

# Sensitive and Rapid Method for the Determination of (*R,R*)-Formoterol and (*S,S*)-Formoterol in Human Plasma Using

## Chiral Liquid Chromatography-Tandem Mass Spectrometry

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### Introduction

Arformoterol, i.e., (*R,R*)-formoterol; (*R,R*)-2'-Hydroxy-5'-[1-(1-hydroxy-2-[[p-methoxy-methylphenyl]-amino]-ethyl]-formanilide), is a selective, potent, and long-acting  $\beta_2$ -adrenoreceptor agonist bronchodilator that was recently approved by the FDA for the long term twice daily 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*)-formoterol. The current commercially available product of formoterol, Foradil<sup>®</sup>, is a racemic mixture of (*R,R*)- and (*S,S*)-formoterol. However, (*R,R*)-formoterol (arformoterol) is the most potent isomer among the four diastereoisomers and the (*S,S*)-formoterol is 1,000-fold less potent as a  $\beta$ -agonist than arformoterol.



In order to study arformoterol tartrate inhalation solution and Foradil<sup>®</sup> in patients with mild to moderate COPD, an open label, randomized, multiple dose, 3-way crossover, multicenter, and inpatient and outpatient study was conducted.

A sensitive and robust LC-MS-MS method for the analysis of (*R,R*)- and (*S,S*)-formoterol in human plasma with calibration range of 0.500-50.0 pg mL<sup>-1</sup> was developed and validated to support this study. An additional objective of this investigation was to determine the potential for *in vivo* chiral inversion from (*R,R*)-formoterol to (*S,S*)-formoterol.

### Experimental

#### Sample preparation

(*R,R*)- and (*S,S*)-Formoterol and the internal standards were extracted from 1.2 mL of stabilized human plasma by solid phase extraction (SPE). The desired methanol (2 x 0.5 mL) extracts were evaporated to dryness under a nitrogen stream and the residue was reconstituted with 160  $\mu$ L of 10 mM ammonium formate buffer.

### Liquid chromatography

LC System: Pump Shimadzu LC-10AD  
 Autosampler Shimadzu SIL-HT  
 System Controller Shimadzu SCL-10A  
 Analytical Column: Astec Chirobiotic T2 column (250 x 2.0 mm)  
 Mobile Phase: Methanol/Acetonitrile/H<sub>2</sub>O containing 10 mM NH<sub>4</sub>COOH: 78/20/2 (v/v/v) with 0.25% CH<sub>3</sub>COOH and 0.006% NH<sub>4</sub>OH  
 Flow rate: 0.2 mL/min (14.5 min), 0.1 min to 0.45 mL/min  
 Injection Volume: 30  $\mu$ L

### Mass spectrometry

MS System: PE SCIEX API-4000  
 Condition: LC(+)-ESI-MS/MS (MRM)  
 The mass spectrometer was set up for the following transitions:  
 (*R,R*)- and (*S,S*)-Formoterol: 345.2 → 149.1  
 (*R,R*)- and (*S,S*)-Formoterol-*d*<sub>6</sub>: 351.3 → 155.2

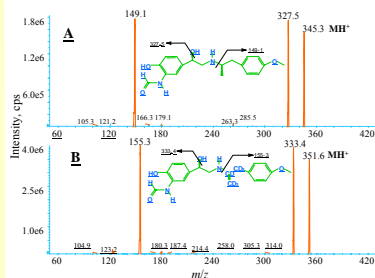


Figure 1. Product ion spectra of (*R,R*)-Formoterol (A) and (*R,R*)-Formoterol-*d*<sub>6</sub> (B)

Table 1. Precision and accuracy of calibration standards (n=3)

	0.500	1.00	2.00	5.00	15.0	25.0	50.0 (pg mL <sup>-1</sup> )
( <i>R,R</i> )-Formoterol							
Mean	0.506	0.982	1.98	5.05	14.9	24.7	51.4
CV	7.91%	6.92%	1.26%	5.52%	2.97%	4.29%	0.64%
RE	1.20%	-1.80%	-1.00%	1.00%	-0.67%	-1.20%	2.80%
( <i>S,S</i> )-Formoterol							
Mean	0.493	1.03	2.00	5.14	15.0	24.3	49.8
CV	2.03%	2.52%	3.85%	1.60%	1.26%	1.86%	1.89%
RE	-1.40%	3.00%	0.00%	2.80%	0.00%	-2.80%	-0.40%

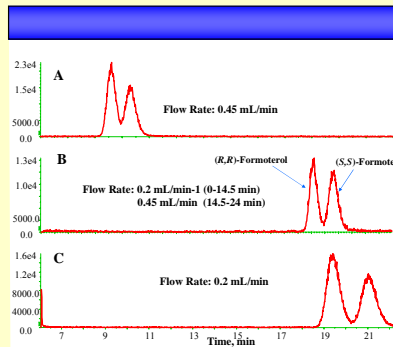


Figure 2. The ion chromatograms of (*R,R*)- and (*S,S*)-formoterol at different LC flow rate. (A) 0.45 mL/min, (B) 0.2 mL/min change to 0.45 mL/min at 14.5 minutes, (C) 0.2 mL/min.

Table 2. Precision and accuracy of quality control samples

	( <i>R,R</i> )-Formoterol (pg mL <sup>-1</sup> )			( <i>S,S</i> )-Formoterol (pg mL <sup>-1</sup> )		
	1.50	10.0	40.0	1.50	10.0	40.0
Intraday (n=5)						
Mean	1.55	10.2	39.8	1.47	10.2	40.7
CV	0.90%	1.97%	1.62%	1.70%	2.62%	1.58%
RE	3.33%	2.00%	-0.50%	-2.00%	2.00%	1.75%
Interday (n=15)						
Mean	1.59	10.1	39.4	1.48	10.1	40.1
CV	3.27%	2.14%	3.10%	4.80%	2.58%	2.21%
RE	6.00%	1.00%	-1.50%	-1.33%	1.00%	0.25%

Table 3. Stability of the samples

Compound	Freeze/thaw (3x)	Bench-top (24 h)	Extracts (n <sub>1</sub> > 2 days)	Extracts (n <sub>2</sub> > 3 days)	Long-term (>70 °C > 1 year)
( <i>R,R</i> )-Formoterol (%)	94.0-96.0	85.3-86.0	87.0-98.0	98.5-101.3	103.9-107.5
( <i>S,S</i> )-Formoterol (%)	93.5-101.3	87.8-94.0	90.3-99.3	96.8-99.3	96.7-102.0

### Results and Discussion

#### HPLC flow rate effect on the chiral separation

It was found that the separation performance varied with the HPLC flow rate for the Chirobiotic T2 column. The lower the flow rate, the better the separation efficiency. As shown in Fig. 2C, acceptable separation was achieved at 0.2 mL min<sup>-1</sup>, but showed band broadening. A better peak shape was obtained at a higher flow rate of 0.45 mL min<sup>-1</sup>, but separation efficiency decreased (Fig. 2A). A combination of low (0.2 mL min<sup>-1</sup>) and high (0.45 mL min<sup>-1</sup>) flow rates resulted in the best separation performance (Fig. 2B).

#### Precision and accuracy

Table 1 shows the validation data on accuracy and precision of each standard concentration. Table 2 presents the inter-day and intra-day accuracy and precision data of QC samples. The data demonstrate that this method is reproducible and reliable with acceptable values of CV and RE.

#### Stability of the analytes

Table 3 shows the stability evaluation data. Three freeze/thaw cycles and ambient temperature storage of the QC samples for up to 24 h prior to analysis, appeared to have little effect on the determination of (*R,R*)- and (*S,S*)-formoterol in human plasma. QC samples stored in a freezer at  $\leq 70$  °C remained stable through about one year. The extracted QC samples were allowed to stand at ambient temperature for two days or refrigeration condition ( $-4$  °C) for three days prior to injection. No effect on quantitation of (*R,R*)- and (*S,S*)-formoterol in human plasma was observed.

#### Clinical Study Design and Results

This was part of an open label, randomized, multiple dose, 3-way crossover, multi-center, inpatient and outpatient study to compare the pharmacokinetic (PK) profile of arformoterol tartrate inhalation solution and Foradil<sup>®</sup> in male and female subjects with mild to moderate COPD.

> Subjects received the following treatments in random order for 13 consecutive days and a single dose on the morning of the 14th day:

- 12 mg of racemic formoterol fumarate (Foradil<sup>®</sup> Aerolizer<sup>TM</sup>) BID
- 15  $\mu$ g of nebulized arformoterol tartrate inhalation solution BID

> PK sampling: pre-first dose, 15, 30 and 45 minutes and 1, 2, 6, 8, 12, 24, 48, 72 and 96 hours post-dose.

The resultant concentration vs. time curves for the isomers contained within the formoterol product are shown in Fig. 3.

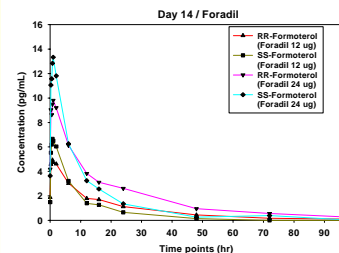


Figure 3. Day 14 concentration versus time (hr) profile after oral inhalation administration of 12  $\mu$ g or 24  $\mu$ g Foradil dose in human

### Study Conclusions

No matrix interference was observed across the elution windows of (*R,R*)-formoterol and (*S,S*)-formoterol, indicating the specificity of the method. Acceptable intra-day and inter-day assay precision ( $\leq 4.8\%$  CV) and accuracy ( $\leq 6.0\%$  RE) were observed over a linear range of 0.5-50 pg/mL. The mean (n=3) correlation coefficients in human plasma were 0.9994 $\pm$ 0.0002 and 0.9985 $\pm$ 0.0005 for (*R,R*)-formoterol and (*S,S*)-formoterol, respectively. The mean extraction recovery was 79.8% for (*R,R*)-formoterol and 82.7% for (*S,S*)-formoterol. The validated method has been successfully employed to quantify (*R,R*)-formoterol and (*S,S*)-formoterol in human plasma collected from the clinical study. No inversion of (*R,R*)-formoterol to (*S,S*)-formoterol was observed.

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