T2318

Quantitative Determination of Bosentan in Human Plasma by LC/MS/MS

Authors:

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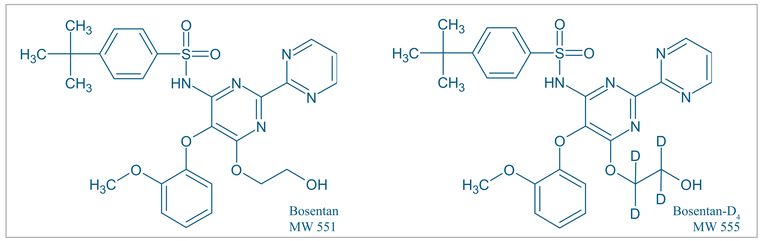
Introduction:

Bosentan is indicated mainly for pulmonary hypertension and is a competitive antagonist of endothelin receptors. Under normal conditions, endothelin-1 binding of these receptors causes pulmonary vasoconstriction. By blocking this interaction, bosentan decreases pulmonary vascular resistance. A bioanalytical assay was developed and validated for bosentan in human plasma to assess the pharmacokinetic properties of bosentan at clinical doses.



Experimental:

Reference Compounds and Chemicals:

Bosentan and bosentan-D_4 internal standard were purchased from Toronto Research Chemicals, Inc. All the chemicals used were AR grade and all solvents were HPLC grade or better.

Standard Solutions:

Stock solutions of bosentan and bosentan-D_4 were prepared in 50 mM sodium carbonate/methanol, 1:1. A series of spiking standard solutions was prepared from dilutions of the stock to cover the quantitation range from 20.0 to 2500 ng/mL.

Quality control (QC) samples were prepared in human plasma at three different concentrations (60.0, 500, and 2,000 ng/mL). These low, medium and high QC samples were stored as 0.300 mL aliquots at -20°C in polyprolylene tubes and were prepared from different stock solutions than the calibration standard samples.

Extraction:

To all samples (0.200 mL) except blank-blank samples, 20 µL of internal standard solution (bosentan-D_4) is added. To all samples 0.200 mL of 0.5% formic acid in water is added and samples were vortexed briefly. Samples are loaded onto 400 mg Biotage supported liquid extraction (SLE) plate. The sample wells are then eluted 4 times with 0.400 mL of methyl-t-butyl ether (MTBE). Elution solvent is evaporated and the dry extract is reconstituted in 0.800 mL of mobile phase.

LC-MS-MS:

The LC-MS-MS system consisted of LC-10AT Shimadzu HPLC pump, Perkin Elmer Series 200 autosampler and SCIEX API 4000 mass spectrometer with a TurboIonSpray interface. The ion transitions monitored were m/z 552→202 for bosentan and m/z 556→202 for bosentan-D_4 (internal standard). Luna C18, 3 micron, 50 x 2.0 mm column (Phenomenex) was used with isocratic mobile phase. The retention time was approximately 1.2 minutes and the run time was 2 minutes.

Mobile Phase consisted of acetonitrile/methanol/5mM ammonium acetate/acetic acid; 38:38:24:1.

Representative Chromatograms:

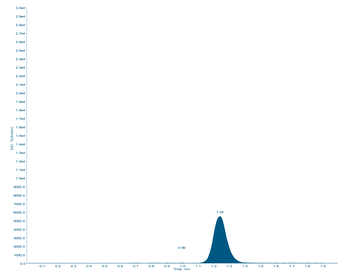


Figure 1. Bosentan LLOQ Sample (20 ng/mL)

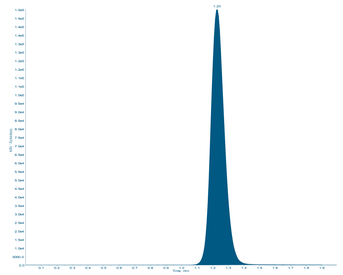


Figure 2. Internal Standard (Bosentan-d4)

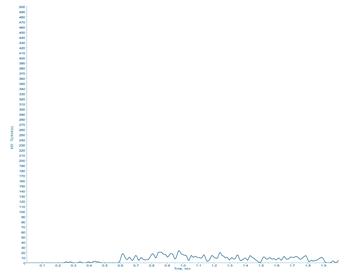


Figure 3. Blank Plasma Sample

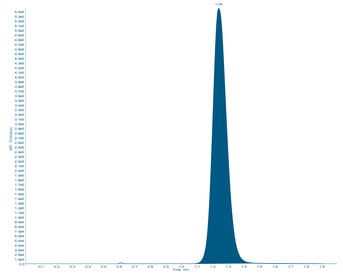


Figure 4. Bosentan ULOQ Sample (2500 ng/mL)

Extraction Graphic

0.200 mL Sample, Spiked as Above
↓
Vortex with 0.200 mL of 0.5% formic acid in water
↓
Load diluted sample to SLE plate (400 mg)
↓
Elute with 4 x 0.400 mL of MTBE
↓
Evaporate to Dryness
↓
Reconstitute in 0.800 mL of mobile phase

Results:

The assay was linear over the range 20.0 to 2500 ng/mL using a plasma volume of 0.200 mL. Three validation runs were performed each on separate days. Precision (%CV) and accuracy (%bias) across all levels of the QC range were within ± 10.5%. The precision and accuracy at the LLOQ level was within 2.0 %. No chromatographic interferences or matrix effects from six different lots of plasma were observed at the retention times of bosentan or the internal standard indicating the specificity of the method. Stability of bosentan in plasma was established for 24 hours at room temperature, 5 cycles of freezing and thawing, and 151 hours in the final extract at room temperature. The long-term stability of bosentan in human plasma was established after 150 days of storage at approximately -20ºC and -70°C.

Standard Curve Samples
Mean of 3 curves over 3 days of Validation

Added (ng/mL)	20.0	40.0	100	250	750	1500	2250	2500
Mean (ng/mL)	20.0	40.3	99.2	253	754	1500	2260	2460
CV (%)	0.3	0.4	1.6	1.9	1.3	2.5	2.5	1.1
Bias (%)	0.0	0.8	-0.8	1.2	0.5	0.0	0.4	-1.6

LLOQ and Quality Control Samples
Mean over 3 days of Validation

	Concentration (ng/mL)			
	2000	500	60.0	20.0
	Intraday			
n	6	6	6	6
Mean (ng/mL)	1940	509	62.0	20.2
CV (%)	10.5	1.6	1.8	1.5
Bias (%)	-3.0	1.8	3.3	1.0
	Interday			
n	18	18	18	18
Mean (ng/mL)	1950	504	61.1	20.1
CV (%)	5.8	2.4	1.8	2.0
Bias (%)	-2.5	0.8	1.8	0.5

Stability Samples

	QC Low	QC High
QC Pool Concentration (ng/mL)	60.0	2000
Mean % Change from QC Pool Concentration		
Benchtop (24 hr)	0.8	-1.2
Freeze/Thaw (5 cycles)	1.3	-1.8
Extract Stability (151 hr)	2.4	0.2
Long-Term Stability (-20°C for 7 days)	0.8	-2.7
Long-Term Stability (-20°C for 150 days)	6.9	0.2
Long-Term Stability (-70°C for 150 days)	5.2	0.7

Conclusion:

Bosentan for a treatment of pulmonary hypertension may improve the ability to exercise and slow the worsening of symptoms in patients. A rugged bioanalytical method to analyze bosentan was successfully validated and the simple and efficient method requires few extraction steps leading to high throughput sample analysis with minimal error.

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