LC-MS/MS Method for the Quantification of Budesonide in Human Plasma

Using the SCIEX Triple Quad™ 5500+ – QTRAP® Ready System

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Glucocorticoids are a type of corticosteroid, which are a class of steroid hormones. Glucocorticoids bind to the glucocorticoid receptor and are part of the feedback mechanism in the immune system. Pharmaceutical products of the glucocorticoids class are anti-inflammatory. Budesonide is one such product used in the treatment of asthma, allergic rhinitis and various skin disorders. Budesonide is formulated as extended release tablets and inhalers and has low bioavailability requiring a sensitive (low pg/mL) bioanalytical method for its accurate quantitation.

In this application note, a bioanalytical LC-MS/MS method is presented for the quantitation of budesonide from human plasma using the SCIEX Triple Quad 5500+ System with a range of 2 to 1024 pg/mL.

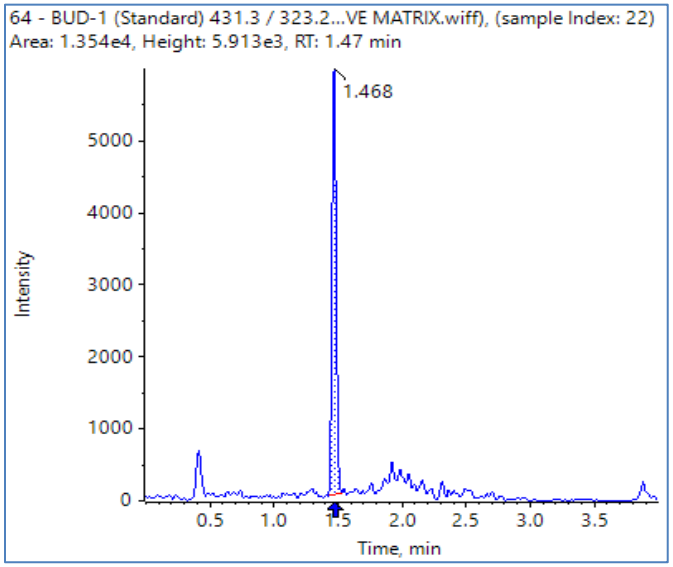

Figure 1. Quantitation of Budesonide in Human Plasma. Example chromatogram of budesonide in human plasma at 64 pg/mL. A single LC-MS/MS peak (1.47 min) was used for generation of the calibration curve as the two epimers were not separated by this chromatographic method.

Key Features of the SCIEX Triple Quad 5500+ System for Bioanalysis

- The SCIEX Triple Quad 5500+ – QTRAP Ready LC-MS/MS System is a quantitative platform with exceptional performance in speed, polarity switching, linear dynamic range, and ease of use data handling with SCIEX OS-Q Software.
- Detector system, with high energy dynode (HED) and multi-channel electron multiplier (CEM) provides up to six orders of linear dynamic range (LDR) for broad and sensitive quantitative performance[1]
- Robustness required for bioanalysis with the Turbo V™ Source and Curtain Gas™ Interface
- Triple quadrupole functionality for quantitation *plus* full scan MS/MS for confirmation and MRM[3] for selectivity with the QTRAP Ready system

Methods

Sample Preparation and Processing: K_2EDTA Human plasma (Bioreclamation IVT) was spiked with budesonide (Sigma-Aldrich) to create a set of calibration standards from 1024 to 2 pg/mL. A 200 µL aliquot of each sample was processed by first diluting with an equal volume of water containing internal standard (budesonide-D8). The diluted samples were loaded on a Phenomenex Strata-X RP SPE cartridge that was conditioned with methanol and water. The SPE cartridge was washed with 5% methanol and then eluted with 100% methanol. The eluent was dried under vacuum and then reconstituted with 40% acetonitrile in water for injection.

Chromatography: Chromatographic separation was performed using the ExionLC™ AD system. Details are outlined in Table 1.

Mass Spectrometry: The SCIEX Triple Quad 5500+ system was operated in positive electrospray ionization mode. The MS and source conditions were optimized for maximum sensitivity and set as follows: positive MRM, curtain gas set at 30; ion source temperature 600°C, ion source gas (GS1) at 60 and drying gas (GS2) at 80; ionization voltage at 3000 V; and dwell time 75 ms for all transitions. The compound dependent parameters for analyte and internal standard were as follows:

Table 2. Mass Spectrometry Conditions. Optimized MRM transitions for the analyte and internal standard for Budesonide**.**

Compound	Q1	Q3	DP	EP	CE	CXP
Budesonide-1	*431.3*	*323.2*	*70*	*10*	*19*	*21*
Budesonide-2	*431.3*	*147.1*	*70*	*10*	*35*	*21*
Budesonide-D8	439.3	323.2	*70*	*10*	*19*	*21*

Data Processing: Each standard was injected in triplicate. Data was acquired in Analyst® Software 1.7.1. Quantitive data was processed in SCIEX OS-Q software and a 1/x2 weighted linear regression was used to calculate the concentrations.

Table 1. Chromatography Conditions.

Column	*C18, 100 x 2.1 mm, 1.7 µm*
Mobile Phase	*A: 5 mM ammonium bicarbonate* *B: CH_3CN*
Flow rate	*500 µL/min*
Column temperature	*50 °C*
Injection volume	*10 µL*

Gradient Profile	Time (min)	% B
	0	*40*
	2.0	*95*
	3.0	*95*
	3.25	*40*
	4.0	*40*

Quantitative Results

The budesonide drug product is a mixture of two epimers (22R and S) and both are active. In this method, the two epimers were not chromatographically resolved and a single LC-MS/MS peak was integrated. An example chromatogram is shown in Figure 1.

Different sample extraction techniques were tried to remove interferences, reduce matrix suppression and achieve the desired LLOQ of 2 pg/mL. The SPE technique outlined above achieved all three. Figure 2 shows an example chromatogram of the IS blank sample (A) without interference at the retention time of budesonide and the LLOQ calibration standard (B, 2 pg/mL).

In the present method, linearity was established in the range of 2.0 to 1024 pg/mL in human plasma. The calibration curve is shown in Figure 3 with correlation coefficient r = 0.99. Table 3 shows the accuracy and precision data of the calibration standards. All are within the acceptance criteria of %CV ±20% at LLOQ level and ±15% at other levels.

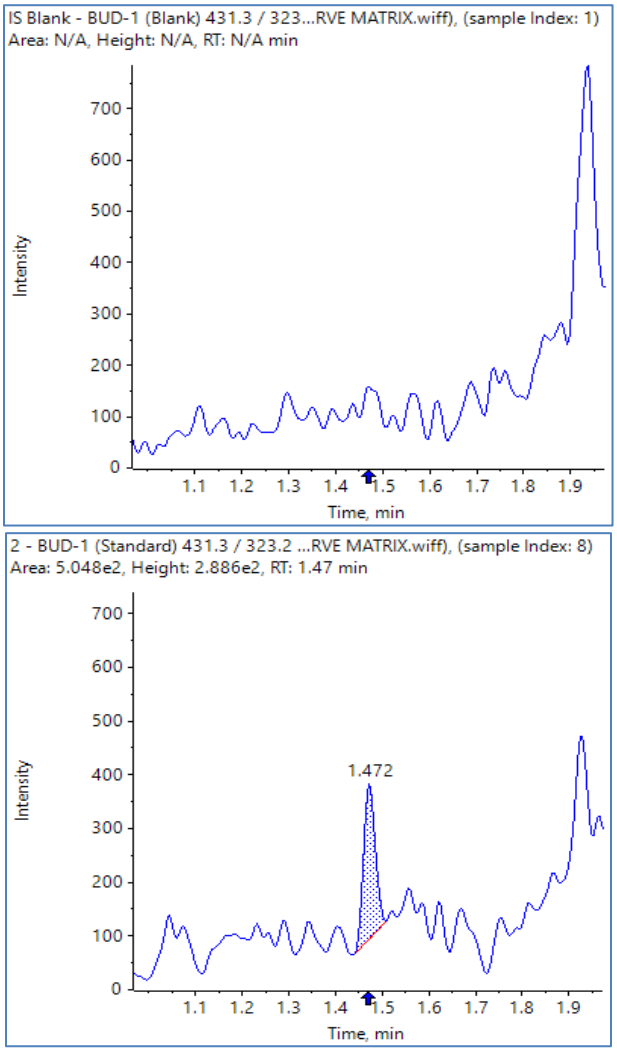

Figure 2. Example Chromatograms. Example chromatogram of the IS blank (A) and LLOQ calibration standard (B, 2 pg/mL) of budesonide in human plasma.

Table 3. Statistics Table For Concentration Curve. The reproducibility and accuracy for the concentrations across the concentration curve for the SCIEX Triple Quad 5500+ System were well within the bioanalytical requirements.

Concentration (pg/mL)	%CV	Accuracy
2.0	*10.01*	*100.17*
4.0	*12.17*	*96.12*
8.0	*7.50*	*102.64*
16	*11.68*	*105.66*
32	*9.98*	*95.48*
64	*7.79*	*101.52*
128	*4.18*	*101.01*
256	*5.80*	*97.76*
512	*4.23*	*97.66*
1024	*1.91*	*99.90*

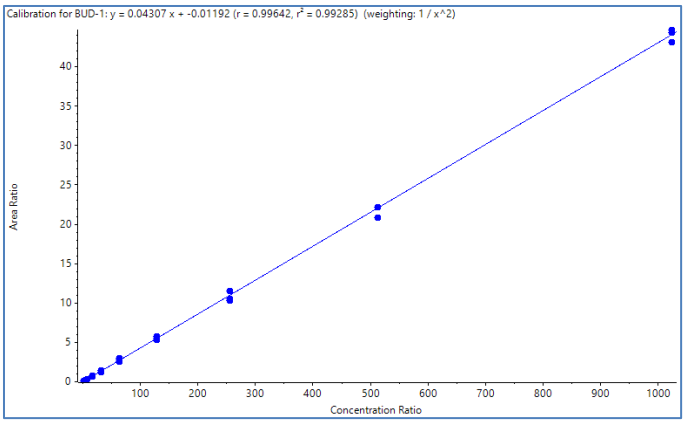

Figure 3. Calibration Curve. A linear calibration curve for budesonide was generated from 2.0 to 1024 pg/mL.

Conclusions

A selective and sensitive bioanalytical method was developed for the detection of budesonide, with an LLOQ of 2 pg/mL in human plasma with the SCIEX Triple Quad 5500+ LC-MS/MS System.

References

1. Collins BA, Dima MA Ivosev G, Zhong F (2013) A high dynamic range pulse counting detection system for mass spectrometry. *Rapid Comm Mass Spect* **28(2)**, 209-216.

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