

# A Highly Sensitive and Specific LC/MS/MS Method (3 pg/mL) for Quantitation of Fluticasone Propionate in Human Plasma

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## Overview

A sensitive liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method capable of quantifying fluticasone propionate down to 3 pg/mL in human plasma is described.

In this method, the drug was extracted from plasma using liquid-liquid extraction. A special mobile phase was employed, so that intense and consistent ion signals were achieved. The method was validated over linear range of 3-1000 pg/mL. Excellent linearity, accuracy, and precision were obtained.

The method has been successfully applied to bioequivalent human plasma sample analysis.

## Introduction

Fluticasone propionate, the active ingredient, is used to relieve the nasal symptoms of seasonal and year-round allergies. It helps reduce the inflammation that leads to symptoms that include congestion, sneezing, and itchy, runny nose. In recent years, many analytical methods have been developed for pharmacokinetic studies or clinical trials. However, to support clinical trials in human plasma, an even lower detection limit is needed by regulatory requirements. We now report a rapid, specific, and highly sensitive liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method capable of quantifying fluticasone propionate from human plasma at levels as low as 3 pg/mL.

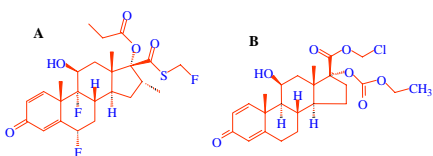


Figure 1. The structure of fluticasone propionate (A), and internal standard (B).

## Experimental

### Sample Preparation

A mixture of human plasma sample and internal standard solution was partitioned with an organic solvent. The organic solvent extract was evaporated to dryness under a nitrogen stream, and the residue was reconstituted with reconstitution solution.

### Liquid chromatography:

LC System: Pump Shimadzu LC-10AD  
Autosampler Shimadzu  
System Controller Shimadzu SCL-10A  
Analytical Column: Ace C18 column, 2.1 x 50 mm, 3 μm  
Gradient  
Flow rate: 0.5 mL/min  
Injection Volume: 35 μL

### Mass Spectrometry

MS System: PE Sciex API-3000  
Condition: LC/(-)ESI-MS/MS (MRM)  
The mass spectrometer was set up for the following transition:  
Fluticasone Propionate: 499.1 → 413.2  
Internal Standard 465.2 → 345.1

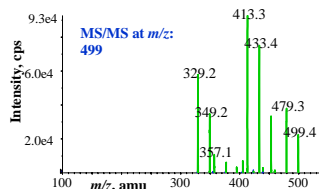


Figure 3. Product ion mass spectrum of fluticasone propionate.

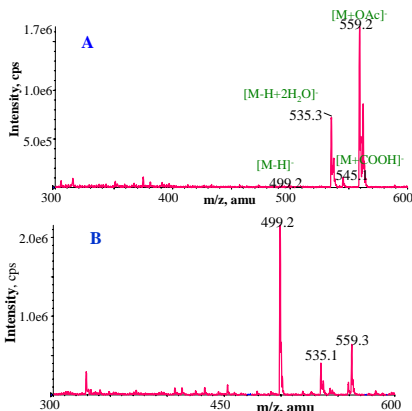


Figure 2. LC/(-)ESI-MS spectra of fluticasone propionate with general mobile phase (A), and that with the mobile phase in this method (B).



Figure 6. LC-MS System

- Excellent linearity was obtained with a correlation coefficient greater than 0.995 (Figure 5). The inter-day precision (CV%) and accuracy (RE%) for all QC samples, including LLOQ were <10% and <7%, respectively (Table I).

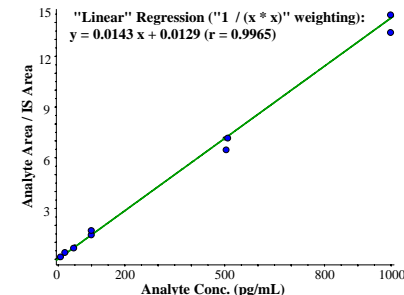


Figure 5. Representative calibration curve for the measurement of fluticasone propionate in human plasma

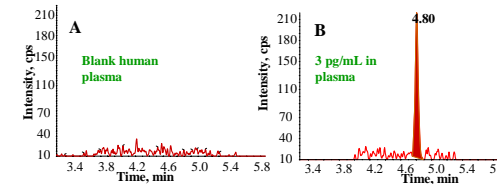


Figure 4. Representative chromatograms of fluticasone propionate in plasma

## Results and Discussion

- In some previous work, using a general LC mobile phase gave an observed ion other than [M-H] as shown in Figure 2A which affected detection limits and caused concern regarding specificity. Our method employed special mobile phases, so that the ion [M-H], became a dominate peak (Figure 2B). Intense and consistent ion signals were achieved.

- APCI ionization mode was used in a previous method based on the molecular structure as shown in Figure 1. In our method, ESI negative mode was used, which dramatically increases the signal intensity (Figure 4).

Calibration Range		3 to 1000 pg/mL	
Correlation coefficient (r, 3 batches)		0.9955 to 0.9974	
Accuracy & Precision		Accuracy	Precision
		RE%	CV%
Inter-Batch (n=18)	QC		
	LLOQ	3	3.33
	Low	8	-6.87
	Medium	200	-6.00
	High	800	-6.87
		Compared with Nominal Value (RE%)	
Storage Stability		Condition	%
Room Temperature		~ 18 hrs	-11.25 to -8.37
Freeze and Thaw		-20C, 3 cycles	3.25 to 5.13
In Process		~ 4C, 3 days	7.38 to 8.50
Long-term		-20C, 77 days	-5.62 to -3.62
Method Recovery			%
Fluticasone Propionate			94.10 to 97.14
IS			83.25 to 90.35

Table I. Human plasma validation results

## Conclusion

The newly developed liquid chromatography-tandem mass spectrometry assay using a special LC mobile phase with API-3000 mass spectrometer substantially improves, sensitivity, accuracy, and precision for the quantitation of fluticasone propionate in human plasma at a concentration range of 3-1000 pg/mL.