A New RP-HPLC Method for Monitoring of Atenolol: Application to Atenolol Metal Interactions Studies

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(Received 13th April 2007, revised 16th June 2007)

Summary: A sensitive high-performance liquid chromatography method was developed for the determination of atenolol in bulk drug and pharmaceutical formulation in the presence of flurbiprofen as internal standard. Separation was carried out on a Poroshper Start, C18 (5 µm, 250 x 4.6 mm) column with MeOH: H2O (80: 20, % v/v) solvent systems and pH was adjusted to 2.85 with ortho-phosphoric acid. Mobile phase was pumped with a flow rate of 1 ml/minute with UV detection at 224 nm. Standard curves were linear over the concentration range of 0.25-30 µg/ml. The inter-day and intra-day coefficients of variation were less than 1 % at different concentrations. The method was then applied to study interaction of atenolol with essential and trace elements. The combination of hydroxy and aminogroups makes atenolol a good chelating ligand. Drug metal interaction studies were carried out at 37 °C to monitor the complexation of drug with metal ions. The order of complexation of atenolol with metals was found to be copper > ferric > chromium > zinc > ferrous > cobalt > nickel > magnesium > manganese > cadmium > calcium.

Introduction

β-adrenergic receptor antagonists have received enormous clinical attention because of their efficacy in the treatment of hypertension, can improve cardiac function, reduce the symptoms of heart failure, improve functional capacity, and enhance exercise tolerance ischemic and certain arrhythmias [1-9].

Atenolol (Fig. 1) or 4-[2-hydroxy-3-[(1-methylethyl) amino] propyl bezenacetamide is α-adrenergic, cardioselective drug having no intrinsic sympathomimetic activity [10]. It is also poorly bound to plasma protein (less 5% of the amount in blood) [11] most of the drug is eliminated, in its unchanged form, by several routes, but prevailing via the kidney [12].

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

Many workers reported assay of atenolol in urine, serum, human plasma, biological fluids and pharmaceutical formulation [13-18]. Some workers reported the determination of derivatized enantiomers of atenolol in whole blood [19-20]. Complexation has often been used to influence biological processes that are metal dependent [21-25].

Nevertheless, many drugs behave as ligands, coordinating such biometals as copper, zinc and iron, which affect their homeostasis. It can be assumed, therefore, that the action of at least some of the drugs used in the treatment of metal-dependent diseases can be explained on these grounds [26-28]. Arterial hypertension represents a good example of this type, being sensitive to copper and zinc concentration levels [29-31].

The β-blockers are among the drugs most widely used in the treatment of hypertension and for that reason we have initiated a study on interaction of atenolol with essential and trace elements as complexation of atenolol with copper, zinc and ferric are reported [32-34].

There are number of interactions reported with cephalosporins [35-36] and antacids containing di and trivalent metal cations [37]. The availability of drugs can be affected by the
concurrent ingestion of drugs containing multivalent cations. It is imperative to be aware of these interactions. In present paper we describe a fast and reproducible method for the quantitation of atenolol and its metal complexes with essential and trace elements, which may either be present in low concentrations in human body or may be ingested as a result of multiple drug therapy.

Results and Discussion

Method Development and Validation

The mobile phase consisted of methanol and water (80: 20) with pH 2.85, but various ratios (80: 20, 75: 25 70: 30, v/ v) were also tested as starting solvent for system suitability study. The variation in the mobile phase leads to considerable changes in the chromatographic parameters, like peak symmetry, retention time, theoretical plates, resolution and capacity factor. When the mobile phase was used without change in pH, peak was broad and unsymmetrical. With 75: 25 and 70: 30 mobile ratios peak tailing increases and theoretical plate decrease. So, 80: 20 ratios with pH 2.85 were found suitable for present work. Flow rate was also varied to 1.5 and 0.5, at flow rate 1.5 ml / min, peak appeared near to 1.0 min. and when flow rate was decreased to 0.5 ml/ min retention time increased to 4.0 but peak was broad. So 1ml/ min flow rate was suitable.

Linearity and Sensitivity

For the determination of linearity, standard calibration curve was used, which was linear over the concentration range 0.25 to 30 μg / ml. The proposed method was evaluated by its correlation coefficient and intercept value, calculated in the corresponding statistic study. Characteristic parameters for regression equation (y = a + bx) obtained by least square treatment of the results were used to confirm linearity of the method developed. Results of calibration curve showed good linearity with coefficient of correlation (r²) of 0.9999 (Table-1).

<table>
<thead>
<tr>
<th>Table-1: Statistical analysis for proposed method.</th>
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<tbody>
<tr>
<td>Concentration range (μg /ml)</td>
</tr>
<tr>
<td>Limit of detection (μg /ml)</td>
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<tr>
<td>Limit of quantitation (μg /ml)</td>
</tr>
</tbody>
</table>

Precision and Accuracy

Both precision and accuracy were determined by analyzing independently prepared solutions of atenolol in triplicates at different concentration levels covering the entire linearity range. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day), which is reported as %C.V. As shown in Table-2, the intra- and inter-day precision did not exceed 2 % R.S.D. at different concentration levels.

<table>
<thead>
<tr>
<th>Table-2: Accuracy and precision of atenolol in pharmaceutical formulation.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. Spiked Conc. Found Accuracy, % Precision</td>
</tr>
<tr>
<td>μg/ml</td>
</tr>
<tr>
<td>0.25</td>
</tr>
<tr>
<td>0.50</td>
</tr>
<tr>
<td>1.0</td>
</tr>
<tr>
<td>2.0</td>
</tr>
<tr>
<td>4.0</td>
</tr>
<tr>
<td>7.5</td>
</tr>
<tr>
<td>15.0</td>
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<tr>
<td>30.0</td>
</tr>
</tbody>
</table>

Limit of Detection and Quantification

The limit of detection (LOD) and limit of quantification (LOQ) of atenolol was found to be 0.40 μg/ ml and 1.10 μg/ ml respectively. While at concentrations where the signal/noise ratios were equal to 3 and 10, respectively.

Selectivity

To evaluate the specificity of the method, atenolol tablets were analyzed and compared with the standard atenolol and excipients chromatograms. The results showed that there is no interference of excipients (Fig. 2). Solutions of atenolol containing aspirin, acetaminophen and caffeine were also prepared and then injected to check for interference from these commonly used drugs. The method demonstrated good resolution between atenolol and found to be free of interferences. Aspirin, acetaminophen and caffeine appeared at retention time of 3-4 min.

Ruggedness

Method ruggedness was demonstrated by having two analysts performing assay on separate
The method did not show any notable deviation in results from acceptable limits.

**Metal Interaction Studies**

The interaction between atenolol and metals is a chelating reaction. It is postulated that the multivalent cations complex with the hydroxy and amino-groups on the atenolol molecule (Fig. 3). Before interaction atenolol appeared at retention time of 2.07 minutes (Fig. 4) but after interactions,

![Proposed monodentate and bidentate complex of atenolol. Where L is atenolol](image)

The chromatogram of atenolol and flurbiprofen (internal standard) is shown in Fig. 4.
complex formed appeared at different retention time as shown in Figures 5 and 6. The interactions between these essential and trace elements and atenolol are shown in Table 3. The maximum complexation occurred with copper, ferric and chromium chloride and complex appeared at retention time of 2.36, 2.54 and 2.55 minutes respectively. The change in retention time was due to formation of complex and %availability of drug also increased. In case of zinc, ferrous and cobalt %availability drug also increased to 876.55, 796.06 and 676.96 respectively. In case of nickel, magnesium, maganese and cadmium %avability of drug was increased to 186.82, 181.68, 180.71 and 129.74 %. In case of calcium only 1.52 % drug was complexed.
Table-3: Quantitation of atenolol metal complexes by the proposed method.

<table>
<thead>
<tr>
<th>Drug Metal Complex</th>
<th>Retention time</th>
<th>AUC</th>
<th>% Drug available</th>
<th>% Drug complexed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>2.07</td>
<td>299605</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>2.63</td>
<td>22108413</td>
<td>7379.18</td>
<td>7279.17</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>2.54</td>
<td>13035289</td>
<td>4350.82</td>
<td>4250.82</td>
</tr>
<tr>
<td>Cr(III)</td>
<td>2.50</td>
<td>6952300</td>
<td>2320.48</td>
<td>2220.48</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>2.60</td>
<td>2626220</td>
<td>876.55</td>
<td>776.55</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>2.43</td>
<td>2385060</td>
<td>796.06</td>
<td>696.06</td>
</tr>
<tr>
<td>Co(II)</td>
<td>2.55</td>
<td>2028221</td>
<td>676.96</td>
<td>576.96</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>2.59</td>
<td>559738</td>
<td>186.82</td>
<td>86.82</td>
</tr>
<tr>
<td>Mg(II)</td>
<td>2.58</td>
<td>544349</td>
<td>181.68</td>
<td>81.68</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>2.59</td>
<td>541420</td>
<td>180.71</td>
<td>80.71</td>
</tr>
<tr>
<td>Cd(II)</td>
<td>2.52</td>
<td>388711</td>
<td>129.74</td>
<td>29.74</td>
</tr>
<tr>
<td>Ca(II)</td>
<td>2.56</td>
<td>295023</td>
<td>98.47</td>
<td>1.52</td>
</tr>
</tbody>
</table>

Assay Procedure

An amount equivalent to 10.00 mg of atenolol was accurately weighed and dissolved in methanol into a 100 ml volumetric flask to produce a standard solution of 100.0 μg/ml. Aliquots were diluted to 50, 30, 15, 7.5, 4, 2, 1, 0.5, and 0.25 μg/ml. 0.5g of metal salt was weighed accurately in 25 ml Erlenmeyer flask. 10 ml of atenolol solution (50 μg/ml) was added to each flask. These flasks were kept in a constant temperature bath at 37°C for one hour with constant stirring. The contents of the flask were filtered through a millipore filter (0.45μ) and injected through a rhodyne of 20-μL loop to HPLC system as described above.

Dosage Sample Solution

Five tablets of atenolol® 100 mg from Bosch Pharma (Pvt) Ltd, were weighed to obtain the average tablets weight, powdered and transferred with 10 ml of methanol to 100 ml volumetric flask. This stock solution was filtered and was diluted to desired concentrations.

Conclusions

A rapid, precise, accurate, low cost and least time consuming RP-HPLC method for the qualitative and quantitative analysis of atenolol in bulk drug and pharmaceutical formulations has been developed and validated. The proposed RP-HPLC method enables simultaneous determination of atenolol and its metals complexes with small retention time of less than 4 minutes. The detector response was found to be linear over a wide concentration range 0.25-30 μg/ml. Results were accurate and precise and were confirmed by the statistical parameters. Reliability, rapidness, sensitivity, economical nature, good recovery and precision of this HPLC method give it an advantage over the other reported HPLC methods.

References