Experimental research for determination of bisoprolol fumarate in human plasma samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique

Received for publication, January 5, 2009
Accepted, March 30, 2010

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Abstract

Liquid chromatography coupled with mass spectrometry detection is one of the most powerful analytical tools for organic compound analysis. The advantages of using LC/MS methods over HPLC methods include: selectivity, chromatographic integrity, peak assignment, structural information, rapid method development.

In this context, a liquid chromatography-tandem mass spectrometry method has been developed and validated for the determination of bisoprolol in human plasma samples, using metoprolol as internal standard.

The assay has proven to be sensitive, specific and reproducible, suitable to determine the bisoprolol concentration, following a single oral administration of a 10 mg bisoprolol tablet in 22 healthy volunteers, in the bioequivalence study of Bisoprolol 10 mg coated tablets, produced by Antibiotice S.A. versus Concor® 10 mg, produced by Merck.

Keywords: Bisoprolol, HPLC, internal standard, bioanalytical method validation.

Introduction

The objective of this work was to develop and to validate a LC-tandem mass spectrometry method for the determination of bisoprolol in human plasma samples. Bisoprolol fumarate is a synthetic cardioselective $\beta_1$-adrenergic blocker. Chemically, bisoprolol fumarate is (±)-1-[4-[[2-(1-methylethoxy)ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino]-2-propanol(E)-2-butenedioate (2:1) [1]. It possesses an asymmetric carbon atom in its structure and is provided as a racemic mixture. The S(-) enantiomer is responsible for most of the beta-blocking activity.

Several assay are used to determine bisoprolol concentrations in biological fluids, including high performance liquid chromatography procedures. Reversed phase column were generally used, with aqueous mobile phases containing different ratio of organic solvents [2-7]. Liquid-liquid extraction was used to separate the active substance from the components of the biological samples. The method described in this paper is based on HPLC with mass spectrometry detection and the plasma samples were prepared using a liquid extraction allowing to detect and quantify concentrations as low as 1 ng/ml.

Materials and Methods

Instruments

High Performance Liquid Chromatograph Agilent 1100 LC/MSD Trap XCT device was employed during this study, with the following system structure: Agilent 1100 Degasser, Agilent 1100 Binary Pump, Agilent 1100 Autosampler, Agilent 1100 Mass Selective Detector. The Bruker Daltonik software was used for system control and data acquisition.
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An analytical balance Mettler-Toledo XP56, a Sigma 2-16 K centrifuge and a Vibramax 110 shaker were used for the sample preparation. The separation was performed using a reverse phase column (Zorbax SB-C18 Solvent Saver Plus, 3 x 100mm, 3.5μm, supplied by Agilent, USA). The chromatography was performed at 40°C temperature, automatically injecting 5 μl into the chromatographic system.

Reagents.
All solvents and other chemicals (acetonitrile, methanol, sodium hydroxide, tert-butyl methyl ether, water, formic acid) were HPLC grade provided by Merck’s Chemical Co., Darmstadt, Germany. The reference substances of bisoprolol and metoprolol (internal standard) were supplied from the USP Pharmacopoeia. The human plasma was obtained from Center for Blood Drawing and Preservation, Iasi, Romania.

Mobile Phase.
The mobile phase 0.1% formic acid solution – acetonitrile (50-50, v/v) was pumped at 0.3 ml/min flow rate into the chromatographic system using the binary pump.

Bisoprolol stock solution.
Bisoprolol was dissolved in methanol, obtaining a bisoprolol stock solution of 500 μg/ml.

Metoprolol stock solution.
The internal standard, metoprolol, was dissolved in methanol, obtaining a metoprolol stock solution of 500 μg/ml.

Solutions for linearity response.
Eight bisoprolol concentrations were prepared in human plasma, covering the expected range of observed concentrations (1–100 ng/ml). The theoretical concentrations of bisoprolol calibration standards were 1.0; 2.0; 10.0; 20.0; 40.0; 60.0; 80.0 and 100.0 ng/ml.

Quality control samples.
Plasma samples having bisoprolol concentrations of 3 ng/ml, 25 ng/ml and 75 ng/ml were considered to be appropriate to be used to validate the bioanalytical method.

Samples for recovery.
In order to determine the analyte and the internal standard recovery from the plasma, water samples containing the same bisoprolol concentrations as the quality control samples were prepared.

Samples preparation.
After alkalization with sodium hydroxide, the 0.100 ml plasma sample was extracted with tert-butyl methyl ether. The solvent was evaporated using a flow air at 40°C. The solid residue was dissolved in a 0.250 ml mixture 0.1% formic acid solution – acetonitrile (50-50, v/v).

Results and Discussions
The method was validated according Guidance for Industry: Bioanalytical Method Validation [8]. Parameters usually examined in the validation process are selectivity, linearity, limit of quantification, accuracy and precision.

Selectivity
The reversed-phase HPLC method described in this paper has been tested for possible interferences from other plasma factors. Plasma aliquots from six different sources were assessed for analysis in order to investigate the plasma components behavior.

As it can be seen in Fig. 1., no overlapping peaks were detected at bisoprolol and internal standard retention time, 1.7 min and 1.9 min, respectively. The bioanalytical method proved to be selective.
Linearity and lower limit of quantification

The linearity was investigated for a bisoprolol concentration range between 1 ng/ml and 100 ng/ml and the calibration curve was derived by plotting the peak-height ratios of the analyte and the internal standard against the concentration of bisoprolol, using linear regression analysis.

The least-square linear regression revealed that the relationship was linear in the investigated domain, with a correlation coefficient of 0.998599, meeting the acceptance criteria ($r^2 \geq 0.990$), as it can be seen in fig. 2.

The lower limit of quantification, i.e. the lowest standard level with a coefficient of variation less than 20%, is for bisoprolol 0.99 ng/ml with 41.433 signal to noise ratio. The bioanalytical method proved to be sensitive, allowing a precise quantification of concentrations as low as 1 ng/ml (see fig. 3.)

Results are presented in table 1.
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Table 1. Lower Limit of Quantification

<table>
<thead>
<tr>
<th>Analyte Concentration (ng/ml):</th>
<th>0.99</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. (ng/ml)</td>
<td>% Nominal</td>
</tr>
<tr>
<td>0.989</td>
<td>99.945</td>
</tr>
<tr>
<td>1.407</td>
<td>142.094</td>
</tr>
<tr>
<td>0.958</td>
<td>96.802</td>
</tr>
<tr>
<td>1.167</td>
<td>117.863</td>
</tr>
<tr>
<td>1.175</td>
<td>118.723</td>
</tr>
<tr>
<td>1.241</td>
<td>125.400</td>
</tr>
</tbody>
</table>

N 6 6 6
Mean 1.15 116.804 41.433
SD (±) 0.166
CV(%) 14.339

Acceptance criteria:
4 out of 6 LLQC must be 100±20% nominal value.
Mean % Nominal 100±20%
CV(%) ≤ 20%
Signal/Noise Ratio ≥ 5

Accuracy and precision

Accuracy of the analytical method represents the degree of closeness of the determined values of an analyte to the nominal/or known true value declared from an individual sample. The accuracy of a bioanalytical method is expressed as a percentage of the nominal value (% Nominal).

Precision of the analytical method represents the degree of dispersal of the values determined of an analyte, from a series of samples processed and analyzed individually from a homogeneous volume of biological matrix. Precision of a bioanalytical method is expressed as the coefficient of variation of the concerned series of determinations, CV (%).

The accuracy and precision of this method were calculated for three concentrations of bisoprolol in serum. Six replicate samples having bisoprolol theoretical concentrations of 3 ng/ml (QC1), 25 ng/ml (QC2) and 75 ng/ml (QC3) were injected into the system. Table 2 summarizes the results obtained for the intra-day parameters. The inter-day precision and accuracy was evaluated also using six aliquots for each quality control sample concentration, prepared and analysed in six different days. The results are presented in table 3. Coefficients of variation no higher than 7% and 8% were obtained for precision and accuracy, respectively, for all three concentrations studied.

Table 2. Evaluation of intra-day precision and accuracy for bisoprolol spiked quality control samples.

<table>
<thead>
<tr>
<th>Cth = 3 ng/ml</th>
<th>Cth = 25 ng/ml</th>
<th>Cth = 75 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cexp (ng/ml) % Nominal</td>
<td>Cexp (ng/ml) % Nominal</td>
<td>Cexp (ng/ml) % Nominal</td>
</tr>
<tr>
<td>1. 2.912 98.391</td>
<td>2.524 91.263</td>
<td>69.203 93.468</td>
</tr>
<tr>
<td>2. 3.003 101.439</td>
<td>24.862 100.737</td>
<td>68.525 92.552</td>
</tr>
<tr>
<td>3. 3.262 110.192</td>
<td>23.762 96.281</td>
<td>66.781 90.196</td>
</tr>
<tr>
<td>4. 2.829 95.568</td>
<td>25.189 102.062</td>
<td>76.691 103.580</td>
</tr>
<tr>
<td>5. 3.018 101.960</td>
<td>21.739 88.085</td>
<td>65.844 88.931</td>
</tr>
<tr>
<td>6. 2.854 96.431</td>
<td>21.371 86.593</td>
<td>74.009 99.958</td>
</tr>
<tr>
<td>Mean 2.980 100.664</td>
<td>23.241 94.170</td>
<td>70.176 94.781</td>
</tr>
<tr>
<td>SD 0.158</td>
<td>1.610</td>
<td>4.268</td>
</tr>
<tr>
<td>CV, % 5.296</td>
<td>6.927</td>
<td>6.082</td>
</tr>
</tbody>
</table>

Cth= theoretical concentration
Cexp= experimental concentration
SD= standard deviation
CV, %= coefficient of variation

Acceptance criteria:
67% Total QC must be 100±15% nominal values
50% QC per level must be 100±15% nominal values
Mean % nominal 100±15%
CV(%) ≤ 15%
Table 3. Evaluation of inter-day precision and accuracy for bisoprolol spiked quality control samples

<table>
<thead>
<tr>
<th>Cth =3 ng/ml</th>
<th>Cth = 25 ng/ml</th>
<th>Cth = 75 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cth = 3 ng/ml</td>
<td>Cexp (ng/ml) % Nominal</td>
<td>Cexp (ng/ml) % Nominal</td>
</tr>
<tr>
<td>1.</td>
<td>3.015</td>
<td>101.860</td>
</tr>
<tr>
<td>2.</td>
<td>2.879</td>
<td>97.276</td>
</tr>
<tr>
<td>3.</td>
<td>3.257</td>
<td>110.048</td>
</tr>
<tr>
<td>4.</td>
<td>2.964</td>
<td>100.136</td>
</tr>
<tr>
<td>5.</td>
<td>2.943</td>
<td>99.439</td>
</tr>
<tr>
<td>6.</td>
<td>3.152</td>
<td>106.480</td>
</tr>
<tr>
<td>Mean</td>
<td>3.035</td>
<td>102.540</td>
</tr>
<tr>
<td>SD</td>
<td>0.142</td>
<td>1.940</td>
</tr>
<tr>
<td>CV, %</td>
<td>4.686</td>
<td>7.860</td>
</tr>
</tbody>
</table>

C\textsubscript{th}= theoretical concentration
C\textsubscript{exp}= experimental concentration
SD= standard deviation
CV, % = coefficient of variation

Acceptance criteria:
- 67% Total QCs must be 100±15% nominal values
- 50% QCs per level must be 100±15% nominal values
- Mean % nominal 100±15%
- CV(%) ≤15%

The assay has proven to be suitable to determine the bisoprolol concentration in the bioequivalence study of Bisoprolol 10 mg coated tablets produced by Antibiotice S.A. (referred to as test drug) versus Concor\textregistered 10 mg coated tablets produced by Merck (referred to as reference drug). In figure 4, average bisoprolol concentrations recorded for 22 volunteers are plotted against time for both test and reference drugs. The bisoprolol concentration reaches the maximum values in two hours after the administration of the investigated medicines. The concentration profiles are similarly, fitting the results obtained for the in vitro dissolution test (see fig. 5)

![Graph showing average bisoprolol concentrations recorded for the test and reference drugs in the bioequivalence study performed on 22 healthy volunteers.](image-url)
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![Graph showing average dissolved bisoprolol recorded for test and reference drugs recorded for the in vitro dissolution test.](image)

**Fig. 5.** Average dissolved bisoprolol recorded for test and reference drugs recorded for the *in vitro* dissolution test.

Based on the determined bisoprolol concentrations, the calculated pharmacokinetic parameters demonstrated that the medicine produced by Antibiotice S. A. is bioequivalent with the one produced by Merck.

**Conclusions**

Under the present HPLC conditions, bisoprolol and the internal standard were well separated without any interfering from other plasma components and the absorbance showed a linear relationship with the analyte concentration, allowing to quantify low concentrations of substances with good precision and accuracy and so, suitable for pharmacokinetic studies.

**References:**

5. LI DING, XIA ZHOU, XIAOFENG GUO, QINXIN SONG, JIAN CHANG HE, GUILI XIU, “LC-ESI-MS method for the determination of bisoprolol in human plasma”, Journal of pharmaceutical and biomedical analysis, 2007, 44, 2, (302);