2.2.3 Method Validation

Validation in this thesis was conducted in accordance with EMEA guidelines of bioanalytical method validation.

2.2.3.1 Intra-day accuracy and precision

Intra-day accuracy and precision were calculated by analyzing six replicates of each QC concentration mentioned in **table 2.3** on three different days accompanied by a calibration curve for each day in order to calculate the concentration per each. Accuracy level was determined by accuracy % while coefficient of variation (CV%) was calculated to determine precision. As mentioned previously in **1.7.1 and 1.7.2** Accuracy % of QC (low, mid and high) should be within 85-115% while LLOQ should be within 85-120%, in regard to CV% value it should not exceed 15% for all QC samples (QC low, mid and high), except for the LLOQ which should not exceed 20%.

Table 2.3 Quality control concentrations used for method validation

QC solution	Final concentration (ng/ml)
LLOQ	**50**
QC low	**150**
QC mid	**1500**
QC high	**2500**

2.2.3.2 Inter-day accuracy and precision

Inter-day accuracy and precision were calculated by using data of measured concentrations obtained from days 1, 2 and 3 to calculate CV% and accuracy % for each QC concentration (LLOQ, QC low, QC mid and QC high) separately.