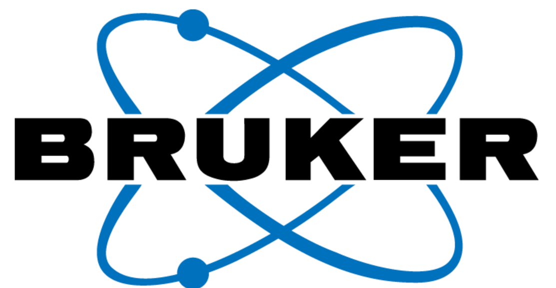
Evaluating performance factors of a targeted label-free protein quantitation approach on an ultra-high resolution API-Qq-TOF



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Bruker Daltonics

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Introduction

Comprehensive quantitation of specific changes in biological systems in response of a certain treatment or perturbation is one of the most important but also among the most challenging tasks in proteomics. We apply an ultra-high resolution API-Qq-TOF in a targeted label-free LC-MS/MS workflow for this purpose (Fig. 1).

Methods

- Model for complex biological sample: Tryptic digest of E. Coli cell lysate spiked with different amounts of BSA digest
- BSA amounts 1, 5, 25, 50, 100, and 500 fmol spiked into 100 ng E.Coli digest per LC run
- 5-7 replicate 1D-LC-MS runs per BSA amount (group) were measured in two measurement series
- LC conditions: Gradient: 90 minutes, flow rate: 300 nl / min
- Source: New nano-ESI sprayer

Results

- Improved intensity stability (*Fig. 2*)
  - Analysis of intensity variations of 7334 peptides in two groups across 7 replicate LC-MS runs revealed an average coefficient of variation (CV) of smaller than 12%
- Improved sequence coverage of low abundant proteins
  - For 5 fmol BSA in 100 ng E.Coli the sequence coverage was increased by a factor of 2 with a targeted LC-MS/MS run
- Optimum sample amount on column
  - Performing separate LC runs for ID and quantitation allows to use higher sample amounts which is optimal for ID and moderate sample amounts which is optimal for quantitation (*Tab. 1*)
- Accurate protein quantitation (*Tab. 1*)
  - Very accurate and precise quantitation results are obtained for regulations in the range of 1 order of magnitude and BSA sample amounts between 50 and 100 fmol
  - Regulations of 2 orders of magnitude as well as low abundant peptides can be reliable quantified
  - At 500 fmol BSA, saturation effects start to influence quantitation
  - 500 E.Coli proteins are identified and quantified with an average protein regulation CV value smaller than 5%

- Many samples in different groups
- Digest proteins
- Separate peptides (LC)
- At least one LC-MS run per sample
- Derive peptide quantitation from LC-MS runs
- Generate multiple Target Lists for MS/MS based on quantitation or compound intensity
- Targeted LC-MS/MS runs for regulated or low abundant precursors
- Data dependant LC-MS/MS
- Combine quantitation with ID
- Proteins identified, proteins quantified
- Report for publication

Fig. 1: Label-free Quantitation Workflow: Peptide quantitation and identification are derived from different LC runs. This allows to select optimal conditions (e.g. sample amount) for quantitation and identification.

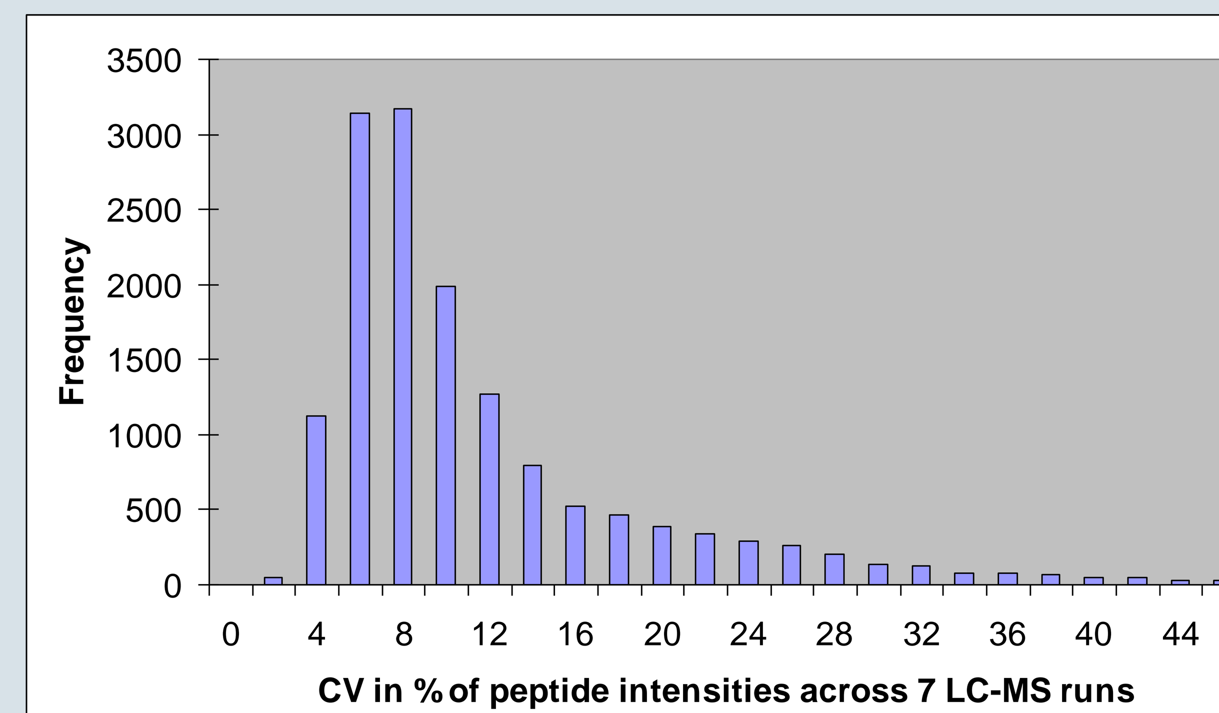

Fig. 2: Distribution of intensity variations of 7334 peptides in 7 replicate LC-MS runs. Two groups of the model system are considered.

BSA Amount / fmol	Expected Ratio	Measured Ratio	No. of Pep. Ratios	CV / %	Error / %
500 / 100	5	5.83	48	36	17
50 / 100	0.5	0.53	37	8	6
25 / 100	0.25	0.30	33	17	20
1 / 100	0.01	0.0115	7	46	15
1 / 5	0.2	0.25	5	22	23

Tab. 1: Regulation ratios of different BSA amounts in 100 ng E.Coli digest.

Summary

- A solution for quantitative proteomics is evaluated using a model system
- Low peptide intensity variations lead to accurate and precise protein quantitation

Conclusions

- A new nano-ESI sprayer leads to improved peptide intensity stability and is the basis for label-free quantitation in high throughput applications
- Accurate quantitation over 2-3 orders of magnitude in a complex biological mixture is achieved
- Targeted LC-MS/MS runs lead to an increase of low abundant biomarker identifications

UHR-TOF

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