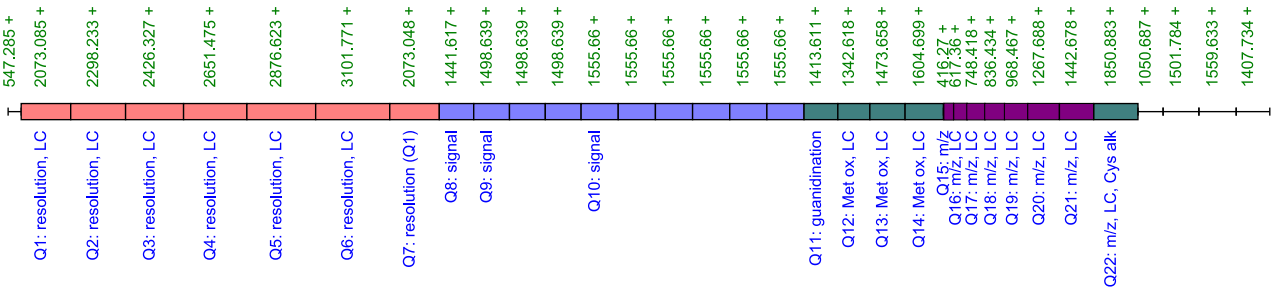
QCAL: a calibration standard for Mass Spectrometry

Calibration standard for MS instrumentation including a range of different hydrophobicities. QCAL also allows assessment of typical chemical modifications like oxidation of methionine, deamidation of glutamine or asparagine and modification of lysine residues by guanidination.



PolyQuant has designed an artificial protein (QCAL) for use in the optimisation and standardisation of all commonly used instrumentation platforms for proteomics (1).

QCAL is a QconCAT (2) (52.2kDa) based calibration standard, comprising 22 unique peptides, with masses ranging from ~ 410 to 3100Da. QCAL has been designed to provide standards for peptide separation by reverse-phase chromatography, to facilitate the assessment and optimisation of instrument resolution and to evaluate the linearity of signal detection in different MS instruments, including MALDI-ToF, ESI MS and FTICR (1). QCAL also contains peptides with amino acid residues which may undergo artifactual modification (oxidation of methionine, deamidation of glutamine or asparagine) and modification of lysine residues by guanidination (3), allowing the routine monitoring of these processes.

Evaluation of peptide separation by reversed-phase chromatography

Digested QCAL provides a stoichiometric collection of peptides covering a broad range of hydrophobicities. QCAL peptides show good chromatographic resolution, eluting between 5 and ~35% acetonitrile (Figure 1), which spans the range of hydrophobicities displayed by the majority of tryptic peptides eluting from C_{18} media. Digested QCAL can be therefore readily used to standardise reverse-phase chromatographic conditions for peptide separation.

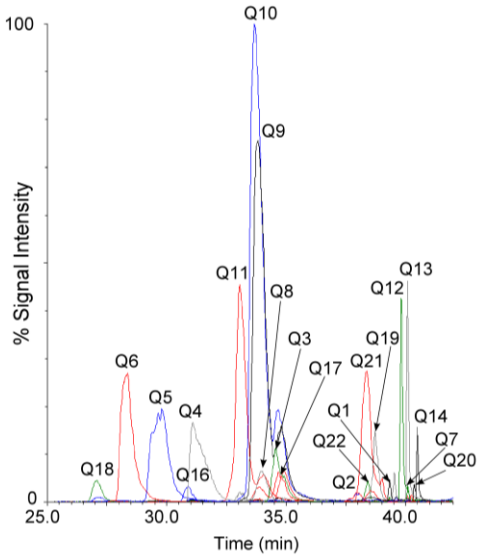


Figure 1. Analysis of digested QCAL by LC-MS/MS. *Extracted ion chromatograms for all Q-peptide eluting from a PepMap™ C18 column (3 µm, 0.075 x 150 mm, 100 Å) from LC Packings (Dionex, CA) at 200 nl/min. The column was equilibrated in 0.2% formic acid (solvent A) and was developed with 90% acetonitrile/0.2% formic acid (solvent B) 0-20% over 30min, 20-60% over 10min and 60-100% over 5min. All peptides are eluted between 5 and ~35% acetonitrile with a peak width of ~20s. Source: Eyers, C. E. et al. (2008).*

Calibration of mass spectrometry

QCAL has been designed to generate tryptic peptides with $[M+H]^+$ m/z values between 400 and 3000 (figure 2). Additionally, the multiple protonated species of these peptides permit calibration of mass spectrometers with an electrospray ionisation source in the m/z range of 600 to 1600. This can be further extended to m/z of 100 if using tandem MS data.

For ESI-MS/MS analyses many laboratories use the [Glu^1]-fibrinopeptide B ("glufib"; EGVNDNEEGFFSAR) to assess instrument sensitivity, and for calibration of fragment ion m/z following collision-induced dissociation. A modified version of this peptide sequence was incorporated into QCAL (Q8) for use in post-fragmentation calibration.

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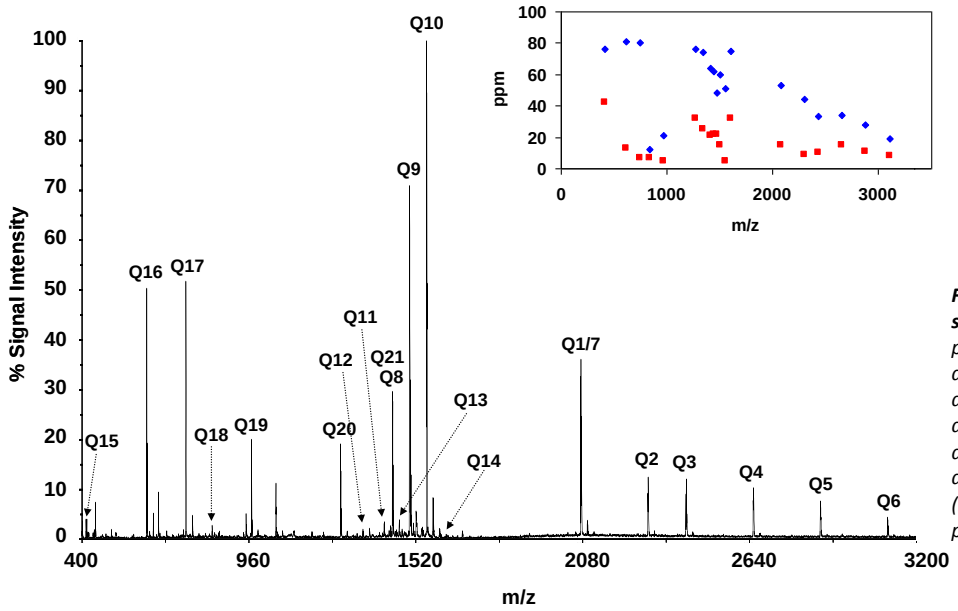

Figure 2: MALDI-ToF mass spectra of digested QCAL. *Limit peptides generated after tryptic digestion of QCAL were analysed following MALDI using a Voyager DE™ STR. Inset demonstrates the ppm mass accuracy before (blue) and after (red) optimisation of acquisition parameters.*

QCAL for the assessment and optimisation of instrument resolution

High-resolution Fourier Transform mass spectrometers, including Ion Cyclotron Resonance (FT ICR) and Orbitrap instruments, perform analytic mass determinations to high accuracy (low or sub-ppm). QCAL can be used with these instruments either as an external calibrant or as an internal calibrant. Peptides Q1 and Q7 of QCAL are identical except for a lysine to glutamine substitution, providing peptides whose masses differ by 0.0364 Da. Discriminating these two peptides requires an instrument resolution of ≥57,000 (FWHM). As shown in Figure 3, these two peptides can be readily distinguished, with peak resolution >105,000 (FWHM) being observed in this instance. Differentiation of these peptides can therefore be used as a benchmark for instrument resolution (figure 3).

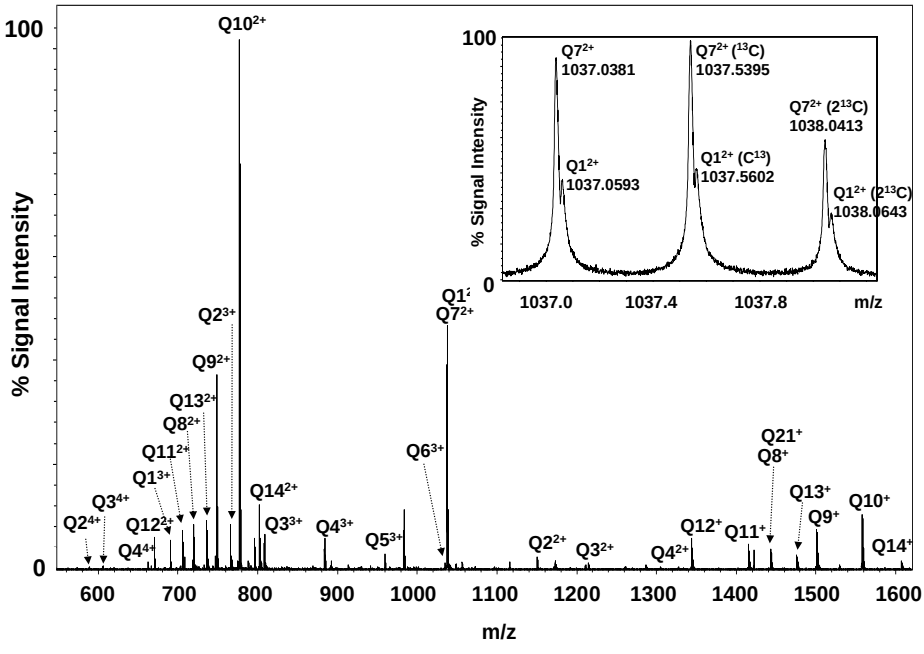

Figure 3. ESI FT ICR mass spectra of digested QCAL. *Inset is a high-resolution mass spectrum depicting the doubly protonated species of Q1 and Q7, where the same peptide sequence contains a K to Q substitution, readily confirming that the instrument resolution in this mode exceeds 57K.*

QCAL for the characterisation of the linear range of peptide ion signal intensity for quantification

Determining the linearity of signal detection is crucial for quantification experiments. QCAL was therefore designed to

comprise multiple copies of two variants of the Glu-fibrinogen derived peptide. The three peptides, Q8, Q9 and Q10, differ only by the number of glycine residues at their amino terminus (one, two and three respectively). There are three copies of Q9, six copies of Q10 and one copy of Q8. Analysis of these three peptides by LC-MS on a quadrupole-time of flight (Q-ToF) instrument demonstrated good linearity of signal, with Q8:Q9:Q10 being detected at a ratio of 1.0:3.1:6.2.

QCAL for the assessment of peptide modification

In addition to the detection of peptide deamidation, QCAL comprises peptide sequences to assess peptide modification such as methionine oxidation (Q12, 13 and 14) and modification of cysteine residues (Q22).

In MALDI-ToF MS, because arginine-terminated tryptic peptides frequently give superior signals to their lysine-terminated counterparts, lysine residues in peptides are often converted to homoarginine. QCAL peptides Q8 and Q11 were therefore included to test the efficiency of C-terminal homoarginine formation by lysine guanidination.

QCAL peptides Q8 and Q11 were therefore included to test the efficiency of C-terminal homoarginine formation (figure 4).

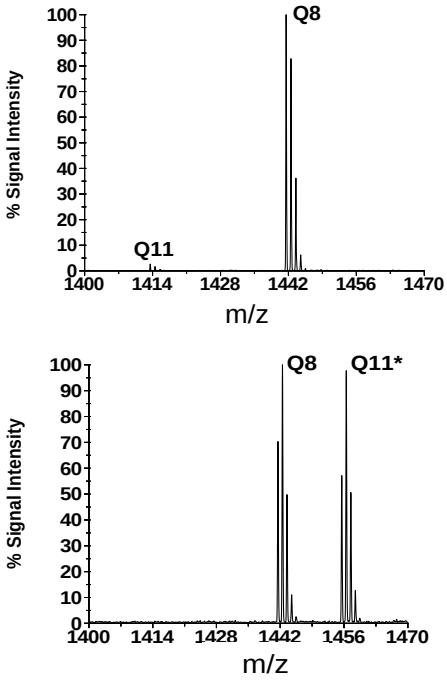

***Figure 4:**. Trypsin hydrolysate of QCAL, analysed in MALDI-ToF (spectra over a m/z range of 1410-1470). Before quanidation (A), Q11, the lysine-terminated counterpart of Q8 almost cannot be detected. After guanidination (B) these peptides are detectable with the same relative signal intensity.*

References

1) Eyers, C.E, *et al.* (2008) QCAL – a novel standard for assessing instrument conditions for proteome analysis. J Am Soc Mass Spectrom
2) Pratt, J.M. *et al.*(2006). Multiplexed absolute quantification for proteomics using concatenated signature peptides encoded by QconCAT genes. Nature protocols, 1, No. 2, 1-15.
3) Brancia, F.L. *et al.* A combination of chemical derivatisation and improved bioinformatic tools optimises protein identification for proteomics. Electrophoresis 22, 552–559 (2001).

Appendix:

Peptide	Retention Time (min)	Relative ψ	$[M+H]^+$: m/z
Q1	39.3	35.98	2073.1015
Q2	37.9	34.51	2298.2492
Q3	34.6	32.52	2426.3442
Q4	31.1	30.39	2651.4919
Q5	29.6	28.23	2876.6397
Q6	28.4	26.66	3101.7874
Q8	34.0	22.50	1441.6342
Q9	33.8	23.20	1498.6557
Q10	33.7	22.92	1555.6772
Q11	33.1	21.97	1413.6281
Q11*			1455.6499
Q12	39.8	36.14	1342.6354
Q13	40.1	38.14	1473.6758
Q14	40.5	40.94	1604.7163
Q15	N.D.	42195	416.2873
Q16	30.9	16.98	617.3776
Q17	34.7	28.17	748.4358
Q18	27.1	17.27	836.4518
Q19	38.7	31.77	968.4842
Q20	40.4	38.27	1267.7051
Q21	38.4	29.19	1442.6957
Q22	N.D.	31.68	1850.8999
Q22*	38.4	-	

Table 1: *Peptide retention times, relative hydrophobicity (ψ) and m/z values*