RESEARCH ARTICLE

NEWER RP-HPLC METHOD FOR THE DETERMINATION OF DOXAZOSIN IN HUMAN PLASMA AND FORMULATION

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A new sensitive, specific, precise and accurate RP-HPLC method has been developed and validated for rapid assay of doxazosin in human plasma and pharmaceutical formulations. Isocratic elution at a flow rate of 1.5 ml/min was employed on Chromosil C18 (250 mm × 4.6 mm, 5 µm) column at ambient temperature. The mobile phase consisted of methanol:water:acetonitrile (25:25:50 v/v), was filtered through 0.45 µm membrane filter and sonicated. The detection was carried out at 280 nm. The injection volume was 20 µl and the total run time was 8 min. The percentage RSD for precision and accuracy of the method was found to be 0.051. The method developed was validated as per the ICH guidelines. The method can be successfully utilized for routine analysis of doxazosin in the rapid and reliable determination of doxazosin in human plasma and pharmaceutical formulations.

Key words: Doxazosin, RP-HPLC, UV detection, Methanol, Acetonitrile.

INTRODUCTION

Doxazosin, an antihypertensive agent, is chemically known as 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-[(2,3-dihydro-1,4-benzodioxin-2-yl]carbonyl]piperazine (Figure 1) and is official in Indian Pharmacopoeia.

![Fig. 1. Structure of doxazosin](image)

Its molecular formula is C₂₃H₂₆N₅O₅ and the molecular weight is 451.47 g/mol (Chien, 1983; 1989; Berner and Dinh, 1992). Literature survey indicated that there are several methods reported for the determination of doxazosin in human plasma and in pharmaceutical formulations (Chien, 1988; Swarbrick and Boylan, 1988; Scott and Hollenbeck, 1991; Mandal and Womack, 1999; Bai et al 2002; Bachy et al 2004). There are RP-HPLC methods for determination of drugs in literature (Prasanthi et al 2011; Bhimavarapu et al 2011) but yet no method is reported for the estimation of doxazosin in formulations. So, an attempt was made to develop and validate a simple, precise, accurate and economical RP-HPLC method as per ICH guidelines for the estimation of doxazosin in pure pharmaceutical dosage form and to apply the developed method to determine the forced degradation compounds.

EXPERIMENTAL

**Preparation of mobile phase solution**

The mobile phase was prepared by mixing methanol, water and acetonitrile (25:25:50 v/v) followed by sonication for 30 min.
Preparation of standard
Stock solution of doxazosin was prepared by dissolving accurately weighed 50 mg of drug in 10 ml methanol (final concentration - 5 mg/ml). The prepared stock solutions were stored away from light. From the stock, standard solutions was freshly prepared during the day of analysis.

Preparation of working standard solution
From the standard stock solution, 2.5 mg/ml solution was prepared by the half dilution.

Preparation of formulation sample solution
Ten milligram tablet powder was accurately weighed from DOXACARD (1 mg formulation) tablets powder and dissolved in 10 ml of mobile phase and injected into HPLC and chromatogram was recorded.

Preparation of serum sample solution
From a local hospital, blood was collected and serum was separated. Half millilitre of the serum was taken in a test tube and to this, 100 µl of diltiazem hydrochloride (1 µg/ml), 0.1 ml of 1 M NaOH and 5 ml of dichloromethane were added. Mixing of contents was done for 20 min in vortex mixer and centrifuged at 3000 rpm for 10 min. From this centrifuged solution, 4 ml of organic layer was separated and evaporated to dryness to get residue. To this residue, 100 µl of 1 M acetic acid, 3 ml of n-hexane were added and mixed for 5 min by vortex mixer and the organic layer was evaporated and finally, the remaining sample was injected into HPLC and chromatogram was recorded.

Linearity and calibration
Linearity was accessed by performing single measurement at several analyte concentrations by varying quantities of standard stock solution diluted with the mobile phase to give a concentration of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml. Injection was made at intervals of 6 min. The linearity was tested for the concentration ranging from 0.5-2.5 mg/ml. The peak area ratio of the drug was plotted against concentration. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision
Reproducibility was performed by injecting three replicate concentrations of standard and sample solutions which were prepared and analyzed by same analyst on same day. Inter-day variations in the peak area of drug solutions and the amount of drug were calculated in terms of percentage relative standard deviation. The sample concentration was 2.5 mg/ml.

Accuracy
Recovery assessment was obtained by using standard addition technique by adding known quantities of pure standards at three different levels in 80%, 100% and 120% to the pre-analyzed sample formulation.

Intermediate precision or Ruggedness
Inter-day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness was also expressed in terms of percentage relative standard deviation.

Robustness
Test for robustness was carried out by varying the two parameters from the optimized chromatographic conditions.

Specificity
The method was determined as specific by comparing test results obtained from analyses of sample solution containing excuse ingredients with that of test results those obtained from standard drug.

System suitability parameter
System suitability tests were carried out on freshly prepared standard stock solutions of doxazosin and it was calculated by determining the standard deviation of doxazosin standards by injecting standards in five replicates at 8 min interval and the values were recorded.

Assay
For the estimation of drug in pharmaceutical dosage forms, DOXACARD (Cipla) tablets of 1 mg strength were evaluated for the amount of doxazosin present in the formulation. Each sample was analyzed in triplicate after extracting the drug. Half of the tablet was weighed and dissolved in 1 ml of mobile phase and injected into HPLC followed by recording of chromatogram. The amount of drug present in the formulation was calculated by comparing the mean peak area from standard.
RESULTS AND DISCUSSION
The reverse phase high performance liquid chromatography method was developed as a stability indicating assay method. Pure drugs chromatogram (Figure 2) was run in different mobile phases containing methanol, acetonitrile, THF and different buffers like potassium dihydrogen phosphate, sodium dihydrogen phosphate, orthophosphoric acid in different volumes ratios using different columns like C8, C18, phenyl, cyano with different dimensions. Then, retention time and tailing factor were calculated. Finally, methanol, water and acetonitrile in the volume of ratio 25:25:50 v/v (pH 5.0) and Kromasil C18 analytical column was selected which exhibited a sharp and symmetrical peak with 1.47 tailing (Table 1).

Table 1. Optical characterization of doxazosin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Doxazosin</th>
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<tbody>
<tr>
<td>Linearity range (mg/ml)</td>
<td>0.5-2.5</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9983</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>223191.2</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.0177</td>
</tr>
<tr>
<td>Limit of detection (LOD; µg/ml)</td>
<td>110</td>
</tr>
<tr>
<td>Limit of quantification (LOQ; µg/ml)</td>
<td>40</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.47</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>4.282</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>6757</td>
</tr>
<tr>
<td>(% R.S.D.)</td>
<td>0.051</td>
</tr>
<tr>
<td>(%) Accuracy</td>
<td>103.12</td>
</tr>
<tr>
<td>The amount of doxazosin present in 5 mg formulation</td>
<td>49.33</td>
</tr>
<tr>
<td>The amount of doxazosin present in 0.5 ml serum</td>
<td>0.248</td>
</tr>
</tbody>
</table>

Calibration graph was found to be linear at range 0.5-2.5 mg/ml. Five different concentrations of doxazosin in range given above were prepared and 20 µl of each concentration injected in HPLC. The slope (m) and intercept (c) obtained were found to be 223191.2 and 0.0177. The correlation of coefficient (r²) obtained was found to be 0.9983. It was observed that the concentration range showed a good relationship. The limit of detection for doxazosin was found to be 110 µg/ml and the limit of quantification was found to be 40 µg/ml. It proved the sensitivity of method. The percentage assay of doxazosin in formulation was found to be 49.33. The relative standard deviation value obtained was below 1 which indicated the preccession of the method. The validation of the proposed method was further verified by recovery studies. The percentage recovery was found to be 96.51% which shows a good index of accuracy of the developed method. The amount of drug present in the human serum sample was calculated from the linearity graph and was found to be 0.248 mg/0.5 ml. Sample and serum HPLC chromatograms are presented in Figure 3 and Figure 4.
**CONCLUSION**

The RP-high performance liquid chromatographic method for analysis of doxazosin from their formulations was found to be accurate and precise. Thus, the proposed HPLC method can be successfully used for the routine quality control analysis of doxazosin formulations.

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