

A Highly Sensitive and Specific LC/MS/MS Method (0.5 pg/mL) for Quantitation of (R,R)/(S,S)-Formoterol and (R,S)/(S,R)-Formoterol in Human Plasma and Urine

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Overview

A sensitive and specific liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method capable of quantifying (R,R)/(S,S)-Formoterol and (R,S)/(S,R)-Formoterol in Human Plasma and Urine is described.

In this method, the drug was extracted from plasma and urine using a solid phase extract method. The chiral separation was performed on a reverse phase C18 column with a special mobile phase, so that the sensitivity was dramatically increased. The use of this mobile phase also avoided the partial binding of the analytes to the column.

The method has been successfully applied to pharmacokinetic studies in human plasma and urine samples.

Introduction

In a recent study for obstructive airway disease, including asthma and COPD, (R,R)-formoterol exhibited a rapid onset of action comparable to the short-acting bronchodilator, VENTOLIN[®], as well as a duration of action of up to 24 hours. Many analytical methods have been developed for pharmacokinetic studies or clinical trials. For racemic formoterol, there is a HPLC method which used a normal phase chiral column for (R,R)-, (R,S)-, (S,R)-, and (S,S)-formoterol separation and UV detections with each run time as long as 60 minutes. However, to support clinical trials, a short run time and lower detection limit are needed. We now report a rapid, specific, and highly sensitive LC/MS/MS method capable of quantifying (R,R)-, (R,S)/(S,R)-, and (S,S)-formoterol at levels as low as 0.5 pg/mL for plasma sample¹. This method includes two parts, the first is to separate and quantify (R,R)/(S,S)- and (R,S)/(S,R)-formoterol as demonstrated in this poster. The other is to separate (R,R)- and (S,S)-formoterol which will be reported later.

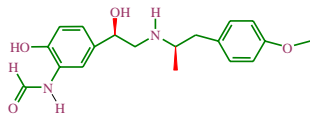


Figure 1. The structure of (R,R)-Formoterol

Experimental

Sample Preparation

The analyte and internal standard were extracted from human plasma and urine using solid phase extraction. The organic solvent extract was evaporated to dryness under a nitrogen stream, and the residue was reconstituted with reconstitute solution.

Liquid chromatography:

LC System: Pump Shimadzu LC-10AD
 Autosampler Shimadzu
 System Controller Shimadzu SCL-10A
 Analytical Column: C18 column, 2.1 x 50 mm, 3 μm
 Gradient
 Flow rate: 0.5 mL/min
 Injection Volume: 20 μL

Mass Spectrometry

MS System: AB Sciex API-4000
 Condition: LC(+)-ESI-MS/MS (MRM)
 The mass spectrometer was set up for the following transition:
 Formoterol: 345.2 → 149.1
 Formoterol-d₆: 351.3 → 155.1

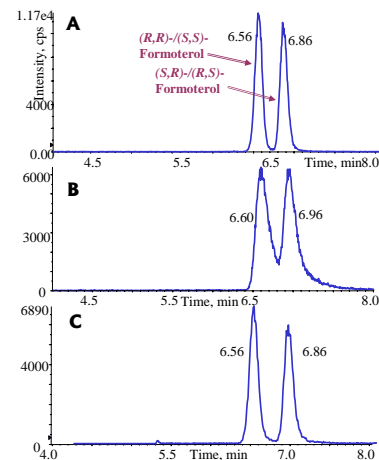


Figure 2. The ion chromatograms of (R,R)/(S,S)-formoterol and (R,S)/(S,R)-formoterol with ACN as mobile phase B at the first sample injection (A), and at the 30th injection (B). (C) The ion chromatogram at the 200th injection with our special mobile phase B.

Plasma	Calibration Range		0.5 to 50 pg/mL			
	Correlation coefficient (r, 3 batches)					
Accuracy & Precision	QC	Conc. (pg/mL)	RR-/SS-Formoterol		RS/SR-Formoterol	
			Accuracy	Precision	Accuracy	Precision
Inter-Batch (n=15)	LLOQ	0.5	0.00	5.60	-3.40	11.59
	Low	1.5	1.33	3.62	-0.67	6.04
	Medium	20	2.00	3.90	5.00	3.80
	High	40	1.25	4.12	2.50	3.83
Method Recovery			%			
RR-/SS-Formoterol			74.16		to 80.80	
RS-/SR-Formoterol			80.16		to 86.63	
Urine	Calibration Range		5 to 1000 pg/mL			
	Correlation coefficient (r, 3 batches)					
Accuracy & Precision	QC	Conc. (pg/mL)	RR-/SS-Formoterol		RS/SR-Formoterol	
			Accuracy	Precision	Accuracy	Precision
Inter-Batch (n=15)	LLOQ	5	-2.00	5.20	-3.40	4.55
	Low	15	0.67	4.33	3.33	4.48
	Medium	450	1.11	4.46	0.67	3.38
	High	900	-2.33	4.21	-2.44	4.78
Method Recovery			%			
RR-/SS-Formoterol			67.15		to 79.66	
RS-/SR-Formoterol			69.37		to 78.91	

Table I. Human plasma and urine validation results

Results and Discussion

In some previous work, a normal phase chiral column was used to separate (R,R)-, (R,S)-, (S,R)-, and (S,S)-formoterol with a long run time (>60 min) and less sensitivity. In this study, we used reverse phase C18 column, so that the sensitivity was dramatically increased.

If acetonitrile is used as HPLC mobile phase B, the chiral separation was getting worse after several ten sample injections (Figure 2). A special solvent was developed in this study to replace acetonitrile, and the peak separation was almost constant after couple hundred injections.

For urine samples, a special buffer solution was applied in the sample clean up. Without this washing step, the column resolution would deteriorate rapidly (Figure 3).

Excellent linearity was obtained with a correlation coefficient greater than 0.996. The inter-day precision (CV%) and accuracy (RE%) for all QC plasma and urine samples, including LLOQ were ≤11.5% and ≤5%, respectively (Table I). Three freeze/thaw cycles and ambient temperature storage QC samples for up to 4 h prior to analysis, appeared to have little effect on the quantitation.

References

- Gu, Z., Zhao, X., Maier, G., Hsu, R. S. H. AAPS Annual Meeting October 27-31 2003, Salt Lake City, Utah.

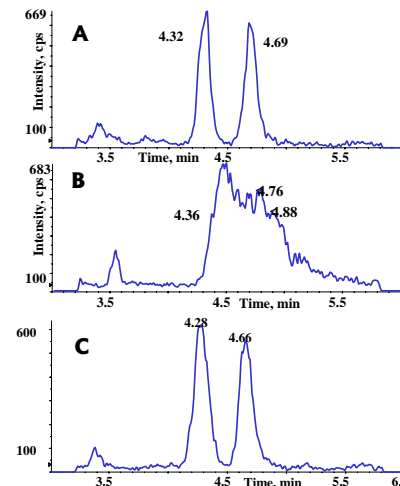


Figure 3. The comparison of the ion chromatograms with and without buffer clean step in the sample preparation. (A) The 1st sample injection without the buffer clean. (B) The 15th injection without the buffer clean. (C) The 35th injection with the buffer clean.

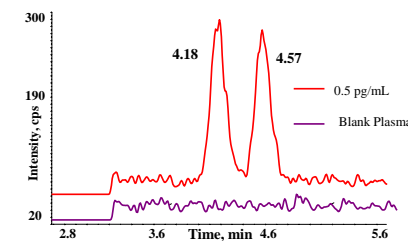


Figure 4. Representative chromatograms of formoterol at the lowest limit of quantitation (LLOQ) in plasma

Conclusion

A rapid and specific LC/MS/MS method was developed and validated for quantifying (R,R)/(S,S)-Formoterol and (R,S)/(S,R)-Formoterol with the lowest limit of quantitative level as low as 0.5 pg/mL for plasma samples and 5 pg/mL for urine samples.