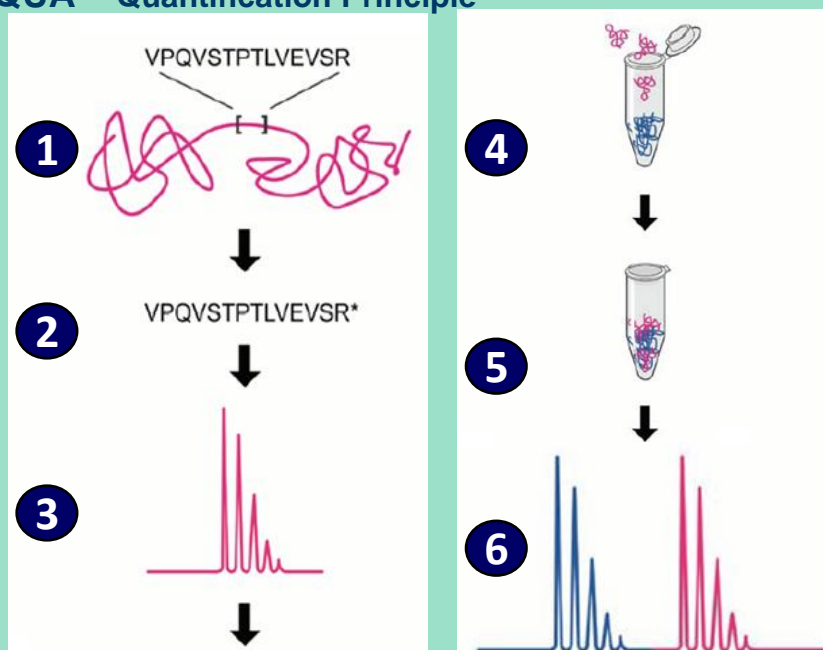


# Alpha-Quant™

Protein specific quantification using stable isotope dilution LC-MS/MS

## AQUA – Quantification Principle



- 1) Select representative signature peptides
- 2) Synthesize AQUA peptides with heavy isotope label ( $C^{13}$ ,  $N^{15}$ )
- 3) Optimize peptide purification and LC MS/MS conditions
- 4) Add known amount of AQUA peptide to sample for quantification
- 5) Digest protein with trypsin and purify peptides
- 6) LC-MS/MS and MRM measurements for quantitative analysis

## Advantages

- Direct measurement of specific analyte peptides
- Does not need time-consuming antibody ELISA development
- Multiplex quantification of several proteins and protein variants is possible
- Applicable to proteins in complex biological matrices and to purified formulated proteins
- Assay development in 6-12 weeks

## Applications

- Recombinant protein production – upstream & downstream process yield determination and optimization
- Drug discovery & clinical development:
  - Pharmacokinetics in humans, animals
  - Biomarker measurements

# Mass spectrometry based protein specific quantification

## Alpha-Quant advantages

▪ **This method is protein specific and general and can be applied in all stages of process development.** It is applicable to early cell line development as well as in-process samples and formulated drug substance. The same quantification method can therefore be applied throughout all process development stages and for final process documentation.

▪ **When antibody ELISA cannot be applied:**

- Specific antibody & qualified assay not available
- Interference with matrix or formulation
- Quantification of specific protein variants with similar epitopes

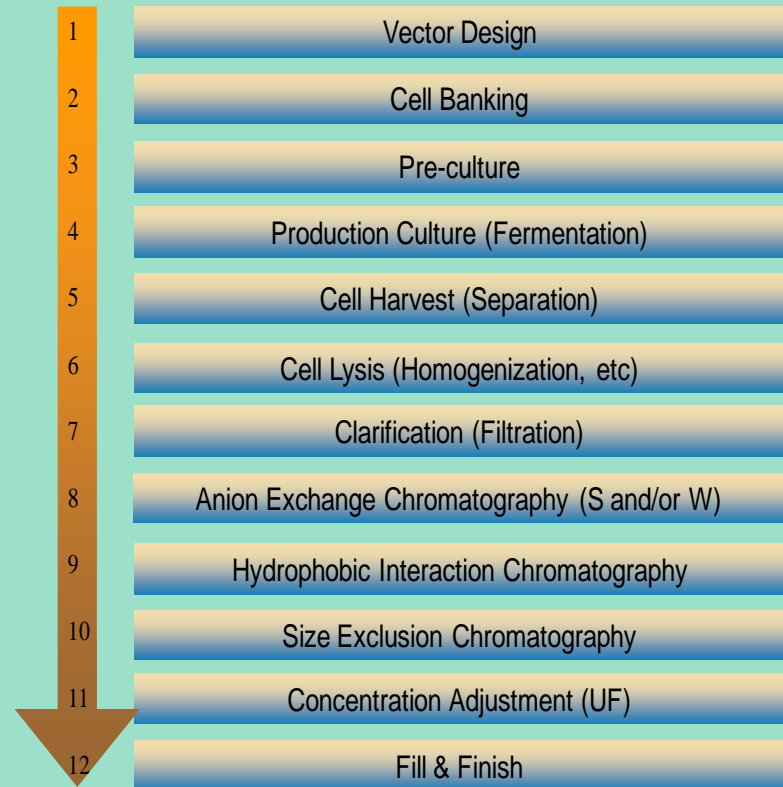
▪ **When Chromatography and colorimetric assay cannot be applied:**

- The protein exhibits low A280 absorbance due to lack of aromatic amino acids in sequence
- The protein is a hydrophobic or lipid protein

▪ **Multiplex analysis possible**— when several proteins or individual protein forms should be quantified in the same sample.

▪ **Application in pre-clinical and clinical pharmacokinetic studies** on serum samples.

### cGMP manufacturing of biologics (proteins) flow chart



## Fast and Easy Access to Protein Analysis

## Benefits of Alpha-Quant Assays

You can stop being troubled when more traditional methods do not quantify your proteins of interest! You can stop wasting your precious time to find out why your colleagues arrive at completely different results than expected even he is only one process step apart! Alphalyse's Absolute Protein Quantification method can give you and your colleagues the right and coherent **answer** from the very beginning. Due to its innovative uniqueness, Alpha-Quant allows you to quantify your protein and disregard any other co-existent proteins in complex biologic samples, a new and valuable approach in strain development, cell bank production (RCB, MCB and WCB), fermentation process development, and early steps of purification. As purification process progresses and as the proteins of interest get increasingly purer, the Alpha-Quant can still remain the main quantifying method or be readily switched to other assays such as A280, BCA and HPLC when appropriate.

## What is Alpha-Quant

Alpha-Quant is a unique solution to help develop your protein production processes with highest possible yield. Learn more about Alpha-Quant in this white paper, or visit our website [www.alphalyse.com](http://www.alphalyse.com)

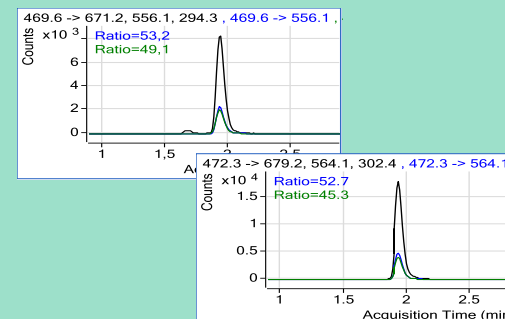
## Case example: Quantification of a recombinant protein vaccine in manufacturing process development

- Recombinant protein vaccine manufactured by CMO for clinical phase I-III studies
- 21 kDa Campylobacter surface protein without Cys residues expressed in E. coli

### Assay use

- Quantification method for absolute content of protein in:
  - Raw fermentation solutions
  - Partly purified material from down stream processing
  - Final drug substance
- Optimize protein yields from individual process steps
- Documentation and in-process control analysis

### 3 MRM transitions per peptide



Fast and Easy Access to Protein Analysis

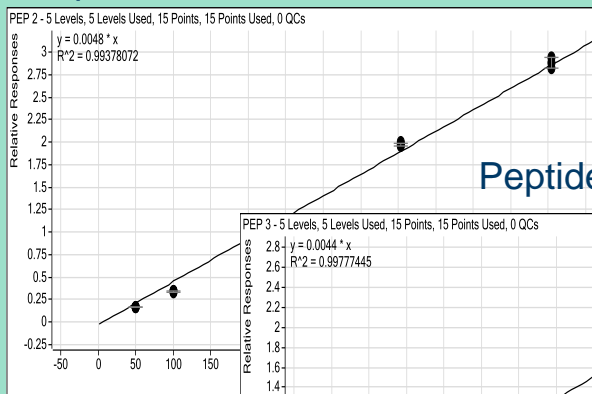


# Mass spectrometry based protein specific quantification

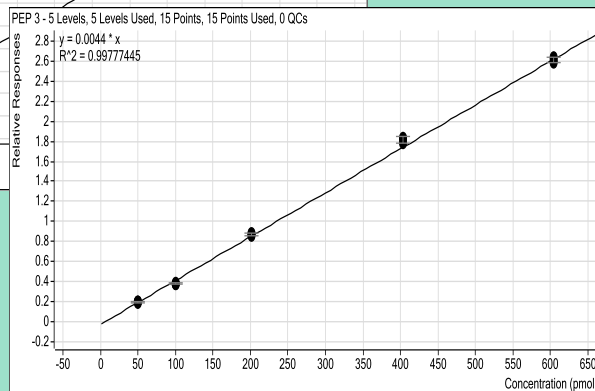
## External calibration curves on same protein

- External calibration curves for Peptide 2 and Peptide 3
- Triplicate analysis, fixed AQUA concentration
- Demonstrates assay linearity and precision within measurement range

Peptide 2.  $R^2 = 0.994$

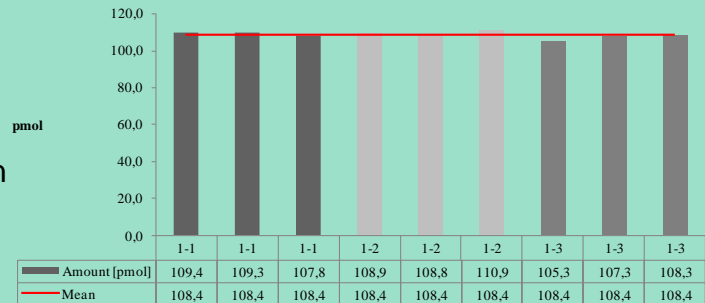


Peptide 3.  $R^2 = 0.998$



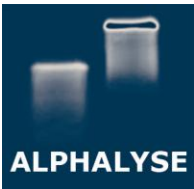
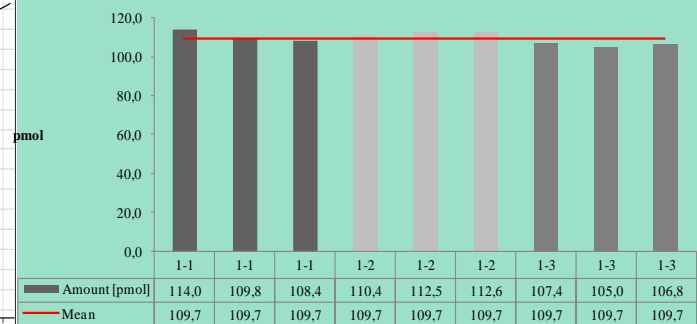
Accuracy (recovery): 98.4%  
Precision: 108.4 pmol +/- 1.6

### Peptide 2



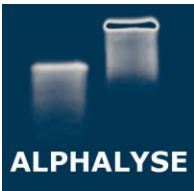
Accuracy (recovery): 99.7%  
Precision: 109.7 pmol +/- 3.0

### Peptide 3



Fast and Easy Access to Protein Analysis

# Mass spectrometry based protein specific quantification



## How We Work

**Goal.** Ensuring your success and fulfilling your objectives are our ultimate service goals. Alphalyse experienced Project Managers will work with you to define project deliverables, budget and timelines.

**IP.** Alphalyse has developed and optimized unique analytical procedures for your disposal. Analytical measurements and results on your samples are yours to keep.

**Quality.** All analysis procedures follow Standard Operating Procedures (SOPs) with relevant Quality Control standards (QC). Personnel are trained in the analytical procedures and documentation. All results and data are documented, stored and backed up on our in-house built Laboratory Information Management System (LIMS). Our laboratory facilities can be audited by customers.

**Confidentiality.** To work under confidentiality on customer proprietary projects is our standard business and our employees are trained and follow all confidentiality measures. Confidentiality Agreement (CDA) are signed upon customer request.

## Service Offering

The Alpha-Quant™ service will be performed in close collaboration between you and Alphalyse. Contact us today for details about assay setup, delivery time and costs.

We would also like to discuss with you about the product and process to be quantified and other relevant sample information, in order to provide you with a detailed project quotation.

## Contact Details

### EUROPE

Alphalyse A/S  
Unsbjervej 4  
DK-5220 Odense  
DENMARK

Tel: +45 6310 6500  
[www.alphalyse.com](http://www.alphalyse.com)

### USA/CANADA

Alphalyse Inc.  
200 Page Mill Road  
Suite 100  
Palo Alto, CA 94306  
USA

Tel: (650) 543-3193  
[info@alphalyse.com](mailto:info@alphalyse.com)

## Fast and Easy Access to Protein Analysis