

Overview

A sensitive and specific liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method capable of quantifying budesonide in human plasma is described.

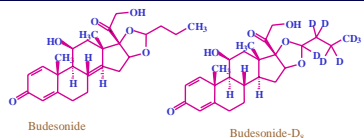
In this method, the drug was extracted from a 0.5 mL sample of plasma using a reverse phase C18 96 well plate solid phase extraction method. Separation was performed on a reverse phase C18 column. Detection was achieved using a AB/SCIEX API-5000 tandem mass spectrometer employing turbo-ion spray ionization in the positive ion mode along with multiple reaction monitoring (MRM). The lower limit of quantitation was 10 pg/mL.

This method has been successfully applied to clinical pharmacokinetic studies.

Introduction

Budesonide, an inhaled glucocorticoid for asthma control, is being investigated for the local treatment of colitis. The drug is efficiently metabolized in the liver after oral administration, resulting in very low plasma levels. For example, maximum plasma levels after oral administration of clinically relevant doses of budesonide are in the lower ng/mL range. To support these clinical trials, a short sample preparation time and a sub ng/mL detection limit are desirable. Current published bioanalytical methods are not capable of quantifying budesonide to 10 pg/mL in human plasma. We now report a rapid, specific, and highly sensitive LC-MS/MS method capable of quantifying budesonide at levels as low as 10 pg/mL from 0.5 mL of human plasma.

Structures



Experimental

Sample Preparation

Budesonide and the internal standard (budesonide-D₃) were transferred onto preconditioned Strata C18-E Polymer 96-well plates (Phenomenex). The plates were preconditioned with 0.8 mL of acetonitrile, 0.8 mL of methanol and 0.8 mL of water. The loaded plates were then washed with 2 mL of water and 2 mL of 20% methanol in water. Both analyte and internal standard were then eluted using 90% acetonitrile in water.

The eluted samples were evaporated to dryness for approximately 30 min in a 96-Well Nitrogen Evaporator (EvapArray™) at approx. 35 °C. The residue was dissolved in 125 µL of 50% methanol in water.

Liquid Chromatography:

HPLC System: Shimadzu LC-10AD
Analytical Column: C8 column, 2.1 x 50 mm, 5 µm
Mobile Phase A) 10 mM ammonium Acetate in water (pH4)
Mobile Phase B) Acetonitrile
Gradient
Flow rate: 0.4 mL/min
Injection Volume: 20 µL

Mass Spectrometry

MS System: AB Sciex API-5000
Condition: LC(+)/ESI-MS/MS (MRM)
MRM Transition:
Budesonide: 431.2 → 323.1
Budesonide-D₃: 439.4 → 323.1

Results and Discussion

Table I. Validation Data Summary

Calibration Range			
10 to 2,000 pg/mL			
Accuracy & Precision	QC Conc. (pg/mL)	Accuracy	Precision
		RE%	CV%
Inter-Batch (n=18)	LLOQ 10	-1.30	14.2
	Low 30	0.33	7.28
	Medium 750	-2.40	3.29
	High 1500	-0.67	3.33
Method Recovery			
Compared with Nominal Value (%)			
≥90.00			
Method Recovery	Condition	Accuracy	
		RE%	
Freeze/Thaw	3 Cycles, -20 °C	<1.33	
Bench-Top	4 hrs, Room Temperature	<6.00	
Autosampler Extract Stability	4 Days, Room Temperature	<12.0	
Long-Term Storage Stability	35 Days, -20 °C	<5.37	

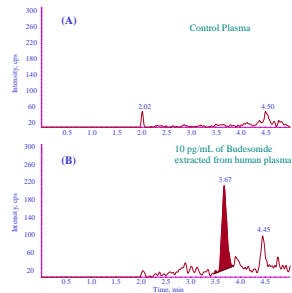


Figure 1. Ion chromatograms of blank plasma (A), and 10 pg/mL budesonide extracted from plasma (B)

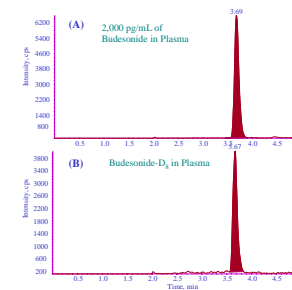


Figure 2. Ion chromatograms of 2,000 pg/mL budesonide extracted from plasma (A), and the internal standard extracted from plasma (B)

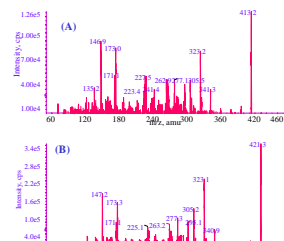


Figure 3. LC(+)-ESI-MS-MS product ion spectra of budesonide (A) and budesonide-D₃ (B)

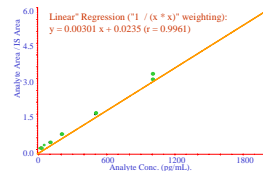


Figure 4. Representative calibration curve for the determination of budesonide in the range from 10 pg/mL to 2,000 pg/mL in human plasma

- In most previous methods, negative electron spray ionization mode was utilized in LC-MS/MS analysis, which restricted the MS detection limit. Larger plasma sample volumes (1-2 mL) were needed in order to achieve a lower detection limit. These sample volumes would make preparation much longer and require large individual SPE cartridges and introduce more error in the preparation procedure.

- The current method employed positive ion mode which dramatically increase the MS sensitivity, and therefore, only 0.5 mL of human plasma was required to reach the requisite sensitivity.

- A 0.5 mL sample volume afforded the use of a 96-well SPE plate format, instead of individual cartridges. In addition, the TOMTEC liquid handling system made sample preparation quick and consistent.

- Excellent linearity was obtained with a correlation coefficient greater than 0.9934. The inter-day precision (CV%) and accuracy (RE%) for all QC plasma samples, including LLOQ were ≤14.2% and ≤0.33%, respectively (Table I). Three freeze/thaw cycles, ambient temperature storage for up to 4 h prior to analysis, and three month long-term storage at -20°C appeared to have little effect on the quantitation.

Conclusions

A rapid and specific LC-MS/MS method has been developed and validated for quantifying budesonide with a lower limit of quantitation of 10 pg/mL from a 0.5 mL plasma sample.