Quantification of Host Cell Proteins using Mass Spectrometry

Analytical Method Qualification Protocol
2-February-2021

Sponsor: Biotech

Testing facility: Alphalyse A/S
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Management Signatures

Analyst Alphalyse:	_____	Date:_____	Name:_____
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Sponsor:	_____	Date:_____	Name:_____

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1. Scope

Present Scope
The present scope is method qualification to support use of the method as a characterization assay in testing of Phase 1 clinical supply. Design of the qualification protocol addresses the validation parameters in the ICH Guideline; Validation of analytical procedures Q2(R1), for intended use in a quantitative test for impurity content.

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Sponsor intends to use the qualified method for analysis and release of DS batches in Phase 1 clinical supply.

2. Summary of test parameters for the qualification

This HCP test is qualified according to the ICH Guideline; Validation of analytical procedures Q2(R1), for intended use in a quantitative test for impurity content. The parameters validated include accuracy, precision (repeatability), specificity, linearity, range LOD and LLOQ. Intermediate precision and Robustness will be validated as development proceeds. As this is a phase 1 qualification, pre-determined acceptance criteria was not applied. Rather the data after qualification was observed to assess whether or not the method is suitable for its intended purpose.

The design of the qualification protocol addresses ICH Q2 parameters as follows:

Precision is determined by performing a 3 X 3 matrix. The sample digestion is repeated three different times (3 different sample preps) and analyzed in triplicate. Precision is assessed by taking 3 proteins found in Drug Substance) 1) **acestrum**, 2) **aligent** 3) **tionem** and comparing the ppm in each of the three preparations. Precision is confirmed if the ppm recovery of the 3 proteins in the three different preparations is acceptable.

Accuracy is determined by spiking seven standard proteins into Drug Substance at 8 different levels (0 ppm, 5 ppm, 10 ppm, 25 ppm, 50 ppm, 100 ppm, 500 ppm, 2000 ppm). Accuracy is confirmed if the recovery of the seven standard proteins is acceptable (in 5 out of the 7 proteins) at levels above the LLOQ.

Specificity is confirmed by comparing the recovery of spiked and unspiked (0 ppm) samples, showing the presence of other proteins does not affect the recovery of common HCPs in Drug Substance.

Linearity and Range are obtained from the accuracy experiment. Linearity is confirmed if the R^2 for all spiked levels above the LLOQ is acceptable. The Range is defined as the concentration range in which the linearity is acceptable.

LLOQ is determined as the lowest level in which the recovery of the seven standard proteins is acceptable (in 5 out of the 7 proteins).

3. Analytical Methods

The qualification run is performed using the Analytical Methods and SOPs implemented during method development.

Analytical method: AM 019-1.0 Quantification of HCPs by SWATH LC MS/MS

4. Reference Standards/samples

Table 1. Reference Drug Substance

Lot no.	Protein Names
123456	Drug Substance

Table 2. Standard Proteins.

Proteins	No	Mass (Da)	Protein Names
Standard 1	S1	34083	occabor
Standard 2	S2	22883	volorrum, et
Standard 3	S3	13239	coneturit
Standard 4	S9	45184	laut repudit
Standard 5	S10	74954	ehendigent
Standard 6	S12	14225	occabor
Standard 7	S13	88064	et laut repudit

Normalization protein:

To ensure good linearity and accuracy in the linearity experiment a normalization protein is added in ≈5000 ppm. This could be sumquas imagni.

5. Specificity

Specificity will be shown by comparing the recovery of the spiked and unspiked samples, showing that the presence of other proteins does not affect the recovery of common HCPs in Drug Substance. Three unspiked samples and three samples spiked with 2000 ppm will be investigated.

6. Limit of detection (LOD)

To determine LOD, seven standard proteins will be used. The Drug Substance sample is spiked with 2000 ppm of each standard protein and is diluted in an un-spiked sample (0 ppm) to 5 ppm (2000, 500, 100, 50, 25, 10, and 5 ppm spike-in). LOD is when the protein is no longer identified by at least 2 peptides by LC-MS/MS.

7. Lower limit of quantitation (LLOQ)

To determine LLOQ, seven standard proteins will be used. The Drug Substance sample is spiked with 2000 ppm of each standard protein and is diluted in an un-spiked sample (0 ppm) to 5 ppm (2000, 500, 100, 50, 25, 10, and 5 ppm spike-in). Calibration curves for each of the seven standard

peptides will be examined. LLOQ is confirmed as the lowest level at which the spiked sample is acceptably recovered (50-200% recovery for 5 out of 7 proteins).

8. Linearity and Calibration curves

To determine linearity, seven standard proteins will be used. The Drug Substance sample is spiked with 2000 ppm of each standard protein and is diluted in an un-spiked sample (0 ppm) giving the concentrations 2000, 500, 100, 50, 25, 10, and 5 ppm.

R squared will be calculated for the calibration curves for each of the seven standard proteins.

9. Accuracy

To determine accuracy and LLOQ, seven standard proteins will be used. The Drug Substance sample is spiked with 2000 ppm of each standard protein and is diluted in an un-spiked sample (0 ppm) giving the concentrations 2000, 500, 100, 50, 25, 10, and 5 ppm. Calibration curves for each of the seven standard proteins will be examined regarding accuracy of the calibration points.

10.Precision

Precision will be tested by doing a 3 X 3 matrix repeating the digestion three different times (3 different sample preps) and then analyze them each in triplicate. Precision will be determined by taking 3 residual host cell proteins found in Drug Substance samples – see table 3. and comparing the ppm in each of the three preps.

Table 3. Selected QC proteins for determination of precision

Protein Names	Uniprot	MW (Dalton)
acestrum	A0A123456	27075
aligent	A0A789101	43744
tionem	A0A234567	33184

11. Range

To determine the range, seven standard proteins will be used. The Drug Substance sample is spiked with 2000 ppm of each standard protein and is diluted in an un-spiked sample (0 ppm) giving the concentrations 2000, 500, 100, 50, 25, 10, and 5 ppm. The range will depend on the LLOQ determined.

12.Sample preparation plan

500 mg of the same Drug Substance lot will be needed for the qualification.

A total number of 13 samples will be prepared and analyzed in triplicate. An analytical method will be prepared to describe the dilution steps in detail. The 13 samples needed for the qualification:

3 samples are spiked with 2000 ppm standards. These three samples are used to determine precision of the quantification of three selected HCPs.

3 samples are prepared without standard spike-in. These samples are used to examine the specificity by comparing with the 2000 ppm samples, showing that the presence of other proteins does not affect the recovery of common HCPs in Drug Substance.

7 samples are spiked with standard proteins and a normalization protein. 2000, 500, 100, 50, 25, 10 and 5 ppm standard proteins and ≈5000 ppm normalization protein.