A Validated RP HPLC Method for Simultaneous Determination of Propranolol hydrochloride and Alprazolam in bulk and in Pharmaceutical formulations

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Revised on: 18-12-2010; Accepted on: 19-01-2011

ABSTRACT

The present work describes a new, simple, precise, and accurate RP-HPLC method for simultaneous estimation of propranolol hydrochloride and alprazolam in bulk and formulation. Chromatographic separation of the drugs was achieved on a Waters C18 column (250 x 4.6 mm, i.d. 5 µ) using acetonitrile: water (adjusted to pH 2.3 with ortho phosphoric acid) in the ratio of 60:40 v/v as mobile phase. Flow rate was 1.0 mL/min and the detection wavelength was 214 nm. The two drugs were satisfactorily resolved with retention time values 1.73 min and 3.81 min for propranolol and alprazolam, respectively. The method was validated in terms of accuracy, precision, linearity, and limit of detection, limit of quantitation, ruggedness, and specificity as per ICH guidelines. Linearity was found to be in the range of 4 -30 µg/mL for propranolol hydrochloride and 0.05 – 0.375 µg/mL for alprazolam with significantly high value of correlation coefficient (r2 = 0.9981 for propranolol hydrochloride and 0.9989 for alprazolam). The accuracy and reliability of the method was assessed by evaluation of precision (intra-day and inter-day precision % RSD was less than 2% for both alprazolam and propranolol hydrochloride), accuracy (100.01 % for propranolol hydrochloride and 99.84 % for alprazolam), and specificity, in accordance with ICH guidelines. The LOD and LOQ were found to be 0.4 µg/mL and 1.2 µg/mL for propranolol hydrochloride and 0.018 µg/mL and 0.05 µg/mL for alprazolam respectively. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Keywords: Propranolol hydrochloride, Alprazolam, and Simultaneous

INTRODUCTION

Propranolol hydrochloride is a non-selective beta-blocker mainly used in the treatment of hypertension. It was the first successful beta blocker developed. Chemically it is (RS)-1-(isopropyl amino)-3-(1-naphthyloxy) propan-2-ol Fig 1a.

Figure 1a. Structure of propranolol hydrochloride

Alprazolam is used to treat the panic and anxiety symptoms associated with panic disorder. Alprazolam belongs to a class of medications called benzodiazepines, which act, on the brain and central nervous system to produce a calming effect. It works by enhancing the effects of a certain natural chemical (GABA) in the body. The chemical name of alprazolam is 8-Chloro-1-methyl-6-phenyl-4H-s-triazolo [4,3-1][1,4] benzodiazepine Fig 1b.

Figure 1b. Structure of alprazolam

Reagents and Chemicals

Analytical pure drugs of propranolol hydrochloride and alprazolam were obtained as gift samples from M/S. Trident pharmaceuticals, Hyderabad, India. The combined tablet formulation ALPRA – P (manufactured by ZENLABS India) with a labeled claim of alprazolam 0.25 mg and 20 mg of propranolol hydrochloride, respectively, were obtained from local drug store. Acetonitrile HPLC grade and analytical grade ortho phosphoric acid were procured from SD. Fine Chemicals, Mumbai. Triple distilled water prepared by all glass distillation apparatus was used.

Mobile phase

Acetonitrile and water (adjusted to pH 2.3 with orthophosphoric acid) in the ratio of 60:40 v/v was used for separation of these drugs after filtering through 0.45 µ membrane filter and sonicating each solvent for 10 min.

METHOD DEVELOPMENT

Selection of wavelength

Wavelength was selected by scanning the standard solutions of both the drugs over 200 nm to 400 nm. Both the components show reasonably good response at 214 nm; therefore wavelength 214 nm was selected for further study.

Chromatographic conditions

Separation was carried out on Waters C18 column (250x4.6 mm, i.d 5µm) at room temperature. The sample was injected by using 20 µL fixed volume loop and effluent was monitored at 214 nm. The elution was carried out isocratically at a flow rate of 1mL/ min using acetonitrile: water (adjusted to pH 2.3 with orthophosphoric acid) in the ratio of 60: 40 v/v and total run time was 10 min.

Preparation of standard stock solution

Standard stock solution (1mg/mL) of propranolol hydrochloride and alprazolam were prepared separately by dissolving 25 mg of each drug in 25 mL of mobile phase.

Calibration curve

The solutions were suitably diluted from stock solution with mobile phase to get mixed standard solutions containing 4, 10, 16, 20, 24 and 30 µg/mL of propranolol hydrochloride with the corresponding respective quantities of alprazolam i.e. 50, 125, 200, 250, 300 and 375 ng/mL. From each mixed standard 20 µL of solution was injected and chromatograms were recorded(n=3). Calibration curves were constructed by plotting the average peak areas versus respective concentrations of propranolol hydrochloride and alprazolam, respectively.

Preparation of sample solution

Twenty tablets (ALPRA- P) each containing 20 mg of propranolol hydrochloride and 0.25 mg of alprazolam were weighed, and powdered. Tablet powder equivalent
**METHOD VALIDATION**

**Linearity and range**
Series of mixed standard solutions of propranolol hydrochloride and alprazolam were prepared in 10 mL volumetric flasks using mobile phase to get final concentration of 4 - 30 µg/mL of propranolol hydrochloride and 0.05 - 0.375 µg/mL of alprazolam. Triplicate injections were made for each concentration and chromatographed under the conditions described above. The plots of average peak area versus respective concentrations of propranolol hydrochloride and alprazolam were found to be linear in the above concentration range with a correlation coefficient of 0.9981 and 0.9982, respectively.

**Precision**
Precision study was performed to find out intra-day and inter-day variations. The percent relative standard deviation for intra-day precision was 0.39 for propranolol hydrochloride and 0.43 for alprazolam and inter-day precision was 0.52 for propranolol hydrochloride and 0.67 for alprazolam. Both the values are well within the limits.

**Accuracy**
The accuracy of the method determined by recovery studies. The recovery studies were performed by standard addition method, at 80%, 100%, 120% level for both the drugs and the percentage recoveries were calculated and found to be 99.84 - 100.01 of alprazolam and propranolol hydrochloride, respectively. The high recovery of propranolol hydrochloride and alprazolam shows that there is no interference from excipients.

**Limit of detection and limit of quantitation**
The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. The limit of quantitation (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were determined by using the formula $3.3\sigma/S$ and $10\sigma/S$ where, $\sigma$ is the standard deviation of $Y$ intercept and $S$ is the slope of the calibration curve. The LOD was found to be 0.4 µg/mL for propranolol hydrochloride and 0.018 µg/mL for alprazolam and LOQ were found to be 1.2 µg/mL for propranolol and 0.05 µg/mL for alprazolam.

**Robustness**
Robustness of the method was determined by making slight changes in chromatographic conditions like 5% change in the ratio of mobile phase compositions, ±1nm change in the detection wavelength, pH by 0.2 and 0.1mL change in the flow rate. It was observed that there were no marked changes in the chromatograms that suggest that the developed method is robust.

**Stability**
In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were kept over a period of 24 hours at room temperature and then analyzed. Sample solution injected after 24 hours of preparation did not show any appreciable change in assay value.

**RESULTS AND DISCUSSION**
UV overlain spectra of both propranolol hydrochloride and alprazolam showed that both the drugs absorb appreciably at 214 nm, so 214 nm was selected as the detection wavelength in liquid chromatography Figure 2. Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase acetonitrile: water (pH 2.3) in the ratio of 60:40 v/v. The system suitability test parameters are shown in Table I. The retention time of propranolol hydrochloride was found to be 1.737 min and that of alprazolam was found to be 3.810 min, respectively Figure.3. Resolution between propranolol hydrochloride and alprazolam was found to be 12.399, which indicate good separation of both the compounds. The asymmetric factor for propranolol hydrochloride and alprazolam was found to be 1.428 and 1.423. The calibration curves for propranolol hydro-

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**Table No. I: System Suitability Parameters of the proposed method**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Propranolol hydrochloride</th>
<th>Alprazolam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical plates (N)*</td>
<td>2225</td>
<td>5888</td>
</tr>
<tr>
<td>Resolution factor (Ry)*</td>
<td>11.613</td>
<td>11.613</td>
</tr>
<tr>
<td>Asymmetric factor*</td>
<td>1.468</td>
<td>1.407</td>
</tr>
<tr>
<td>Retention time (Rt)*</td>
<td>1.737</td>
<td>3.810</td>
</tr>
</tbody>
</table>

* Average of six determinations

**Table No. II: Validation and regression parameters of the proposed method**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Propranolol hydrochloride</th>
<th>Alprazolam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity and Range (µg/mL)</td>
<td>4 - 30</td>
<td>0.05 - 0.375</td>
</tr>
<tr>
<td>Regression Equation</td>
<td>$Y=192.19x+0.267$</td>
<td>$Y=0.1182x+0.0394$</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>192.19</td>
<td>0.1182</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.267</td>
<td>0.0394</td>
</tr>
<tr>
<td>Intra day (n=3)</td>
<td>0.39</td>
<td>0.43</td>
</tr>
<tr>
<td>Inter day (n=3)</td>
<td>0.52</td>
<td>0.67</td>
</tr>
<tr>
<td>LOD (µg/mL)</td>
<td>0.4</td>
<td>0.018</td>
</tr>
<tr>
<td>LOQ (µg/mL)</td>
<td>1.2</td>
<td>0.05</td>
</tr>
</tbody>
</table>

$Y$ - is the area under the curve and $c$ - is the concentration

**Table No. III: Assay and recovery results of the proposed method**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount mg/tablet</th>
<th>% Label claim</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propranolol hydrochloride</td>
<td>20</td>
<td>100.02</td>
<td>100.01</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>0.25</td>
<td>99.60</td>
<td>99.84</td>
</tr>
</tbody>
</table>

* Average of six determinations

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**Fig. 2 Overlay spectra of propranolol hydrochloride, alprazolam and mixture**

**Fig. 3 A typical chromatogram of propranolol hydrochloride and alprazolam**
be estimated accurately. The validation parameters are summarized in Table II. The results for stability studies revealed that the retention time and peak area of propranolol hydrochloride and alprazolam remained almost unchanged and no significant degradation was observed within the indicated period. The recovery of propranolol hydrochloride and alprazolam were found to be 100.01% and 99.84%. The proposed liquid chromatographic method was applied for the determination of propranolol hydrochloride and alprazolam in tablet formulations and the assay values for propranolol hydrochloride and alprazolam were comparable with the corresponding labeled amount Table III.

CONCLUSION
The proposed method for the determination of propranolol hydrochloride and alprazolam in combined dosage form is accurate, precise, linear, robust, simple, and rapid. Hence the present RP-HPLC method is suitable for ascertaining the quality control of the raw materials, formulations in combined dosage forms and dissolution studies.

ACKNOWLEDGEMENT
Authors wish to thank M/S Trident Pharmaceuticals, Hyderabad, for providing the gift samples of propranolol hydrochloride and alprazolam for this work. We are also thankful to the Principal, S.K.Gulati, HOD of Pharmaceutical chemistry, Dr. K.Rama Subba Reddy and the management of Sarojini Naidu Vanitha Pharmacy Mahavidyalaya for providing the required facilities and for their constant encouragement.

REFERENCES

Source of support: Nil, Conflict of interest: None Declared