

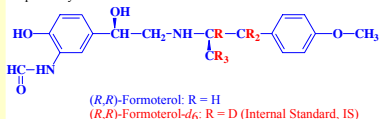
Determination of In Vivo Chiral Inversion of (*R,R*)-Formoterol to its Stereoisomers, (*S,S*)-Formoterol, (*R,S*)-Formoterol, and (*S,R*)-Formoterol, by Highly Sensitive and Specific LC/MS/MS Methods in Human Plasma

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Introduction

Arformoterol (*(R,R)*-formoterol, (*R,R*)-2'-Hydroxy-5'-(1-hydroxy-2-[*p*-methoxy-methylphenyl]-amino)-ethyl]-formanilide) is a selective, potent, and long-acting β_2 -adrenoreceptor agonist currently under FDA review for the long term maintenance treatment of bronchoconstriction in patients with 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*)-formoterols. Arformoterol is the most potent isomer among the four diastereoisomers. The (*S,S*)-formoterol is 1,000-fold less potent as a β -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. One method was used to separate (*R,R*)/(*S,S*)- and (*R,S*)/(*S,R*)-formoterols and the other to separate (*R,R*)- and (*S,S*)-formoterols. The analytes were monitored by liquid chromatography-tandem mass spectrometry (LC/MS/MS) with the Limit of Quantitations (LOQs) at 0.5 and 1 pg/mL respectively.



Experimental

Human Plasma Samples:

Selected plasma samples from four clinical studies, titled

1. The pharmacokinetics and safety of a single dose of 50 μ g (*R,R*)-formoterol in healthy elderly subjects - (*R,R*)-formoterol tartrate inhalation solution (Sepracor Study No. 091-013).
2. A double-blind, double-dummy, randomized, placebo and active-controlled, multicenter, parallel-group study of (*R,R*)-formoterol in the treatment of subjects with chronic obstructive pulmonary disease (Sepracor Study No. 091-050).
3. Pharmacokinetics of (*R,R*)-Formoterol Tartrate Inhalation Solution in Subjects with Renal Insufficiency (Sepracor Study No. 091-014).

Experimental (Cont.)

4. A safety, efficacy and tolerability study of multiple once-daily doses of (*R,R*)-formoterol tartrate inhalation solution in subjects with asthma. (Sepracor Study No. 091-004).

Extraction Procedures:

The analytes and internal standards were quantitatively extracted from human plasma using solid phase extraction (SPEC PLUSTM C18). The organic solvent extract was evaporated to dryness under a nitrogen stream. The residue was reconstituted with CH₃OH-H₂O (3:7, v/v).

Mass Spectrometry:

MS System: AB Sciex API 4000 tandem mass spectrometer coupled with Turbo-Ion Spray interface using multiple reaction monitoring (MRM) detection under positive ion mode

Ion Transitions: Formoterols: *m/z* 345 \rightarrow *m/z* 149
Formoterols-*d*₆ (IS): *m/z* 351 \rightarrow *m/z* 155

Liquid Chromatography:

LC System: Pump – Shimadzu LC-10AD
System Controller – Shimadzu SCL-10A

HPLC Method 1 (Achiral, Basic LC)

Column: Zorbax Extend C18, 3 μ m, 50 \times 2.1 mm
Mobile Phase: A. 0.03%DEA in H₂O,
B. CH₃CN-CH₃OH (7:3, v/v)

Gradient: A:B=92:8 (0.2 min), 2.8 min to A:B=86:14 (2 min),
0.3 min to 90% B (0.2 min), 0.2 min to initial

Flow Rate: 0.5 mL/min

Injection Vol.: 30 μ L

Analytes: (*R,R*)/(*S,S*)- and (*R,S*)/(*S,R*)-formoterols

Quantitation: 0.5 – 50 pg/mL

HPLC Method 2 (Chirabiotic-T, Chiral)

Column: Chirobiotic T2, 250 \times 2 mm
Mobile Phase: CH₃OH containing 0.2% CH₃COOH and
0.006% NH₄OH

Flow Rate: 0.3 mL/min

Injection Vol.: 35 μ L

Analytes: (*R,R*)- and (*S,S*)-formoterol

Quantitation: 1 – 50 pg/mL

Results and Discussion

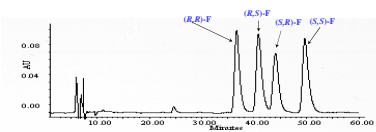


Figure 1. Normal Phase Chiral HPLC Chromatogram of four formoterol isomers

1 Base-line separation of the four formoterol isomers can be achieved under the normal phase (NP) chiral-HPLC conditions (Figure 1). However, due to long run time (>60 min), insufficient sensitivity, and the unstable chromatographic conditions, two HPLC/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*)-formoterols 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.

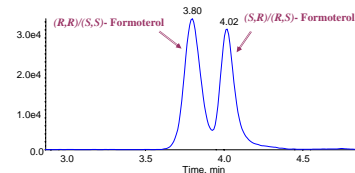


Figure 3. Mass ion chromatogram of four formoterol isomers eluted from a C18 HPLC column under basic LC conditions (HPLC/MS Method 1)

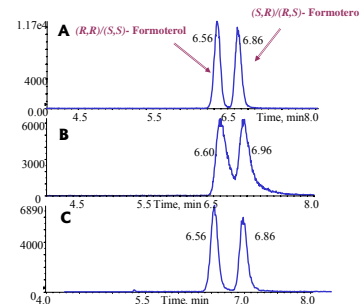


Figure 4. The ion chromatograms of (*R,R*)/(*S,S*)-formoterol and (*R,S*)/(*S,R*)-formoterol with CH₃CN as mobile phase B from the first sample injection (A) and the 30th injection (B). (C) The ion chromatogram at the 200th injection with CH₃CN:CH₃OH (7:3) as mobile phase B. (HPLC/MS Method 1)

3 (*R,R*)-Formoterol and (*S,S*)-formoterol were resolved using a chirobiotic T2 column (Figure 5). A highly sensitive HPLC/MS method was developed and validated with LLOQ at 0.5 pg/mL human plasma for each isomer.

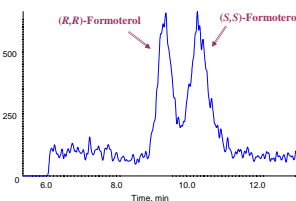


Figure 5. Mass ion chromatogram of (*R,R*)-formoterol and (*S,S*)-formoterol at LLOQ (0.5 pg/mL, HPLC/MS Method 2)

4 Selected plasma samples were obtained from single and multiple (steady state) dose studies with healthy (adult, elderly) subjects as well as diseased state (COPD, Asthma, renal impaired) subjects. Plasma samples were also selected from various time points (up to 6 hours postdose) and pooled from various subjects (up to 10 subjects) for both healthy and diseased subjects. The pooled samples were processed and analyzed by the two validated HPLC/MS/MS methods. No mass ion peaks corresponding to (*R,S*)-formoterol, (*S,R*)-formoterol, (*S,S*)-formoterol, were observed in any pooled human plasma samples (Figure 6).

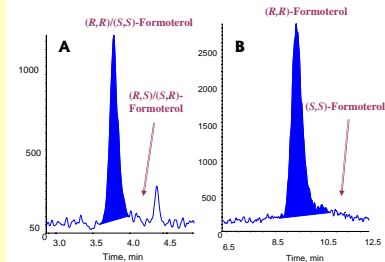


Figure 6. Mass ion chromatogram of (*R,R*)-formoterol in a pooled 6 h COPD patient plasma (3.0 pg/mL) analyzed by HPLC/MS Methods 1 (A) and 2 (B)

Conclusion

The results indicated that there was no evidence of *in vivo* chiral inversion from arformoterol to (*R,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.

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