Bosentan is indicated mainly for pulmonary hypertension and is a competitive antagonist of endothelin receptors. Under normal conditions, endothelial cell binding of these receptors causes pulmonary vasorelaxation. By binding to this interaction, Bosentan decreases pulmonary vascular resistance. Although the half-life is very different and validated for bosentan in human plasma to convert the pharamaceutical properties of bosentan at clinical doses.

The LC-MS-MS system consisted of LC-10AT Shimadzu HPLC pump, Perkin Elmer Series 200 autosampler and SCIEX API 4000 mass spectrometer. The retention time was approximately 1.2 minutes and the run time was 2 minutes.

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

Figure 2. Internal Standard (Bosentan-d4)

Figure 3. Blank Plasma Sample

Extraction Graphic

| Concentration (ng/mL) | Precursor ion M/Z | 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 |
|----------------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 10                  | 500              | 2500            | 5000            | 10000           | 20000           | 40000           | 80000           | 160000          | 320000          | 640000          | 1280000         |
| 20                  | 100              | 500             | 1000            | 2500            | 5000            | 10000           | 20000           | 40000           | 80000           | 160000          | 320000          |
| 30                  | 50               | 250             | 500             | 1000            | 2500            | 5000            | 10000           | 20000           | 40000           | 80000           | 160000          |
| 40                  | 25               | 125             | 250             | 500             | 1000            | 2000            | 4000            | 8000            | 16000           | 32000           | 64000           |
| 50                  | 12.5             | 62.5            | 125             | 250             | 500             | 1000            | 2000            | 4000            | 8000            | 16000           | 32000           |

Results:
The assay was linear over the range 20 to 2500 ng/mL, using plasma volume of 0.200 mL. These validation runs were performed on different days. The percentage CV and % bias were calculated at the Low, Medium and High QC concentrations for each level. The interday and intraday precision and accuracy at each QC level were within ± 10.5% and ± 15%, respectively.

Conclusion:
Bosentan for a treatment of pulmonary hypertension may improve the ability to exercise and slow the worsening of symptoms in patients. A bioanalytical assay was developed and validated for bosentan in human plasma to assess the pharmacokinetic properties of bosentan.


dry extract is reconstituted in 0.800 mL of mobile phase.

Extraction:
The calibration standard samples.

Standard Solutions:
All solvents were HPLC grade or better.

Bosentan and bosentan-D4, editorial (4 mg.Bottle) were purchased from Toronto Research Chemicals, Inc. All the chemicals used were AR grade and internal standard were purchased from Toronto Research Chemicals, Inc.

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

Worldwide Clinical Trials