T1 LC-MS/MS Quality Assurance Parameters

Parameter to Monitor	CLSI-C62A Recommendation	Possible Patterns/Trends	Possible Causes
SST	• Acceptance criteria should be set for variables such as Rt, peak height and width, ion ratio, and signal to noise (S/N) ratio • A minimum of three samples should be evaluated prior to batch analysis and after instrument maintenance • Ion ratio of replicates should have a CV <6% • S/N ratio >10:1 for SST at extracted LLMI	• Shift in Rt or RRt • Peak asymmetry • Change in peak intensity • Detection of additional peaks • Ion ratio change • Decrease in S/N ratio	• Mobile phase change/degradation/ evaporation • LC-MS system malfunction/failure • LC column change/deterioration • Temperature fluctuations • New interferent in system • MS maintenance/cleaning required
Calibrator Accuracy and Calibration Curve Slope	• Allowable bias +15% for all calibrators above the LLMI, +20% for LLMI • Calibration slope r2 > 0.995	• Nonlinearity or change in appropriateness of linear fit • Unacceptable bias for one calibrator or multiple calibrators	• Calibrator deterioration • Loss of detector sensitivity • Insufficient volume of injection • Pipetting/sample preparation error • Poor preparative recovery
IS Peak Area	• Acceptable range for IS peak area should be defined during method validation • IS peak areas should be comparable across calibrators and controls in the same run	• Sporadic IS shift throughout run or for individual samples • Gradual shift in IS peak area • Drastic shift in IS peak area (within or between batches)	• Instrument drift/charging • Poor preparative recovery • Failure to precisely aliquot IS • Unacceptable ionization suppression/ enhancement from matrix effects • Insufficient volume of injection • Degradation of IS
QC	• A minimum of three QC concentrations tested in duplicate per batch • Acceptable QC mean and SD should be established by repetitive analysis, not manufacturer provided ranges • New lots of QC should be evaluated according to CLSI C24 • All failed QC must be investigated and corrective action documented	• Random QC failure in batch • Gradual QC shift oaver time • Drastic QC shift	• QC deterioration • Loss of detector sensitivity • Insufficient volume of injection • Pipetting/sample preparation error • Poor preparative recovery
Rt and/or RRt to Internal Standard	• Rt or RRt for sample should be within +2.5% of the mean Rt/RRt of the calibrators in the same batch (and between batches)	• Sporadic shift in Rts or RRts • Gradual shift in Rt or RRt • Drastic shift in Rt or RRt (within or between batches)	• Mobile phase change/degradation/ evaporation • LC pump malfunction/failure • LC column change/deterioration • Temperature fluctuations
Ion Ratio	• Acceptable range for ion ratio should be determined during method validation • Mean ratio of the calibrators should not alter significantly within or between runs • If signal of qualifier ion is >50% that of the quantifier ion, the ion ratio in the patient samples should be +20% from that of the mean ratio of the calibrators	• Ion ratio outside of acceptable range for individual patient sample • Significant change in ion ratio mean between runs • Ion ratio outside of acceptable range for samples with analytes near the LLMI	• Integration failure of precursor or product ion • Interfering substance in an individual patient sample • Reagent or system change resulting in new interfering substance throughout a batch • Loss in assay sensitivity resulting in inadequate signal for qualifier ion

SD, standard deviation
S/N, signal to noise
IS, Internal standard
QC, quality control
Rt, analyte retention time
RRt, relative retention time
LLMI, lower limit of the measuring interval
SST, System suitability test
CV, coefficient of variation