2.2.3.3 Linearity

Each calibration curve contained seven standard concentrations (50, 100, 200, 500, 1000, 2000, and 3000 ng/ml). Six calibration curves were performed throughout method validation (three from intra-day 1, 2 and 3 validations while the remaining three were obtained from stability tests). Depending on the data obtained from those six calibrations; mean of measured concentrations as well as mean ratios of standard points for each level (concentration) were calculated followed by accuracy % and CV% calculation which should be within the acceptance criteria of EMEA guidelines **(sections 1.7.1 and 1.7.2)**. Linearity test was performed to determine the acceptability of linearity data by plotting a seventh calibration curve (linearity curve) which was obtained by using the mean ratios of the six calibration curves versus each concentration level to obtain R^2 value.

2.2.3.4 Stability

Freeze and thaw 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 stored and frozen at -20°C for 24 hours.