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Highly sensitive quantitative analysis of budesonide from plasma using LC/MS/MS

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Introduction

Budesonide is a glucocorticoid used in the management of asthma, treatment of various skin disorders, and allergic rhinitis. Budesonide is provided as a mixture of two epimers (22R and 22S). Interestingly, the 22R form is two times more active than the 22S epimer. The two forms do not interconvert. [1]

Inflammation is an important component in the pathogenesis of asthma. Corticosteroids like budesonide have been shown to have a wide range of inhibitory activities against multiple cell types (e.g., mast cells, eosinophils, neutrophils, macrophages, and lymphocytes) and mediators (e.g., histamine, eicosanoids, leukotrienes, and cytokines) involved in allergic and non-allergic-mediated inflammation. These anti-inflammatory actions of budesonide contribute to their efficacy in the aforementioned diseases. Budesonide undergoes significant first-pass elimination and its bioavailability is 10 %, which demands its low level quantitation in plasma for bioanalysis. Budesonide is formulated as an extended release tablet and inhalers. [2] Here, an LC/MS/MS method has been developed for highly sensitive quantitation of budesonide (as shown in Figure 1) from plasma using LCMS-8060, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan.

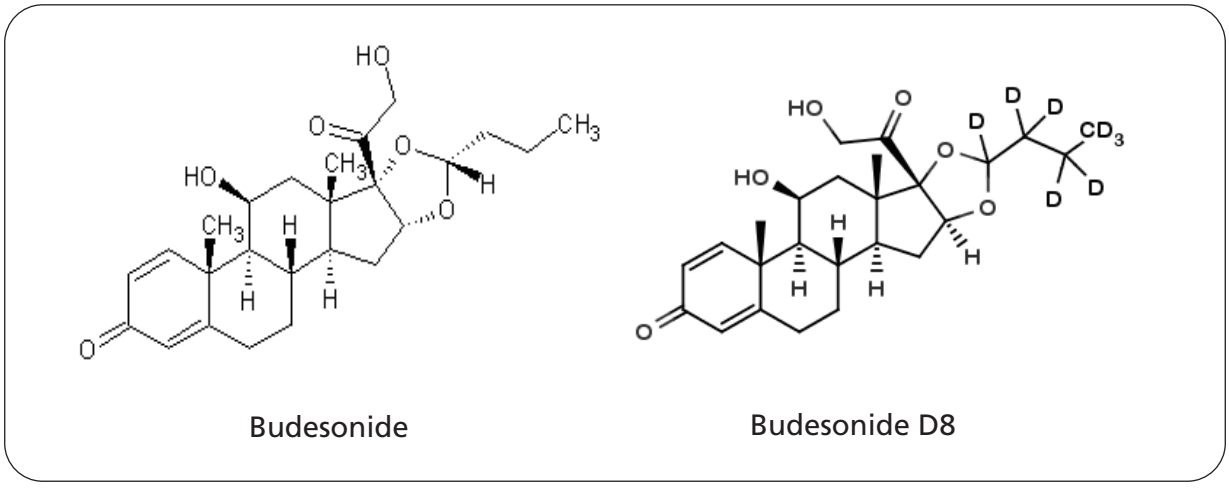

Figure 1. Structure of budesonide and budesonide D8

Materials and method

Sample preparation

- **Preparation of spiked calibration standards and quality control (QC) samples**

Budesonide (procured from TRC) calibration standards at concentration levels of 2 pg/mL, 5 pg/mL, 10 pg/mL, 20 pg/mL, 50 pg/mL, 100 pg/mL, 150 pg/mL, 200 pg/mL and quality control samples at concentration levels of LQC (7.5 pg/mL), MQC (75 pg/mL) and HQC (175 pg/mL) were prepared in plasma.

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• **Sample extraction**

Spiked calibration standards and quality control samples in plasma were taken in 4 mL RIA vials to internal standard solution (Budesonide D8 procured from TRC) was added except in blank. Then above plasma was centrifuged at 4500 rpm for 5 mins.

• **The samples were extracted by solid phase extraction (SPE) technique as followed:**

1. Conditioning (1mL methanol followed by 1 mL water)
2. Loading (entire plasma sample)
3. Washing (1 mL water followed by 1 mL 5 % methanol)
4. Elution (1 mL methanol)

SPE eluent was evaporated at 50 °C for 25 minutes in low pressure nitrogen evaporator. The residue was reconstituted in 200 µL mobile phase, vortexed and filled in HPLC vials for injection.

LC/MS/MS analysis

Figure 2. Nexera with LCMS-8060

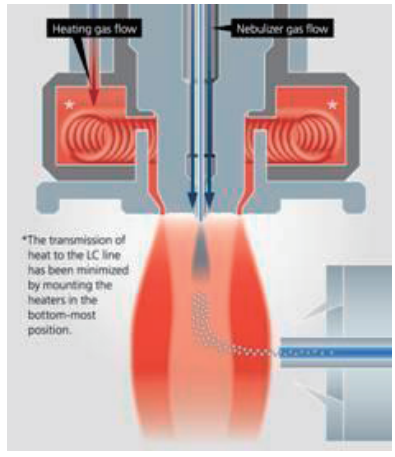

Figure 3. Heated ESI probe

LCMS-8060 triple quadrupole mass spectrometer by Shimadzu (shown in Figure 2), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.
In order to improve ionization efficiency, the newly developed heated ESI probe (shown in Figure 3) combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This development allows high-sensitivity analysis of a wide range of target compounds with considerable reduction in background.

The details of analytical conditions are given in Table 1.

UHPLC conditions (Nexera X2 system)		
Column	:	C18 column100 mm×3 mm, 2.1 um
Mobile phase A	:	Buffer
B	:	Acetonitrile
Flow rate	:	0.4 mL/min
Time program	:	B conc. 40% (0.01 min) - 90% (0.01-1min) - 90% (1.01-2.50min) - 40% (2.51-4.00min)
Injection vol.	:	20 uL
Column temperature	:	40 °C
MS conditions (LCMS-8060)		

Table 1. MRM transitions

Compound	Polarity	MRM transition
Budesonide	positive	431.10 > 323.20
Budesonide D8	positive	439.10 > 323.20

MS interface	:	Electro Spray Ionization (ESI)
Nitrogen gas flow	:	Nebulizing gas- 3 L/min; Drying gas- 10 L/min
Zero air flow	:	Heating gas- 10 L/min
MS temperatures	:	Desolvation line- 250 °C; Heating block- 400 °C Interface- 300 °C

Results

LLOQ of 2 pg/mL was achieved for budesonide in plasma. Overlay of MRM chromatograms of blank and LLOQ for budesonide spiked standards are shown in Figure 4A. Similarly, overlay of the MRM chromatograms of blank and zero standard (Blank+IS) for budesonide D8 are shown in the Figure 4B. No interfering peaks were seen in blank plasma at the retention time of these compounds which confirms the absence of any interference.

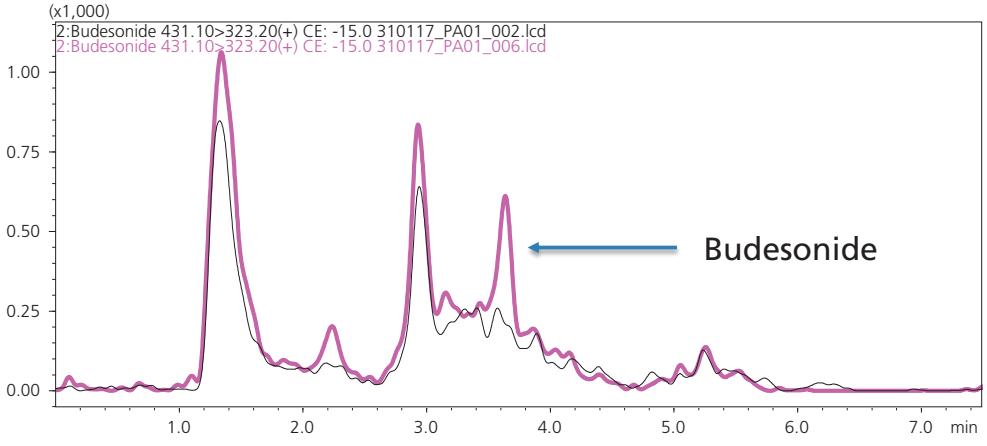

Figure 4A. Overlay of MRM chromatograms of blank and 2 pg/mL for budesonide spiked standard

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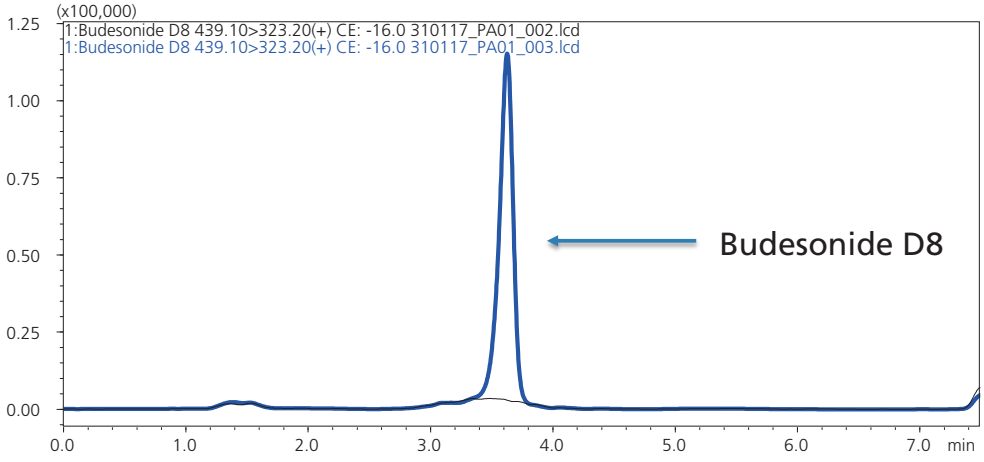

Figure 4B. Overlay of MRM chromatograms of blank and zero standard for budesonide D8

Linearity test was carried out using internal standard calibration method with correlation coefficient of 0.9992 for budesonide as shown in Figure 5.

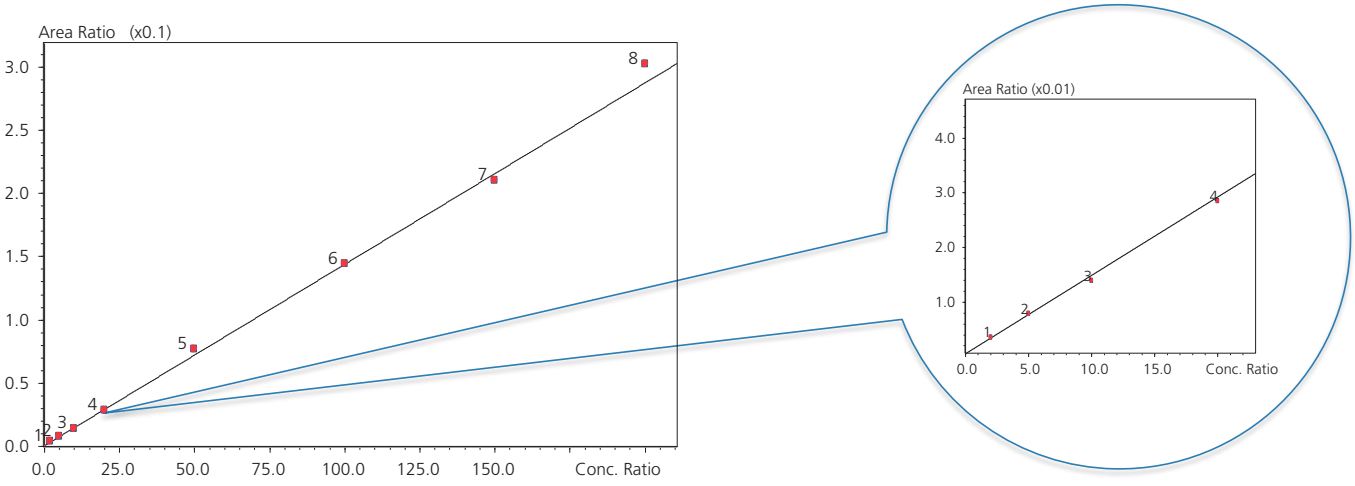

Figure 5. Calibration curve for budesonide spiked standards

Precision and accuracy batch of budesonide meets the bioanalytical acceptance criteria. Results are shown in table 2A and 2B.

Table 2A. Results of accuracy for budesonide spiked calibration standards

Name of compound	Standard concentration (pg/mL)	Calculated concentration from calibration graph (pg/mL)	% accuracy
Budesonide	2	1.997	99.9
	5	5.164	103.3
	10	9.448	94.5
	20	20.088	100.4
	50	51.995	104.0
	100	98.700	98.7
	150	148.574	99.0
	200	202.831	101.4

Table 2B. Results of accuracy and repeatability for budesonide quality control samples

Name of compound	Standard concentration (pg/mL)	Calculated average concentration from calibration graph (pg/mL)	Average % accuracy (n=6)	Average % RSD for area counts (n=6)
Budesonide	7.5 (LQC)	7.264	96.9	5.74
	75 (MQC)	75.285	100.4	6.39
	175 (HQC)	176.429	101.0	4.36

Conclusions

- Ultra-fast and highly sensitive quantitative analysis of budesonide from plasma was developed on LCMS-8060 system.
- Heated ESI probe of LCMS-8060 helped in achieving LLOQ of 2 pg/mL for budesonide with considerable reduction in background. Hence, LCMS-8060 system from Shimadzu gives a complete solution for bioanalysis.

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References

[1] Deventer K., Mikulcikova P., Van Hoecke H., Van Eenoo P., Delbeke F.T. Detection of budesonide in human urine after inhalation by liquid chromatography–mass spectrometry. J. Pharm. Biomed. Anal. 2006;42:474–479.
[2] Streel B., Cahay B., Klinkenberg R. Using total error concept for the validation of liquid chromatography-tandem mass spectrometry method for the determination of budesonide epimers in human plasma. J. Chromatogr. B.

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