'Web course "LC-MS Method Validation"

University of Tartu

https://sisu.ut.ee/lcms_method_validation/

NB! "This table is meant as a general "big picture" comparison between the different guidelines.

The wording in this table does not necessarily exactly match the wording in the guidelines.

For the definitive wording the user must consult the original documents."

Validation parameter	FDA 2018 bioanalysis [1]	EMA 2022 [2]	ICH 2005 [3]	Eurachem 2014 [4]
Selectivity	Blank from at least 6 individual sources; **Acceptance Criteria:** Blank and zero: no interference at analyte and IS RTs. Spiked samples: ± 20% LLOQ. Blank: IS response < 5% of Cals and QCs average IS responses.	Blank from at least 6 individual sources; **Acceptance Criteria:** Analyte response < 20% of LLOQ; IS response <5%		Test samples and RMs Candidate and other independent methods Also test samples with suspected interferences
Specificity	Assess for interference by cross-reacting molecules, concomitant medications, bio-transformed species, etc. **Acceptance Criteria:** Same as Selectivity.		Blanks, matrix-matched samples. **Impurities, if applicable:** Spiking blanks with impurities and/or excipients. Degradation experiments: light, heat, humidity, acid/base hydrolysis and oxidation	
Carryover	Assess impact of carryover on accuracy. **Acceptance Criteria:** < 20% of LLOQ	Inject blank after a high concentration sample or calibration; **Acceptance Criteria:** Blank response: < 20% of LLOQ IS response: < 5%		
Linearity / Calibration Curve	Matrix-matched, Blank, zero, 6 level (inc. LLOQ), **Acceptance Criteria:** LLOQ ± 20%,	Matrix-matched, Blank, zero, 3 runs x 6 levels (inc. LLOQ and ULOQ) (optional, 2 parallels), **Acceptance Criteria:**	≥ 5 levels **Range:** assay: from 80 to 120 % of target conc.;	Instrument and method working range. **Range of interest:** Blank, 6 - 10 levels evenly spaced

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	others ± 15%, 75% (or min. 6 calibrator levels) should meet the criteria, Cal runs need to be reproducible	LLOQ ± 20%, others ± 15%, 75% (min. 6 calibrator levels) should meet the criteria 50% per level should meet the criteria	content uniformity: from 70 to 130 % of target conc., dissolution testing: +/-20 % over the specified range; impurity: from reporting level to 120% of the specification;	expected range ± 10 % / ± 20 %. **Linear range:** Blank, 2-3 parallels x 6-10 levels evenly spaced **Determine if linear range is fit:** Blank, reference materials or spiked sample blanks, 2-3 parallels x 6-10 levels evenly spaced regression plot, residuals plot, regression statistics
Accuracy and Precision (A & P)	3 runs x 4 levels (LLOQ, L, M, H QC) x 5 parallels, Over several days, **Within-run and between runs** **Accuracy:** LLOQ: ± 20% from nominal conc. others: ± 15% from nominal conc. **Precision:** LLOQ: ± 20% RSD others: ± 15% RSD	**Within-run QCs** 4 levels (LLOQ, L, M, H) x 5 parallels LLOQ: ± 20% others: ± 15% (mean conc. from nominal value or RSD) **Between runs QCs** 3 runs (LLOQ, L, M, H) 2 different days LLOQ: ± 20% others: ± 15% (mean conc. from nominal value or RSD)	**Repeatability:** 3 levels x 3 parallels OR 6 determinations at target conc. **Intermediate precision:** Several days, analysts, equipment, etc. Not necessary to study effects individually. Experiment design is encouraged.	**Accuracy:** Blanks, CRMs and/or spiked samples (if RM not available). 10 parallels per level Alternatively: RM/test sample using candidate method and alternative method. **Precision:** RMs, surplus test samples or spiked sample blanks at various levels **Repeatability:** Same analyst, equipment, laboratory, short timescale. 6-15 parallels **Intermediate precision:** Different analysts, equipment, same laboratory, extended timescale. 6-15 parallels for each material. **Repeatability and intermediate precision in one study:**

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				Different analysts, equipment, same laboratory, extended timescale. 6-15 runs x 2 parallels ANOVA to calculate repeatability standard deviation and intermediate precision standard deviation
Matrix effect	Matrix effects should be assessed and eliminated.	≥ 6 lots of individual blank matrices. 3 parallels. Pooled matrix should not be used. CV: ≤ 15 % at L and H levels		
Recovery	QC (L, M, H) extracted samples vs blank extracts spiked post extraction	typically at 3 conc. (L, M, H)		
Stability	Stock solution, freeze-thaw, bench-top, long-term, processed sample, auto-sampler 3 parallels at L and H **Acceptance Criteria:** Accuracy: ± 15% of nominal conc.	Stock and working solution, freeze and thaw, short term, long term, processed sample, on-instrument/auto-sampler, At L and H levels **Acceptance Criteria:** Mean conc. at each level: ±15% of the nominal conc.		
Sensitivity / LLOQ	LLOQ: Lowest non-zero standard **Acceptance Criteria:** Response at LLOQ ≥ 5 x zero response Accuracy ± 20% & Precision ≤ 20% (3 runs x ≥ 5 parallels)	Lowest calibration standard **Acceptance Criteria:** Accuracy ± 20% and precision ≤20%	**LoD and LoQ:** Visual evaluation OR LoD: S/N of 3 or 2:1 LoQ: S/N of 10:1 OR Based on response SD and Slope: $LoD = 3.3 \times \frac{\sigma}{slope}$ $LoQ = 10 \times \frac{\sigma}{slope}$ σ determined from: 1) standard deviation of several blanks	**CC_α, CC_β** refer to EU Commission Decision 2021/808/EC and ISO 11843-2:2007 **LoD and LoQ:** Blank samples, test samples or spiked samples, concentrations of analyte close to or below the expected LOD 6 - 15 parallels $LoD = 3 \times s_{0'}$ $LoQ = k_Q \times s_{0'}$ For calculation of $s_{0'}$(modified standard deviation) refer to the guide, Other alternatives suggested as well.

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			2) calibration graph in LoD region, residual standard deviation or y-intercept standard deviation	
Robustness / Ruggedness			System suitability parameters should be established. **Examples for study:** - stability of analytical solutions; - extraction time. - influence of variations of pH in a mobile phase; - influence of variations in mobile phase composition; - different columns (different lots and/or suppliers); - temperature; - flow rate.	Variables with significant effects must be identified, RMs or test samples, Most effective with experimental designs: e.g. Plackett-Burman experimental design for start. Rank the variables in order of the greatest effect on method performance. Significance tests to determine whether observed effects are statistically significant.
Other Validation Runs	3 QCs (L, M, H) in duplicates **Run Acceptance Criteria:** Cals: Same as calibration curve. QCs: ≥ 67% of QCs ± 15% ≥ 50% of QCs per level ± 15%			
Quality Controls (QC)	**Accuracy and Precision:** 4 lvls: LLOQ, L, M, H 3 runs x 5 parallels **Other runs:** At L, M, and H levels QCs in duplicates Nr of QCs: 5% or 6, whichever is higher **Acceptance Criteria**: ≥ 67% of QCs ± 15% ≥ 50% of QCs per level ± 15%	**All runs (also after validation):** Blank, zero Cals: 6 levels QC: ≥ 3 levels (L, M, H) x 2 parallels or ≥ 5% of study samples, whichever is higher **Acceptance Criteria:** **Cals:** LLOQ ± 20% Other: ± 15% 75% (min. 6 levels) of Cals **QCs:** ≥ 67% of QCs ± 15% ≥ 50% of QCs per level ± 15%		Every batch should have QCs, stable test samples, blanks and/or standard solutions, control charts are recommended,

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Dilution	QCs for planned dilutions, 5 replicates per dilution factor, A & P: ± 15% of nominal conc. or RSD	Cover the dilution applied to the study samples. Spiking matrix above the ULOQ and diluting with blank matrix (≥ 5 determinations per dilution factor). **A & P**: ±15% of nominal conc. or RSD		
Incurred Sample Reanalysis (ISR)	Must reanalyze samples for control: first 1000: 10% remaining: 5% **Sample selection:** C_{max} and in the elimination phase **Acceptance Criteria**: 67% of repeated samples within ±20% of the mean	Must reanalyze samples for control: first 1000: 10% remaining: 5% **Sample selection:** C_{max} and in the elimination phase **Acceptance Criteria:** 67% of repeated samples within ±20% of the mean		
Repeat Analysis	No re-analysis of individual calibrators and QCs is permitted. Reanalysis should be based on reasons At least the same number of replicates for repeats as originally tested	Example cases: Run did not fulfil the acceptance criterias, IS response significantly differing from cal. or QCs response (if criteria pre-defined), Improper sample injection or malfunction of equipment, Obtained concentration above ULOQ or below LLOQ, Analyte levels in blanks too high, Poor chromatography		

Parallels – Samples that have been taken through the entire measurement procedure (each has had independent sample pretreatment)
EMA: LLOQ, L: within three times the LLOQ (low QC), M: around 30 - 50% of the calibration curve range (medium QC), H: at least at 75% of the upper calibration curve range (high QC).
FDA: LLOQ, low (L: defined as three times the, LLOQ), mid (M: defined as mid-range), high (H: defined as high-range)

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References:
1. U.S. Department of Health and Human Services Food and Drug Administration, Bioanalytical Method Validation, Guidance for Industry, 2018: https://www.fda.gov/media/70858/download
2. ICH guideline M10 on bioanalytical method validation and study sample analysis. European Medicines Agency 2022. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m10-bioanalytical-method-validation-step-5_en.pdf
3. ICH harmonized tripartite guideline: validation of analytical procedures: text and methodology Q2(R1), International Conference of harmonization of technical requirements for registration of pharmaceuticals for human use 2005: https://database.ich.org/sites/default/files/Q2_R1__Guideline.pdf
4. B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods - A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014): https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf