1.7 Bioanalytical HPLC method validation parameters definitions (EMEA)

1.7.1 Accuracy

The accuracy of an analytical method describes the closeness of the mean of test value obtained by the method to the actual concentration of the analyte (expressed in percentage) (FDA 2013). Any bias or systematic error in the method is usually indicated by the accuracy (Bliesner 2006). Accuracy samples and quality control samples (QC samples) should be spiked with a specified amount of analyte and the resultant concentrations of the analyzed samples will be compared with the actual value. Accuracy evaluation should be done for values of QC samples obtained within a single run (intra-day accuracy or within run accuracy) and in different runs (inter-day accuracy or between-run accuracy) (EMEA 2011).

Accuracy % is calculated as follows:

$$\text{Accuracy\%} = \frac{\text{Measured (calculated) concentration}}{\text{Theoretical (true) concentration}} \times 100\%$$

Intra-day accuracy (within-run accuracy) **s**hould be measured using a minimum of five samples per level for each single run with a minimum of four concentration levels which are covering the calibration curve range: the lower limit of quantification (LLOQ), within three times the LLOQ (QC low), around 50% of the calibration curve range (QC mid), and at least at 75% of the upper calibration curve range (QC high). On the other hand, inter-day accuracy (between-run accuracy) is measured using **s**amples of LLOQ, QC low, mid and