

Recommendations for Validation of LC-MS/MS Bioanalytical Methods for Protein Biotherapeutics

Recent aapsj publication

*Summary tables of proposed recommendations
for method validation parameters and sample
analysis for protein LC-MS/MS*

**Jean Lee, Ph.D., FAAPS
BioQualQuan, LLC
ljean095@gmail.com**

**6th Japan Bioanalysis Forum Symposium
February 26, 2015**

White Paper

**Recommendations for Validation of LC-MS/MS Bioanalytical Methods
for Protein Biotherapeutics**

**Rand Jenkins,¹ Jeffrey X. Duggan,² Anne-Françoise Aubry,³ Jianing Zeng,³ Jean W. Lee,⁴ Laura Cojocaru,⁵
Dawn Duffield,⁶ Fabio Garofolo,⁷ Surinder Kaur,⁸ Gary A. Schultz,⁹ Keyang Xu,⁸ Ziping Yang,¹⁰ John Yu,²
Yan J. Zhang,³ and Faye Vazvaei^{11,12}**

Received 13 August 2014; accepted 7 October 2014; published online 13 November 2014

Reprint request: faye.vazvaei@roche.com

Abstract

- Consensus views of a cross-section of companies and organizations from US & Canada, prepared under the auspices of the AAPS Bioanalytical Focus Group's Protein LC-MS Bioanalysis Subteam.
- Focus on validation and application of LC-MS/MS methods for bioanalysis of protein biotherapeutics in regulated studies. Intended to serve as a guide to drive harmonization of best practices within the bioanalytical community and provide regulators with an overview of current industry thinking on applying LC-MS/MS technology for protein bioanalysis.

Abstract

- For simplicity, scope limited to the most common approach: Indirect quantification of the protein by LC-MS/MS measurement of one or more of its surrogate peptide(s) produced by proteolytic digestion. We considered a range of sample preparation approaches from simple in-matrix protein denaturation and digestion to complex procedures involving affinity capture enrichment.
- LC-MS/MS methods for protein bioanalysis require different considerations than small molecules. Method development and validation plans need to be tailored to the particular assay format, considering the intended use, test species or study population, characteristics of the biotherapeutic, its similarity to endogenous proteins, potential interferences, and the nature, quality and availability of reference and internal standards.

Table 1 Comparison of conventional method validation parameters for protein LBA and small molecule LC-MS/MS, with proposed recommendations for protein LC-MS/MS

Parameter	Protein LBA	Small Molecule LC-MS/MS	Protein LC-MS/MS, using a Surrogate Peptide (Recommended)
Calibration Curve Regression Function	Non-linear with 4 or 5 parameter logistic Anchor points may be used	Linear preferred, non-linear with justification	Linear recommended when possible; non-linear models may be acceptable with some affinity-capture methods
Lower Limit of Quantification (RE, CV)	Within $\pm 25\%$	Within $\pm 20\%$	Within $\pm 25\%$
Calibration Standards (RE, CV)	Within 20% (except LLOQ and ULOQ)	Within 15% (except LLOQ)	Within 20% (except LLOQ)
Accuracy & Precision (RE, CV)	Within 20% (LLOQ/ULOQ QCs within 25%) Min. 6 runs	Within 15% (LLOQ QC within 20%) Min. 3 runs	Within 20% (LLOQ QC within 25%) Min. 3 runs
Dilutional Integrity/Linearity	RE, CV within 20%	RE, CV within 15%	RE, CV within 20%
Parallelism	Dilution series CV within 30% using incurred samples	NA	NA; may be used for troubleshooting affinity capture methods

Table 1 (continued 1)

Parameter	Protein LBA	Small Molecule LC-MS/MS	Protein LC-MS/MS, using a Surrogate Peptide (Recommended)
Selectivity/Specificity Non-specific matrix-related interferences Using individual matrix lots, analyzed as blanks and fortified at the LLOQ level	10 lots LLOQ: Accuracy within 25% for 80% of fortified lots	6 lots Blanks: <20% of LLOQ <5% of IS LLOQ: Accuracy within 20% for 80% of fortified lots	6-10 lots Blanks: <20% of LLOQ <5% of IS LLOQ: Accuracy within 25% for 80% of fortified lots
Specific Interferences Using LLOQ (and sometimes ULOQ for LBAs) QC samples	Fortified with available material: ADA, soluble target, catabolites or concomitant drugs (large molecule) Accuracy within 25%	Fortified with available metabolites or concomitant drugs, as appropriate Accuracy within 20%	Fortified with available material: ADA, soluble target, catabolites or concomitant drugs, as appropriate Accuracy within 25%
Matrix Effect on MS Ionization Using individual matrix lots Also evaluate hemolyzed, lipemic, or relevant disease population samples	NA	MF in 6 lots IS-normalized CV within 15 % across lots	MF in 6-10 lots Compare surrogate and SIL-IS peptides in processed matrix and reagent blanks IS-normalized CV within 20 % across lots Alternatively: Compare individual QC samples prepared from multiple lots Accuracy (CV) within 20 % (or 25% LLOQ) across lots

Table 1 (continued 2)

Parameter	Protein LBA	Small Molecule LC-MS/MS	Protein LC-MS/MS, using a Surrogate Peptide (Recommended)
Recovery	NA	Extraction recovery should be reproducible	Overall recovery including digestion should be reproducible Recoveries for individual steps may be evaluated for troubleshooting
Matrix Stability	Within 20% of nominal Determine at each storage temperature	Within 15% of nominal Temperature bracketing approach may be used	Within 20% of nominal Determine at each storage temperature
Processed Sample Extract Storage Stability Determine at intended storage temperature	NA	Within 15% of nominal Stored QC extracts measured against freshly prepared curve	Within 20% of nominal Stored QC extracts measured against freshly processed curve, or original curve when justified
Stock and Working Solution Stability	May not be required if covered by COA	Compare old vs. freshly prepared solutions Mean values within 5-7% typical	Compare old vs. freshly prepared solutions Protein must be digested; mean values within 10% recommended

Table 1 (continued 3)

Parameter	Protein LBA	Small Molecule LC-MS/MS	Protein LC-MS/MS, using a Surrogate Peptide (Recommended)
Run Size	NA	Validate maximum anticipated	Validate maximum anticipated
Carryover (Blank following a ULOQ sample)	Generally NA, some assay technologies may need to assess (e.g., Gyros, MSD and others) minimize and mitigate	Prefer <20% LLOQ response Minimize and mitigate	Prefer <20% LLOQ response, may need to accept higher with justification Minimize and mitigate
Change in Critical Assay Reagents	May need revalidation	NA	Primarily a concern for protein reagents Confirm by acceptable accuracy and precision in at least one run; more extensive testing sometimes needed
Critical Assay Reagent Stability	Appropriate testing /stability programs for LBA reagents may be required	NA	Stability is demonstrated by prevalidation testing and acceptability of validation and analytical runs; longer term testing sometimes needed

Table 3. In-Run Considerations for Sample Analysis

Process or Criteria	Considerations for LC-MS/MS assays of protein drugs
1. Number of matrix blanks and calibration standards in a run	At least one blank matrix sample; at least one zero sample (blank matrix with IS); a minimum of 6 non-zero calibration standards
2. Acceptance criteria for Calibration Standards	RE < 25% for LLOQ standard; RE < 20% for remaining standards; ≥75% of the total number of standards must meet the criteria; each curve should contain a minimum of 6 accepted levels
3. QCs and acceptance criteria	Include QCs at the following concentrations: Low QC, near the LLOQ, up to 3x the LLOQ; Mid QC, midrange of the calibration curve; High QC, near the high end of the range. At least 6 QCs per batch (2 at each level) or 5% of the total number of unknown samples. Acceptance criteria: RE < 20%; 2/3 of the total number of QCs must meet acceptance criteria, with a minimum 50% of replicates passing at each concentration level
4. Sample analysis	Chromatographic assays can generally be run with a single determination without replicate analysis
5. Sample Dilutions	When sample dilutions are anticipated to bring the analyte concentration into the assay range, an appropriate dilution factor is evaluated during validation using the same sample matrix as diluent. Typically, a Dilution QC is prepared in the same matrix at a concentration above the curve and analyzed using the same matrix as diluent. Dilutional accuracy can be verified in the sample run by analysis of the Dilution QC applying the same dilution factor that will be used for the samples. Alternatively, if multiple dilutions are required within a run, the highest dilution factor should be evaluated. Acceptance criteria: RE < 20%, with a minimum 50% of replicates passing at each concentration level

Table 3. In-Run Considerations for Sample Analysis (continued)

Process or Criteria	Considerations for LC-MS/MS assays of protein drugs
6. Multiple analytes in a run	Where multiple analytes are analyzed in a single run, acceptable data for one analyte should not be rejected based upon failure to meet acceptance criteria for another analyte in a given sample; when reanalyzing a sample for a failed analyte, it is not necessary to re-quantify the previously accepted analyte; however, the source data for that analyte should be retained.
7. Rejected runs	Data from rejected runs need not be reported, but the fact that a run was rejected and the reason for rejection should be reported.
8. Incurred Sample Reproducibility (ISR)	ISR should be run for nonclinical and clinical studies. For toxicology studies, ISR will be run for one study per species per matrix. For clinical studies, ISR should be run at least for first time in human, bioequivalence, special populations and proof-of-concept studies. The proportion of samples that should be re-analyzed for ISR will depend on the applicable regulatory guidance being followed. If possible, two samples will be chosen from each selected subject, one near C _{max} and the other in the late elimination phase. An ISR assay result is acceptable if at least two thirds of the re-analyzed incurred samples have %Difference ≤ 30.0 between the re-assayed value and the original assay value