<td>1.831 2.741</td>
<td>6.174</td>
<td>1.394 1.278</td>
<td>2548 6363</td>
</tr>
<tr>
<td>Methanol (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>1.995 3.002</td>
<td>6.577</td>
<td>1.362 1.306</td>
<td>2738 6188</td>
</tr>
<tr>
<td>75</td>
<td>1.985 3.007</td>
<td>6.912</td>
<td>1.374 1.229</td>
<td>2862 6325</td>
</tr>
<tr>
<td>77</td>
<td>1.995 3.000</td>
<td>6.813</td>
<td>1.338 1.320</td>
<td>2732 6900</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>220</td>
<td>1.985 3.007</td>
<td>6.421</td>
<td>1.380 1.301</td>
<td>2418 6629</td>
</tr>
<tr>
<td>225</td>
<td>1.985 3.007</td>
<td>6.912</td>
<td>1.374 1.229</td>
<td>2862 6325</td>
</tr>
<tr>
<td>230</td>
<td>1.985 3.007</td>
<td>6.125</td>
<td>1.380 1.300</td>
<td>2411 6557</td>
</tr>
<tr>
<td>Initial time</td>
<td>Final time</td>
<td>Recovery (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution</td>
<td>stability</td>
<td>CTS PRM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 hr</td>
<td>24 h</td>
<td>99.31</td>
<td>99.83</td>
<td></td>
</tr>
</tbody>
</table>

Robustness

The robustness of the method was studied by very deliberately changing the flow rate of the mobile phase, detection wavelength and composition of organic phase. A series of system suitability tests like retention time, resolution, theoretical plates and tailing factor were calculated to evaluate the robustness as per ICH requirements (Guideline on Validation of Analytical Procedures: Text and Methodology Q2-R1, 2005). Stability of the drug in the used mobile phase was determined by carrying out bench top stability of the drug solution by determining the amount of drugs recovered after 24h of the bench-top stand time. The method was found to be robust in accordance with deliberate changes in the mobile phase flow rate (±0.1ml/min), composition of organic phase (±2%) and detection wavelength (±5nm). The study result confirms that CTS and PRM solutions were stable for 24 h at ambient conditions without any significant degradation of the analyte. The results are shown in Table 4.

Limit of detection and Limit of quantitation

The limit of detection and limit of quantitation were separately determined based on the Signal to Noise ratio. For limit of detection, the S/N ratio was taken as 3:1. For limit of quantitation, the S/N ratio was taken as 10:1. The limit of detection values for CTS and PRM were 0.25µg/ml and 0.28µg/ml, respectively. The limit of quantification values for CTS and PRM were 0.81µg/ml and 0.93µg/ml, respectively.

Specificity

Specificity was determined by checking the possible interference from the placebo. The commonly used excipients were added into the standard solutions, and the interference was monitored. No interference was noticed due to the presence of excipients, which indicates that the method is specific.

Application to combined tablet dosage form

The developed method was applied for determination of CTS and PRM in their combined tablet dosage form. Twenty tablets were weighed and powdered finely. A quantity of tablet powder equivalent to 50 mg of CTS and 80mg PRM was accurately weighed and transferred into a 50 ml volumetric flask, containing 20 ml of mobile phase and ultrasonicated for 20 min; the volume was made up to the mark and mixed well. The solution was filtered through a 0.2µm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the mobile phase for analysis as already described. The amount of drugs present in the sample solution was calculated by using the calibration curves. The higher percentage of recovery and non interference of the formulation excipients in retention time of the drugs show the selectivity of the method for estimation of both drugs in their combined tablet dosage form. The result of the assay (n=3) for both the drug yielded 102.32% (SD=±0.130) and 102.12% (SD=±0.138) for CTS and PRM, respectively from the tablet dosage form.

Conclusion

A validated RP-UFLC method has been developed for determination of CTS and PRM in their combined tablet dosage form. The retention times of 1.985 min and 3.007 min for CTS and PRM in tablet dosage form demonstrates a unique advantage for their rapid analysis by RP-UFLC. The results obtained by validation study of the drugs shows that the method is simple, accurate, precise, specific, sensitive and robust. The method was successfully applied for the determination of both the drugs in combined tablet dosage form. Further this method may be applied for routine analysis of both the drugs in API, formulations, dissolution medium, and biological fluids.
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References


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