After 24 hours (Cycle 1): Following the first 24 hours of freezing, samples were thawed at room temperature (25°C) which was the sample processing temperature during analysis stages. After complete thawing, samples were refrozen again for 24 hours.

After 48 hours (Cycle 2): Following the second 24 hours of freezing samples were thawed at room temperature 25°C then were refrozen for the last 24 hours analysis.

After 72 hours (Cycle 3): Finally, the spiked samples were thawed after the last 24 hours of freezing then extracted to be analyzed and the resultant concentrations were calculated.

Sample stability after preparation at room temperature (bench-top stability)

Six samples for each (QC low and QC high) were spiked properly in serum or Krebs buffer without carrying out the extraction procedure.

At zero time: With corresponding calibration curve, three samples for each (QC low and QC high) were extracted then injected to be analyzed and the resultant concentrations were calculated, the remaining three spiked samples for each (QC low and QC high) were left on the bench.

After 24 hours Samples were left on the bench at room temperature 25°C, they were then extracted and injected to be analyzed. The resultant concentrations were calculated.

Autosampler stability

At zero time: With corresponding freshly prepared calibration curve three samples for each (QC low and QC high) were prepared with sufficient volume for the test. They were spiked properly in serum or Krebs buffer, extracted then analyzed and the resultant concentrations were calculated.