LC-MS/MS system calibration using peptide reference standards

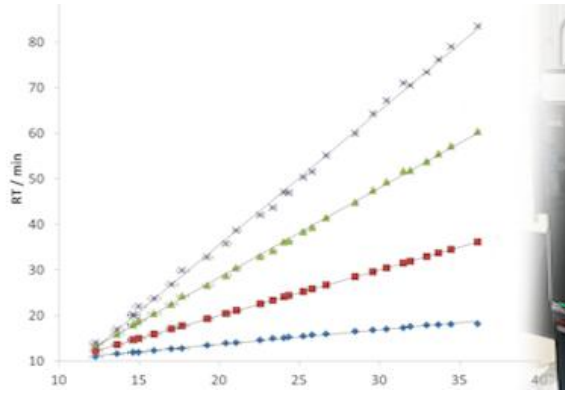
Introduction

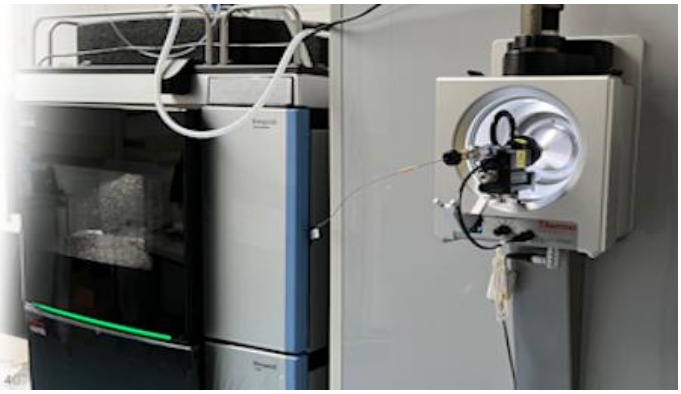
Protein analytics using mass spectrometry has become increasingly important in the past decades, especially the application in biopharmaceutical research, development and production. In May 2025, this development was acknowledged with the addition of a new chapter in the US Pharmacopeia (USP<1132.1> “Residual Host Cell Protein Measurement in Biopharmaceuticals by Liquid Chromatography-Mass Spectrometry) providing detailed information for MS-based workflows, including sample preparation and validation requirements.

Depending on the intended application, proteinaceous samples can be - individually or in combination - analysed using two methods:

- Discovery proteomics: enabling the identification of thousands of proteins present in a sample but with limited and unprecise quantitative information
- Targeted proteomics: allowing more in-depth and quantitative analysis of a limited number of proteins, usually facilitated by addition of heavy isotope-labelled reference standards

Most importantly for analysis of biotherapeutic products, generation of reliable data depends on the optimal performance of mass spectrometry instrumentations. For monitoring sample preparation artifacts, instrument performance and chromatographic retention time, PolyQuant provides specifically designed peptide calibration standards.





QCAL – mass spectrometry calibration standard

PolyQuant's QCAL peptide calibration standard is a highly versatile standard for routine monitoring of mass spectrometry instrument performance as well as for controlling of sample preparation artifacts.

Comprising of 22 peptides with selected physical and chemical properties, QCAL can be used to **optimize and standardize** commonly used mass spectrometry instrumentation platforms.

- Instrument resolution
- Linearity of signal detection
- m/z calibration

Being based on QconCAT technology, QCAL is available as peptide mix but also as complete synthetic protein.

Assess peptide modification

Co-digesting the complete QCAL protein with your sample allows controlling for sample processing and sample preparation artifacts e.g. methionine oxidation, deamidation of gln/asn and modification of cysteine residues.
QCAL also contains peptides enabling the monitoring of C-terminal homoarginine formation.

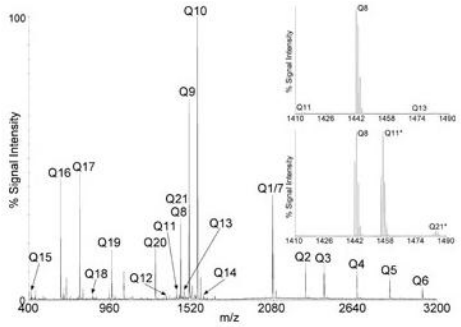


Fig 1: Source [QCAL – a novel standard for assessing instrument conditions for proteome analysis]

RePLiCal – retention time calibration standard

RePLiCal is a HPLC calibration standard comprising of unique, easy to detect peptides with selected chemical properties facilitating retention time standardization and normalization in reverse-phase LC-MS analysis.

The 27 lysine-terminating peptides do not contain modifiable amino acids like methionine, tryptophan, cysteine, and N-terminal glutamine. Also, asp/pro, asn/pro and asn/gly motifs are absent.

Being detectable at low amounts, addition of RePLiCal does not increase the complexity of the sample.

RePLiCal can be applied to calibration of LC gradients and micro and nano (u)HPLC instruments using trap-elute configurations and to benchmark RP LC instrument performance across laboratories.

- Enhanced normalization of reverse-phase chromatography separation between LC-MS runs
- Accurate prediction of normalized retention time, even over extended analysis time
- Assessment of the trapping column performance
- Recalibration of the m/z scale post-acquisition

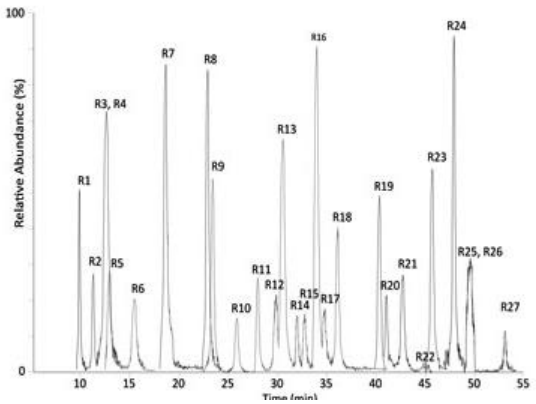


Fig 2: Elution profile of RePLiCal on a 60 min gradient, 8-38% solvent B (0.1% formic acid in acetonitrile)

Summary

PolyQuant's peptide calibration standards are specifically designed for routine monitoring of key instrument parameters of LC-MS and mass spectrometry instrumentations.
They are available as peptide mix or complete protein and ideally complement each other. Used in combination, our standards enable monitoring of workflow performance from sample preparation to data acquisition.

	QCAL	**RePLiCal**
Order number	PQ-CS-5730 ff	PQ-CS-1560 ff
Recommended use	Mass spectrometry instrument calibration	Retention time standardization
Number of peptides	22	27
Package size	25 µg (479 pmol)	1 µg (25,5 pmol/25510 fmol)
Applications	50-500 injections	250-500 injections
Recommended amount per injection	1-10 pmol	50-100 fmol
Peptides terminate with	K/R	K
Peptide size	418 Da to 3101 Da	435 Da to 3372
Non-natural peptides		☺
Co-digestion with sample	☺	☺
Minimal interference with analyte sample		☺
Monitoring of sample preparation artifacts	☺	
Robust and reproducible detection	☺	☺
Monitor highly hydrophilic and hydrophobic peptides		☺
Test Trap Column		☺
Test signal linearity	☺	
Test instrument resolution (MS)	☺	
Implemented in Skyline		☺
iRT calculation		☺