RP-UFLC Method for Estimation of Propafenone in Tablets

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ABSTRACT

A simple, precise and accurate RP-UFLC method was developed for determination of propafenone hydrochloride. Separation was carried on an Enable C18G column (250 mm × 4.6 mm i.d., 5 μm) using methanol: 10 mM TBAHS (95:05, v/v) as mobile phase at flow rate of 1.0 ml/min in isocratic mode. The PDA detection wavelength was 247 nm. The retention time of propafenone was 2.692 min. The method was validated for linearity, specificity, precision, accuracy and robustness as per ICH. The method was linear in the concentration range of 10 – 250 μg/ml with correlation coefficients of 0.999. The LOD and LOQ were 4.5 μg/ml and 9.75 μg/ml, respectively. Average recoveries for recovery study were found to be in the range of 99.73-100.58. R.S.D. values for intraday, interday and system precision were found to be less than 2%. Specificity was established after forced degradation was performed by using HCl, NaOH, H2O2, thermal and UV radiation. The method was applied successfully for estimation propafenone in tablet formulation.

KEYWORDS: Propafenone; Reverse phase; Liquid chromatography; Forced degradation; Tablets.

Introduction

Propafenone hydrochloride (PFH) is a class Ic antiarrrhythmic cardiovascular drug with negative inotropic and beta-adrenoreceptor blocker action (Sweetmann, 2009; Cappuci and Boriani, 1995; Harron and Brogden, 1987). It is indicated for supraventricular and ventricular arrhythmias (Reimold et al., 1998). Chemically, propafenone is 2’-(2-hydroxy-3-propylamino-propoxy)-3-phenylpropiophenone hydrochloride (Fig. 1).

Fig. 1. Chemical structure of Propafenone hydrochloride

The various reported analytical methods for determination of propafenone includes conductometric (Ayad et al., 2012), UV spectrophotometry (Dhandapani et al., 2010; Ayad et al., 2013; Ayad et al., 2013), NMR spectroscopy (Holzer, 2001), flow injection analysis (Hu et al., 2009), TLC (Huang et al., 1997), HPTLC (Jadhav and Tambe, 2013), capillary electrophoresis (Chankvetadze et al., 2001; Afshar and Thorman, 2009), HPLC (Brode et al., 1988; Kunicki et al., 1992; Kern, 1994; Bohm et al., 1995; de Abreu et al., 1999; Bonato et al., 2000; Wu et al., 2004; Afshar and Roumi, 2004; Flore-Perez et al., 2005; Lamprecht and Stoschitzky, 2009), GC-MS (Koppel et al., 1991; Leoux and Maes, 1991), GC-ECD (Chan et al., 1987) and LC-MS methods (Tan et al., 1998; Zhong and Chen, 1999; Hofmann et al., 2000; Buszewski et al., 2009; Sorensen, 2012). Ultra-fast liquid chromatography (UFLC) is a time efficient and cost-effective analytical tool then compared to conventional HPLC to estimate drugs in dosage forms, hallucinogenic agents in drug products, drug in micro dialysis samples, and drugs in skin diffusate samples (Bandarkar and Khattab, 2010; Min et al., 2008; Sun et al., 2010; Gannu et al., 2010). No reverse phase ultrafast liquid chromatographic (RP-UFLC) method using mobile phase of methanol: 10mM TBAHS (95:05, v/v) is reported for determination of propafenone in pharmaceutical dosage form. In this study, we made an attempt to develop and validate a simple, fast, precise, specific and accurate RP-UFLC method for determination of propafenone in tablet dosage form.

Materials and Methods

Chemicals and reagents

All the chemicals used were of analytical reagent grade. HPLC grade methanol was purchased from Merck Ltd., Mumbai, India. Sodium hydroxide, hydrochloric acid and hydrogen peroxide were procured from S.D. Fine Chem. Ltd., Mumbai, India. The water for HPLC was obtained by using TKA Water Purification System, Germany. Analytical Grade PFH was kindly supplied by Glenmark Generics Ltd, India. The purity of PFH was...
evaluated by obtaining the melting point. Tetra butyl ammonium hydrogen sulfate (TBAHS) was purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. The tablet formulation containing 150 mg of PFH 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 20 µl 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 x 4.6 mm i.d., particle size 5 µm) was used for separation. Chromatographic analysis was carried out at ambient temperature on the column using the methanol: 10 mM TBAHS (95:05, v/v) as mobile phase at a flow rate of 1.0 ml/min in isocratic mode. The 10 mM TBAHS solution was prepared by accurately weighing 3.3954 gms of TBAHS salt and dissolving it in 1000 ml of HPLC grade water. Afterwards both the methanol and TBAHS were ultrasonicated (Enertech, India) up to 20 min for degassing before use. Methanol and TBAHS solution were filtered using 0.45 µ filter paper. The PDA detection was carried out at 247 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 solution of PFH was prepared by dissolving 10 mg of the drug in 5 ml of mobile phase and was ultra-sonicated for 5 min. Finally, the volume was made up to 10 ml with mobile phase, which gave 1000 µg/ml solution.

**Method Validation**

The developed method was validated statistically for specificity, linearity, precision, accuracy, LOD, LOQ and robustness as per the ICH Q2 (R1) guideline.

**Results and Discussion**

**Optimization of the chromatographic conditions**

Optimization of mobile phase was carried out based on system suitability results obtained for PFH. During the trial runs different mobile phase compositions like methanol: water, methanol: 10 mM TBAHS, at various ratios (50:50, 60:40, 70:30, 90:10, 95:05; v/v) and flow rates (0.9, 1.0 and 1.1 ml/min) were tested for selection. The mobile phase consisting of methanol: 10 mM TBAHS (95:05, v/v) at a flow rate of 1.0 ml/min was selected which gave sharp and symmetric peak for PFH. Retention time for PFH was 2.692 min. The run time was set at 5 min. Response was measured at 247 nm using PDA detection. The separation was carried out at room temperature. Fig. 2 and Fig. 3 represent the typical chromatograms of PFH in standard solution and sample solution, respectively.

**Specificity**

Specificity of the method was determined by checking any interference because of possible degradation products produced during stress studies. The degradation samples were prepared by taking suitable aliquots of the drug solution, and then subjecting each of these aliquots to different stress conditions. After the fixed time period the treated drug solutions were diluted with mobile phase. For every stress condition a solution of concentration 100 µg/ml of PFH was prepared.
Acidic degradation: Acidic degradation was carried out by adding 1 ml of 0.001M HCl, and after 15 min neutralizing the mixture by adding 0.001M NaOH.

Alkaline degradation: Alkaline degradation was carried out by adding 1 ml of 0.001M NaOH, and after 15 min neutralizing the mixture by adding 0.001M HCl.

Peroxide oxidation: Oxidative degradation was performed by exposing the drug solution to 1 ml of 3% (v/v) H2O2 for 45 min.

Thermal degradation: Thermal degradation was performed by heating the drug solution at 50°C on a thermostatically controlled water bath for 45 min.

Photolytic degradation: Photolytic degradation was achieved by exposing the drug solution to UV radiation (365 nm) inside an UV chamber for 45 min.

PFH shows complete degradation in the applied acidic (0.1M HCl) and alkaline (0.1M NaOH) conditions. So the applied acid and alkaline stress conditions were optimized to get a mild to moderate degradation of the drug. The changed acid and alkaline stress was applied by using a 0.001M solution of HCl and NaOH with a reduced exposure time of 15 min. PFH shows degradation in the final applied stress conditions in the order alkaline > photolysis > thermal > oxidation > acid stress condition. Fig. 4-7 represents the typical chromatograms of untreated drug, peroxide oxidation, thermal and photolysis degraded drug. The run time for each stressed drug solution was increased from 5 min to 10 min to detect any extra peak because of the possible degradation. The results for forced degradation study are summarized in Table 1.
Fig. 7. Typical chromatogram of PFH after photolysis.

Table 1. Results of forced degradation study

<table>
<thead>
<tr>
<th>Stress applied</th>
<th>Retention time (min)</th>
<th>Degradation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001M HCl</td>
<td>2.577</td>
<td>0.17</td>
</tr>
<tr>
<td>0.001M NaOH</td>
<td>2.579</td>
<td>26.22</td>
</tr>
<tr>
<td>3% H2O2</td>
<td>2.667</td>
<td>0.83</td>
</tr>
<tr>
<td>50 ºC</td>
<td>2.683</td>
<td>1.5</td>
</tr>
<tr>
<td>UV radiation (365 nm)</td>
<td>2.681</td>
<td>23.36</td>
</tr>
</tbody>
</table>

Table 2. Results of Precision Study

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Concentration taken (µg/ml)</th>
<th>Peak area ± R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>System precision</td>
<td>50</td>
<td>1335662.33 ± 0.23</td>
</tr>
<tr>
<td>Intraday precision</td>
<td>50</td>
<td>1329675.5 ± 0.60</td>
</tr>
<tr>
<td>Interday precision</td>
<td>50</td>
<td>1338868.83 ± 0.73</td>
</tr>
</tbody>
</table>

*Average of six determinations

*Relative standard deviation

Table 3. Results of recovery study

<table>
<thead>
<tr>
<th>Different concentration level comparing to sample concentration (%)</th>
<th>Amount added Pure Drug (µg/ml)</th>
<th>Recovery (%) ± R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>32</td>
<td>99.73 ± 1.3</td>
</tr>
<tr>
<td>100</td>
<td>40</td>
<td>100.31 ± 0.79</td>
</tr>
<tr>
<td>120</td>
<td>48</td>
<td>100.58 ± 1.1</td>
</tr>
</tbody>
</table>

*Average of three determination

Robustness

Robustness of the method was studied by very deliberately changing the flow rate of mobile phase, detection wavelength and composition of organic phase. A series of system suitability parameters like retention time, 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 drug recovered after 24 hr 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 (± 1%) and detection wavelength (± 5nm). The study result confirms that PFH solution was stable for 24 hr at ambient conditions without any significant degradation of the analyte. The results are shown in Table 4.

Table 4. Robustness Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Retention time (min.)</th>
<th>Theoretical plates (N)</th>
<th>Tailing factor (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate (mL/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>2.992</td>
<td>3126</td>
<td>1.741</td>
</tr>
<tr>
<td>1.0</td>
<td>2.713</td>
<td>2975</td>
<td>1.789</td>
</tr>
<tr>
<td>1.1</td>
<td>2.468</td>
<td>2654</td>
<td>1.785</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>242</td>
<td>2.713</td>
<td>2967</td>
<td>1.780</td>
</tr>
<tr>
<td>247</td>
<td>2.713</td>
<td>2975</td>
<td>1.789</td>
</tr>
<tr>
<td>252</td>
<td>2.713</td>
<td>2951</td>
<td>1.809</td>
</tr>
<tr>
<td>Methanol (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94</td>
<td>2.739</td>
<td>2867</td>
<td>1.720</td>
</tr>
<tr>
<td>95</td>
<td>2.713</td>
<td>2975</td>
<td>1.789</td>
</tr>
<tr>
<td>96</td>
<td>2.684</td>
<td>2544</td>
<td>1.793</td>
</tr>
<tr>
<td>Solution Stability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial Time</td>
<td></td>
<td>Final Time</td>
<td>Recovery (%)</td>
</tr>
<tr>
<td>0 hr</td>
<td></td>
<td>24 hr</td>
<td>99.98</td>
</tr>
</tbody>
</table>
Limit of detection and Limit of quantification

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 and limit of quantification values were 4.5 μg/ml and 9.75 μg/ml, respectively.

Application to tablet formulation

The developed method was applied for determination of PFH in tablet dosage form. Twenty tablets were weighed and powdered finely. A quantity of tablet powder equivalent to 10 mg of PFH was accurately weighed and transferred into a 10 ml volumetric flask, containing 5 ml of mobile phase and ultrasonicated for 20 min; the volume was made up and mixed well. Solution was filtered through 0.2 μm filter to ensure the absence of particulate matter, if any. The filtered solution was appropriately diluted with the mobile phase for analysis as already described. The amount of drug present in the sample solution was calculated by using the calibration curve. The higher percentage of recovery present in the sample solution was calculated by using the stress study shows the method is specific. The method is non-interference of the formulation excipients in the calibration curve. The limit of detection and limit of quantitation were 9.75 μg/ml and 9.75 μg/ml, respectively.

Conclusions

A validated RP-UFLC method has been developed for determination of propafenone in tablet dosage form. The retention time of 2.681 min for propafenone in tablet dosage form demonstrates a unique advantage for rapid analysis by RP-UFLC. The results obtained by the stress study shows the method is specific. The method is environmental friendly as it produces less mobile phase to be disposed in to the environment. 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 propafenone in tablet dosage form. Further this method may be applied for routine analysis of propafenone in API, formulations and dissolution medium.

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References


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