Determination of In Vivo Chiral Inversion of (R,R)-Formoterol to its Stereoisomers, (S,S)-Formoterol, (R,S)-Formoterol, and (S,R)-Formoterol, by Highly Sensitive and Specific LC/MS/MS Methods in Human Plasma

Zhe-ming Gu1,2, Dawei Zhou1, Mei Huo1, Roger Hsu2, Robert Hsu1, and Gary Maier2
1XenoBiotic Laboratories, Inc., 107 Morgan Lane, Plainsboro, NJ 08536; 2 Sepracor Inc., 84 Waterford Drive, Marlborough, MA 01752

Introduction

Formoterol (1R,3R)-formoterol, (1R,3S)-hydroxy-S′ -(1-hydroxy-2-[3-methyl-phenyl][1-[amino]-3-ethyl]formamide) is a selective, potent, and long-acting β2-adrenoceptor agonist currently under FDA review for the long-term maintenance treatment of chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema. Formoterol contains two chiral centers with four stereoisomers, i.e., (R,R)-, (R,S)-, (S,R)-, and (S,S)-formoterol. Arformoterol is the most potent isomer among the four diastereoisomers. The (S,S)-formoterol is 1,000-fold less potent as a β2-agonist than arformoterol. In the current study, in vivo chiral inversion from arformoterol to the other stereoisomers was investigated. Two HPLC methods were established for separation of the four formoterol isomers in human plasma. Each method was used to separate (R,R)-, (R,S)-, (S,R)-, and (S,S)-formoterol and the other to separate (R,R)- and (S,S)-formoterol. The analysis was monitored by liquid chromatography-tandem mass spectrometry (LC/MS/MS) with the lower limit of quantitation (LOQ) at 0.5 and 1 pg/mL, respectively.

Experimental (Cont.)

Human Plasma Samples:


Results and Discussion

1. Baseline separation of the four formoterol isomers can be achieved under the normal phase (NP) chiral-HPLC conditions (Figure 1). This study, due to long run time (>10 min), insufficient sensitivity, and the unstable chromatographic conditions, two HPLC/MS/MS methods were developed and validated for the determination of in vivo chiral inversion of (R,R)-formoterol to the other three isomers. The HPLC Method 1 was used to determine (R,S)- and (S,R)-formoterol in the plasma sample, subsequently, (S,S)-formoterol was determined by HPLC Method 2.

2. The two pairs of enantiomers, i.e., (R,R)(S,S)-formoterol and (R,S)(S,R)-formoterol, were separated using a regular C18 column under basic HPLC mobile phase conditions (Figure 3). A high LC/MS sensitivity was achieved under positive ion mode, at ca. pH 9 LC conditions. Reproducible separation was obtained under mixtures of acetonitrile and methanol (Figure 4). Acceptable precision and accuracy were achieved with an LLOQ at 0.5 pg/mL human plasma for each component.

Conclusion

The results indicated that there was no evidence of in vivo chiral inversion from arformoterol to (S,S)-formoterol, (S,R)-formoterol, or (S,S)-formoterol in humans after single dose and multiple doses at steady state from healthy (adult, elderly) subjects and diseased (COPD, Asthma, Renal impaired) subjects after inhalation administration of (R,R)-formoterol.

Support for this study provided by Sepracor Inc.