

b. Post-Preparative Stability {Auto-sampler Stability}

The stability of processed samples, including the resident time in the auto sampler, was determined. The stability of the drug and the internal standard were assessed over the anticipated run time for the batch size in validation samples by determining concentrations on the basis of original calibration standard.

Three samples with concentrations {(QC Low: 150, QC Mid: 4000, and QC High: 6000) ng/ml} were prepared as mentioned in the sample preparation procedure together and kept at the auto sampler at 15°C for 0.0 and 24.0 hours. The accuracy determined at each concentration level should not exceed 15%.

c. Freeze and Thaw stability over three cycles

Analyte stability was determined after three freeze and thaw cycles.

Three samples with concentrations {(QC Low: 150, QC Mid: 4000, and QC High: 6000) ng/ml} were stored at -30° C for 72 hours and thawed unassisted at room temperature. When completely thawed. The samples were refrozen for 24 hours under the same conditions. The freeze-thaw cycle should be repeated two more times then analyzed after each cycle. If an analyte is unstable at the intended storage temperature, the stability sample should be frozen at -70° C during the three freeze and thaw cycles.

Three samples with concentrations {(QC Low: 150, QC Mid: 4000, and QC High: 6000) ng/ml} were prepared and kept at - 30° C. Each concentration was analyzed at zero time and 72 hours.