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Quantification of Budesonide Using UPLC and Xevo TQ-S

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Abstract

This work demonstrates the benefits of the Regulated Bioanalysis System Solution from Waters, which combines solid phase extraction methodology, UPLC chromatography, and an advanced tandem quadrupole MS. The system is used for the development of a highly sensitive method for quantification of budesonide in plasma that also offers high selectivity and throughput.

Benefits

Addresses the challenges including instrutment's high throughput capability, robustness, ability to address upcoming analytical demands, and ability to address regulatory guidelines.

Introduction

Budesonide is a glucocorticoid steroid used for the treatment of asthma and noninfectious rhinitis (including hay fever and other allergies), and also for treatment and prevention of nasal polyposis. In addition, it is used for Crohn's disease (inflammatory bowel disease). Budesonide, in comparison with prednisolone, has been associated with fewer bone density losses and, unlike other corticosteroids, has little influence on hypothalamic-pituitary-adrenal axis, which also limits the need of tapering before discontinuation. Overall, Budesonide has a lower incidence of systemic manifestations than similar medications.

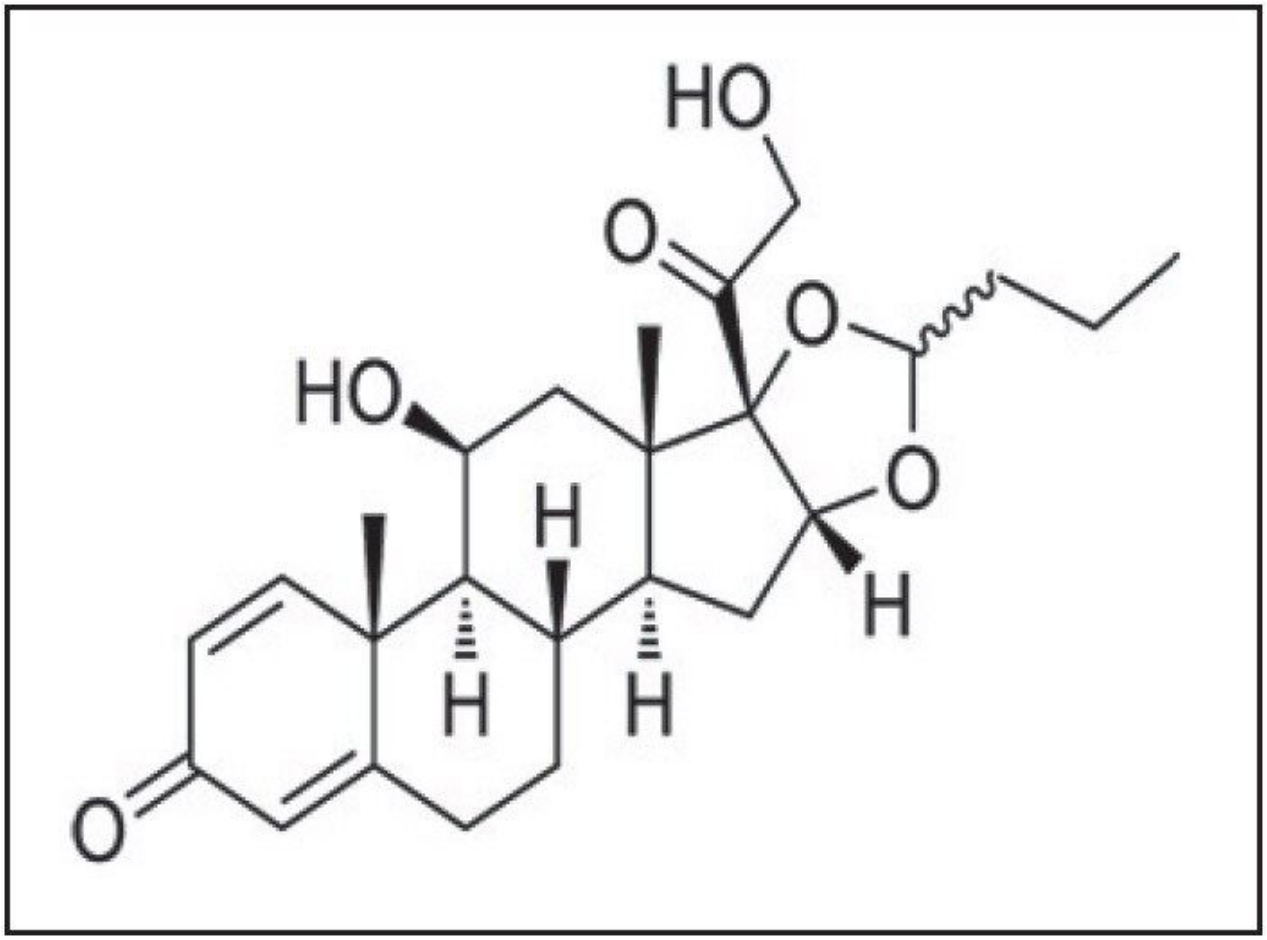

Figure 1. Molecular structure of Budesonide.

Estimating Budesonide at low levels in complex matrices, such as human plasma, is a challenge due to the extremely low circulatory levels in plasma (10% bioavailability) and high affinity to bind proteins. In this application note, we successfully report an LC-MS/MS analysis of Budesonide with an LLOQ of 2 pg/mL.

Experimental

The samples were isolated using solid phase extraction with a Waters Sep-Pak C_{18} (1 cc, 50 mg) Cartridge. A 500 µL aliquot of plasma was precipitated with zinc sulfate, then diluted with ammonia and loaded onto the SPE cartridge previously conditioned with organic solvent and water. The plasma solution was then washed with water followed by an organo-aqueous solution and elution in solvent. The eluted samples were

evaporated to dryness and reconstituted in 50% acetonitrile. Zinc sulfate and acetonitrile were purchased from Fluka (MO, USA).

Results and Discussion

The chromatographic method utilized an ACQUITY UPLC System with an ACQUITY UPLC BEH C_{18} 150 mm, 1.7 µm, 2.1 x 150 mm Column; this provided excellent resolution for the Budesonide analyte from the endogenous components in the samples. Budesonide eluted at 2.71 min with a peak width of 12 s at the base. The data illustrates both the blank signal and the signal obtained from the lower limit of quantification (LLOQ) of Budesonide in human plasma, shown in Figure 2.

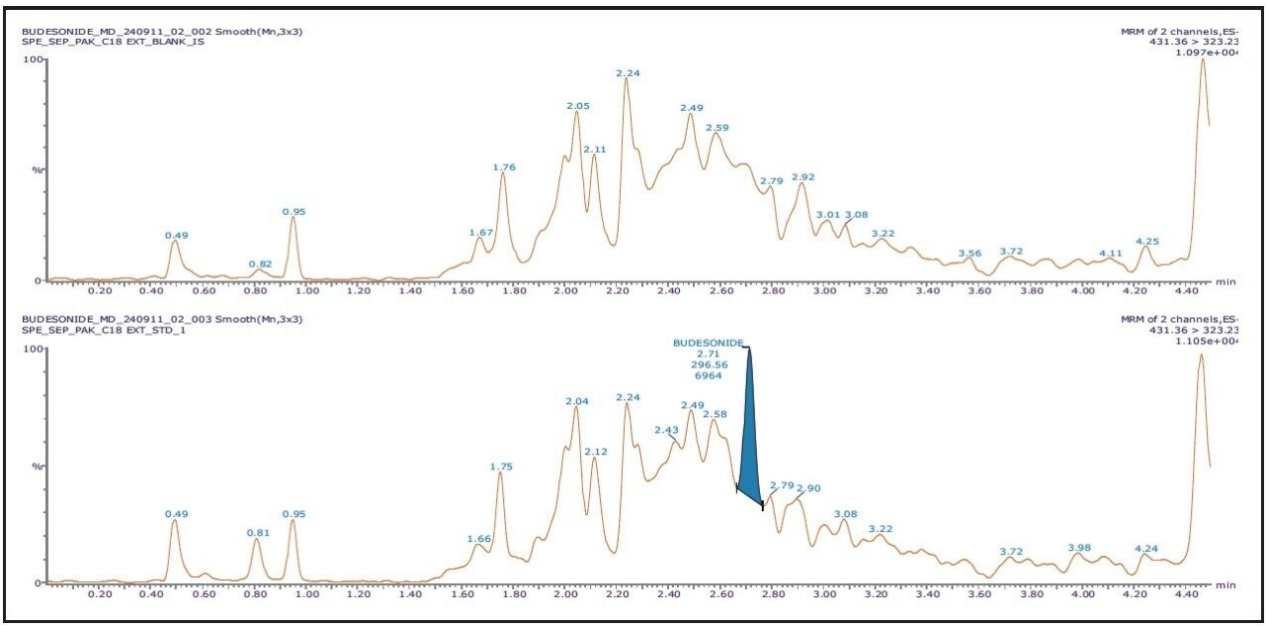

Figure 2. Chromatogram of blank sample (top) and LLOQ concentration (2 pg/mL) of Budesonide (bottom) obtained using an ACQUITY UPLC System coupled with a Xevo TQ-S MS.

As seen in Figure 2, the retention time of Budesonide does not interfere with the background; the signal corresponding to the analyte of interest can be easily observed even at the LLOQ level. The Xevo TQ-S MS is equipped with a novel StepWave ion guide, which when combined with the high-resolution chromatography obtained from the ACQUITY UPLC System, results in successful completion of extremely sensitive applications to be performed with high reproducibility. As observed from Figure 2, the benefits of Xevo TQ-S

along with outstanding sensitivity observed from UPLC allows detection of Budesonide at a concentration of 2 pg/mL, with a signal-to-noise ratio of 35:1.

The assay in this report showed linear calibration over the range of 2 to 256 pg/mL with an excellent r^2 value of 0.992, shown in Table 1 and Figure 3. The back-calculated concentration of the standard was found to be within ±12% of the nominal concentration (Table 1), and an excellent degree of accuracy was achieved for each sample. This assay was performed with a 7 min injection-to-injection time scale highlighting the capability of Waters Bioanalysis System solution to deliver highly sensitive and specific results while maintaining desired precision and high throughput value.

Sample	Type	Nominal	Area	Area Ratio	IS Area	Accuracy
BLANK	Blank		25	0.01433	206	102.56
External Standard 1	Standard	2	315	0.05235	6013	97.9
External Standard 2	Standard	4	418	0.09869	4233	88.54
External Standard 3	Standard	8	857	0.17741	4833	109.5
External Standard 4	Standard	16	1860	0.43676	4259	107.41
External Standard 5	Standard	32	3610	0.8556	4219	89.69
External Standard 6	Standard	64	6597	1.42784	4620	99.49
External Standard 7	Standard	128	13065	3.1663	4126	104.91
External Standard 8	Standard	256	27649	6.67609	4142	102.56

Table 1. Calibration data of Budesonide over the range of 2 to 256 pg/mL.

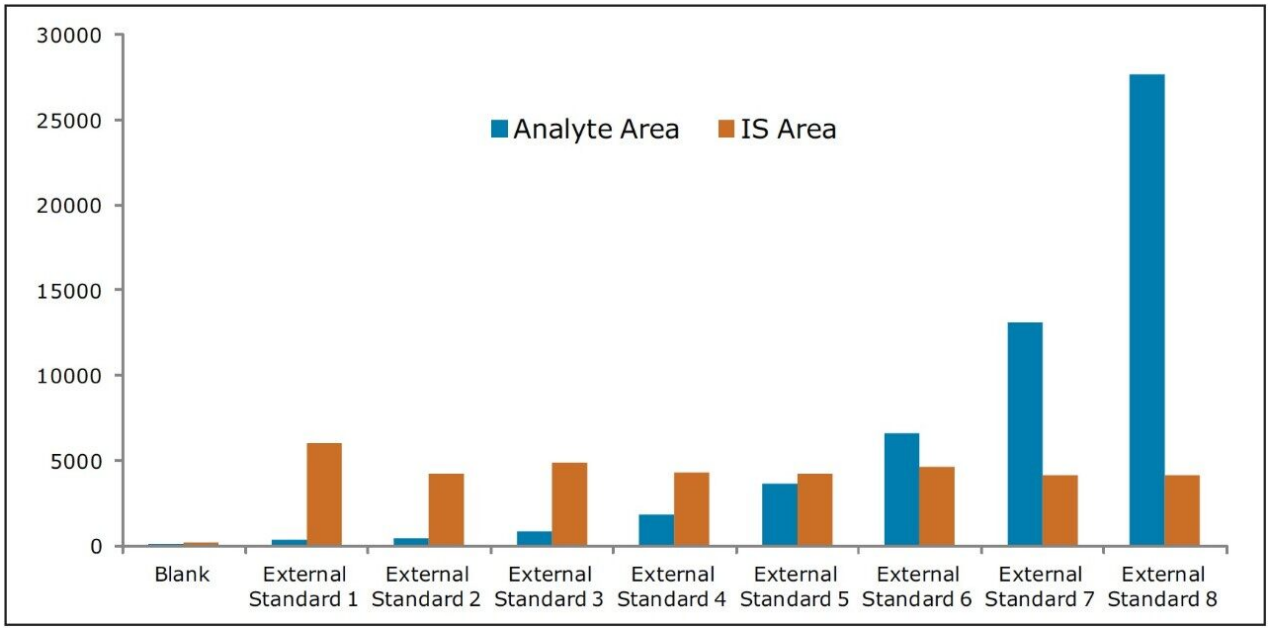

Figure 3. Comparison of area under curve for Budesonide (analyte) and IS for the concentration range of 2 to 256 pg/mL.

Recovery of the analyte and internal standard (IS) was performed by comparison of extracted QC samples against six post-extracted samples and was found to be approximately 75% at LQC, MQC, and HQC levels for both analyte and IS (Figure 4 and Table 2). The %CV for repeat batches were found to be within 10% of LLOQQC and varied between 1% to 3% for all QC levels.

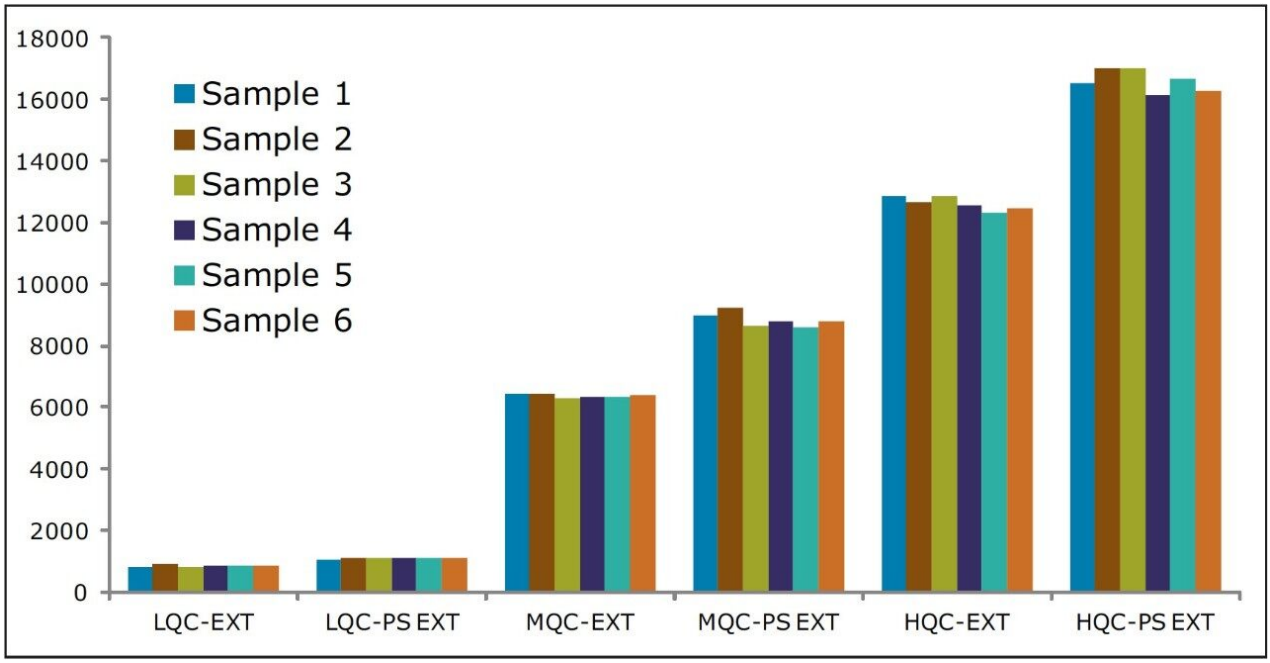

Figure 4. Analyte recovery (area under the curve) from six samples of Budesonide at LQC, MQC, and HQC concentrations.

LQC	MQC	HQC
77.1	72.1	76
Mean Analyte Recovery (%) = 75		

Table 2. Mean analyte recovery (%) of Budesonide at LQC, MQC, and HQC levels.

As can be observed from the data shown in Figure 4, the analyte recovery values for the six samples for all three concentration levels (LQC, MQC, and HQC) did not vary significantly. In addition, as detailed in Table 2, the mean analyte recovery for the three concentration ranges was well within acceptable limits.

For a comparison of samples within the global batches, three separate batches were prepared with six samples in each batch for LLOQQC, LQC, MQC, and HQC concentration levels. The data showed excellent agreement between the six samples in all the three batches (Table 3). The mean accuracy obtained for all the sample levels was found to be > 94% for every concentration (Table 3). This outstanding quality of data was

achieved using the Regulated Bioanalysis System solution.

GLOBAL DATA												
P-A-Batch-01	LLOQQC_1	2	2.280	LQC_1	8	7.460	MQC_1	64	57.933	HQC_1	128	129.695
	LLOQQC_2	2	2.230	LQC_2	8	7.329	MQC_2	64	58.336	HQC_2	128	125.022
	LLOQQC_3	2	2.323	LQC_3	8	7.027	MQC_3	64	59.956	HQC_3	128	130.657
	LLOQQC_4	2	1.968	LQC_4	8	7.571	MQC_4	64	59.211	HQC_4	128	131.516
	LLOQQC_5	2	2.193	LQC_5	8	7.543	MQC_5	64	56.85	HQC_5	128	128.242
	LLOQQC_6	2	1.958	LQC_6	8	7.245	MQC_6	64	56.642	HQC_6	128	128.428
P-A-Batch-02	LLOQQC_1	2	1.966	LQC_1	8	7.891	MQC_1	64	65.914	HQC_1	128	119.348
	LLOQQC_2	2	1.596	LQC_2	8	8.172	MQC_2	64	64.218	HQC_2	128	122.465
	LLOQQC_3	2	2.163	LQC_3	8	7.597	MQC_3	64	64.024	HQC_3	128	119.825
	LLOQQC_4	2	1.932	LQC_4	8	8.026	MQC_4	64	66.349	HQC_4	128	122.48
	LLOQQC_5	2	1.803	LQC_5	8	7.774	MQC_5	64	64.09	HQC_5	128	123.182
	LLOQQC_6	2	1.845	LQC_6	8	7.951	MQC_6	64	64.28	HQC_6	128	120.825
P-A-Batch-03	LLOQQC_1	2	1.978	LQC_1	8	7.033	MQC_1	64	56.47	HQC_1	128	124.581
	LLOQQC_2	2	1.912	LQC_2	8	7.776	MQC_2	64	58.221	HQC_2	128	123.702
	LLOQQC_3	2	1.727	LQC_3	8	6.708	MQC_3	64	56.233	HQC_3	128	124.916
	LLOQQC_4	2	1.958	LQC_4	8	7.243	MQC_4	64	56.18	HQC_4	128	124.972
	LLOQQC_5	2	1.514	LQC_5	8	7.199	MQC_5	64	57.332	HQC_5	128	121.007
	LLOQQC_6	2	2.007	LQC_6	8	7.255	MQC_6	64	57.967	HQC_6	128	122.278
	Mean		1.884			7.902			64.813			121.354
	SD		1.886			0.200			1.0349			1.58
	%CV		10.010			2.530			1.60			1.30
	Accuracy		94.210			98.770			101.27			94.81

Table 3. Comparison of the three separate batches, each containing six Budesonide samples at the LLOQQC, LQC, MQC, and HQC concentration levels.

Conclusion

Budesonide is a glucocorticoid steroid used for the treatment of asthma and rhinitis. However, due to the extremely low circulatory levels in plasma (10% bioavailability) and high affinity to bind proteins, estimation of Budesonide at low levels in complex matrices like human plasma is a challenging task. In this application note and with the successful use of Waters Regulated Bioanalysis System solution, we report an LC-MS/MS analysis of Budesonide with an LLOQ of 2 pg/mL. In addition to achieving the sensitivity demands, there are several other challenges that affect today's biopharmaceutical companies. These challenges include the instrutment's high throughput capability, robustness, ability to address upcoming analytical demands, and

ability to address regulatory guidelines. The results detailed in this application note successfully address each of these challenges.

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Regulated Bioanalysis Platform Solution with UNIFI <https://www.waters.com/134613325>

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