Proteored Multicentric Experiment 8: Quantitative Targeted Analysis in Proteomics. An Assessment Study. (PME8-QTAPAS)

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STUDY DESIGN AND OBJECTIVE

In order to evaluate the robustness and reproducibility, within and across laboratories, of the SRM and pseudo-SRM quantification methodology, we set up a multi-centric study (PME8) carried out at 27 laboratories, including ProteoRed-ISCIII network of proteomics facilities in Spain, several EuPA members, and other laboratories worldwide.

Each participant laboratory received a set of 5 different samples A-E, prepared by spiking different amounts of the Sigma-Aldrich MSQC1 standard into a yeast lysate digest.

The five samples were analyzed in triplicate by SRM or pseudo-SRM using similar chromatographic and spectrometric conditions at the different laboratories and with different instruments. Each laboratory reported results on relative quantification (fold changes between A-E samples) and absolute quantification based on the labeled peptide standards

Analysis conditions

- 3 replicate nLC-MS runs, 90 min gradient , batchwise (3 x [A-E])
- SRM: Monitoring (14 +14) peptides x 3 transitions = 72 transitions
- MPM: Monitoring (14+14) peptides, 30 precursor ions

SAMPLE SET

		fmol / µg yeast digest				
Tier	Protein	sample A	sample B	sample C	sample D	sample E
1	CAH1	20	40	100	200	1000
	CAH2	20	40	100	200	1000
2	NADPH	4	8	20	40	200
	CRP	4	8	20	40	200
3	PPIA	0.8	1.6	4	8	40
	CATA	0.8	1.6	4	8	40

- Set of 5 different samples A-E, prepared by spiking different amounts of the Sigma-Aldrich MSQC1 standard into a yeast lysate digest.
- The samples contain tryptic digests of 6 human proteins, distributed in three concentration tiers, as shown in the table. Amounts indicated as **fmol** spiked protein/ **microgram** of yeast lysate
- Additionally, the samples contain isotopically labeled peptides for each of the human proteins, in different ratios to the corresponding unlabeled peptides, ranging form 1:0.2 to 1:50, as indicated in the table.

Tier	Peptide	ratio L/H
1	CAH1_GGPFSDSY[R]	1
	CAH1_VLDALQAI[K]	2
	CAH2_AVQQPDGLAVLGIFL[K]	10
	CAH2_SADFTNFDP[R]	50
2	NADPH_EGHLSPDIVAEQ[K]	1
	NADPH_ALIVLAHSER[R]	2
	CRP_ESDTSYVSL[K]	10
	CRP_GYSIFSYAT[K]	50
3	PPIA_FEDENFIL[K]	0.5
	PPIA_VSFELFAD[K]	1
	PPIA_TAENF[R]	2
	CATA_GAGAFGYFEVTHDIT[K]	0.2
	CATA_FSTVAGESGSADTV[R]	10
	CATA_NLSVEDAA[R]	50

Participant Laboratories

- **20 SRM Triple Quadrupole Data Sets**
- **3 Q-TOF + 7 ORBITRAP MPM Data Sets**

RESULTS. ABSOLUTE QUANTIFICATION. INTER- LABORATORY/MEASUREMENT TYPE VARIABILITY

TRIPLE-QUADRUPOLE

Sample	A	B	C	D	E	A	B	C	D	E
Peptide	Average fmol measured					CV(%)				
theor. fmol	20	40	100	200	1000					
CAH1_GGPFSDSYR	20.50	40.96	102.29	204.21	1024.68	5.42	5.31	5.26	5.33	5.38
CAH1_VLDALQAIK	21.01	41.29	99.34	205.31	1004.96	13.47	5.82	12.17	4.68	9.64
CAH2_AVQQPDGLAVLGIFLK	15.71	33.27	89.06	168.98	844.86	40.58	24.68	25.32	9.58	9.14
CAH2_SADFTNFDPR	20.69	44.12	114.38	228.20	1154.97	11.26	13.75	9.99	4.11	4.57
theor. fmol	4.00	8.00	20.00	40.00	200.00					
NADPH_EGHLSPDIVAEQK	3.80	7.38	19.10	35.85	173.37	24.82	19.31	15.22	12.91	12.23
NADPH_ALIVLAHSER	2.07	3.89	9.55	19.59	95.10	13.57	16.00	15.81	12.06	6.00
CRP_ESDTSYVSLK	3.83	7.40	18.79	37.06	187.42	17.24	10.46	6.62	5.15	6.82
CRP_GYSIFSYATK	2.17	4.38	13.17	29.62	149.62	52.60	30.63	13.75	13.10	9.50
theor. fmol	0.80	1.60	4.00	8.00	40.00					
PPIA_FEDENFILK	0.51	0.90	2.15	4.29	21.32	18.58	16.83	13.64	13.98	9.71
PPIA_VSFELFADK	0.72	1.06	2.38	4.47	22.30	41.29	31.94	19.19	15.42	15.93
CATA_GAGAFGYFEVTHDITK	1.12	0.47	1.24	2.25	8.04	80.74	46.49	79.39	46.25	25.30
CATA_FSTVAGESGSADTVR	0.62	0.90	2.27	4.79	22.02	113.39	18.95	23.42	17.60	12.70
CATA_NLSVEDAAR	0.30	0.69	2.24	4.95	27.12	76.51	59.91	44.70	33.85	8.64

ORBITRAP

Sample	A	B	C	D	E	A	B	C	D	E
Peptide	Average fmol measured					CV(%)				
theor. fmol	20	40	100	200	1000					
CAH1_GGPFSDSYR	20.01	39.82	101.79	198.71	1001.52	1.84	10.81	4.09	3.48	1.37
CAH1_VLDALQAIK	18.22	39.73	86.40	181.99	986.23	11.41	9.08	11.49	10.75	11.83
CAH2_AVQQPDGLAVLGIFLK	19.66	29.31	73.73	124.25	934.06	65.94	29.58	17.19	29.67	17.50
CAH2_SADFTNFDPR	15.13	29.72	90.46	186.49	1002.49	37.49	18.12	29.96	24.88	27.64
theor. fmol	4.00	8.00	20.00	40.00	200.00					
NADPH_EGHLSPDIVAEQK	3.06	5.79	22.60	27.55	161.07	10.04	21.75	53.26		15.11
NADPH_ALIVLAHSER	1.33	3.29	8.59	18.44	88.68	17.52	7.65	13.20	10.16	17.06
CRP_ESDTSYVSLK	3.69	7.49	15.61	32.95	167.97	9.04	49.12	8.07	17.33	9.53
CRP_GYSIFSYATK	1.97	4.54	14.65	25.03						
theor. fmol	0.80	1.60	4.00	8.00	40.00					
PPIA_FEDENFILK	0.41	0.96	2.05	4.48	24.72	6.74	18.29	10.26	5.88	24.20
PPIA_VSFELFADK	0.83	0.97	2.96	4.66	22.04	70.22	57.51	41.07	14.31	14.98
CATA_GAGAFGYFEVTHDITK	0.79	0.45	1.56	2.40	9.19	56.87	14.18	73.57	29.09	21.71
CATA_FSTVAGESGSADTVR	0.37	0.80	2.98	3.94	22.38	12.72	9.71	41.03	25.04	8.84
CATA_NLSVEDAAR	0.14	0.40	2.91	5.30	24.86	109.32	71.70	32.27	10.70	21.96

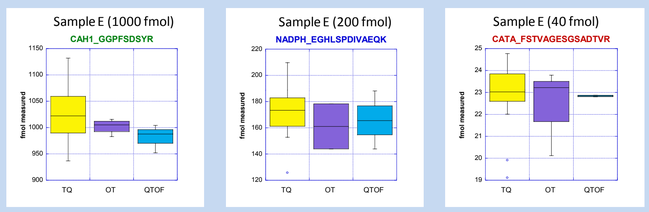
Q-TOF

Sample	A	B	C	D	E	A	B	C	D	E
Peptide	Average fmol measured					CV(%)				
theor. fmol	20	40	100	200	1000					
CAH1_GGPFSDSYR	20.44	40.20	102.97	200.38	981.40	3.27	3.00	3.97	3.47	2.71
CAH1_VLDALQAIK	21.19	41.07	95.07	204.44	977.43	3.65	1.80	14.84	4.31	19.57
CAH2_AVQQPDGLAVLGIFLK	19.40	43.28	107.15	139.73	792.83	7.23	30.28	32.62	27.68	4.19
CAH2_SADFTNFDPR	20.71	43.44	90.52	212.91	1182.04	21.38	9.12	31.40	0.71	11.31
theor. fmol	4.00	8.00	20.00	40.00	200.00					
NQO1_EGHLSPDIVAEQK	3.63	7.54	18.53	35.77	179.64	58.66	23.84	16.67	13.41	6.86
NQO1_ALIVLAHSER	1.68	3.56	8.28	18.56	89.12			3.04		3.18
CRP_ESDTSYVSLK	4.86	11.05	19.15	37.98	188.45	48.40	37.45	15.39	5.60	7.70
CRP_GYSIFSYATK	2.13	4.96	14.12	24.56	148.97	16.25	18.92		21.92	7.51
theor. fmol	0.80	1.60	4.00	8.00	40.00					
PPIA_FEDENFILK	0.51	1.14	3.16	4.65	24.84		24.77	39.59		25.76
PPIA_VSFELFADK	0.42	0.94	3.32	4.26	22.47					7.97
CATA_GAGAFGYFEVTHDITK	2.70	1.10	2.25	5.01						
CATA_FSTVAGESGSADTVR	0.28	1.16	3.54	5.02	22.84	97.18	93.92	49.89	14.23	0.20
CATA_NLSVEDAAR	0.28	0.75	1.81	4.39	31.48					20.08

Tables summarizing the measurements of absolute concentration for each peptide and sample, averaged for each group of instruments, as indicated, and across all 30 datasets. The %CV of variation of the measurements are shown on the right. Red scale highlights %CV values above 20%.

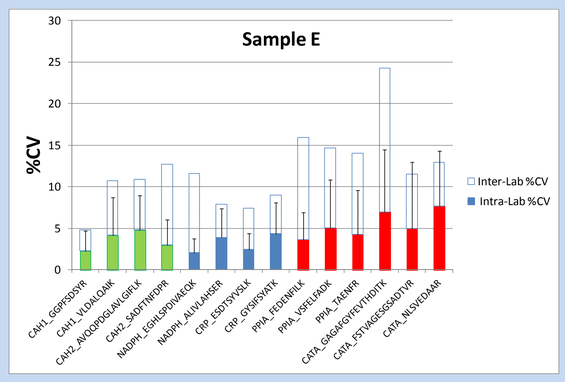
ALL MEASUREMENTS

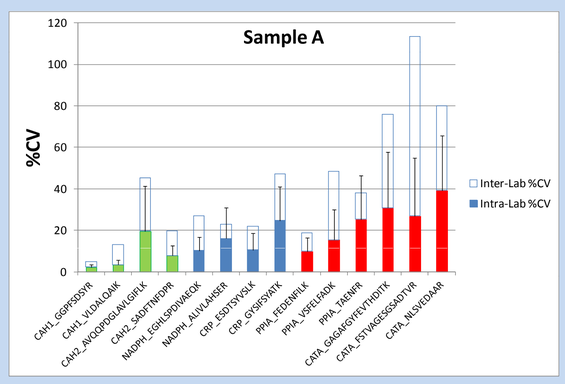
Sample	A	B	C	D	E	A	B	C	D	E
Peptide	Average fmol measured					CV(%)				
theor. fmol	20	40	100	200	1000					
CAH1_GGPFSDSYR	20.41	40.65	102.30	202.87	1015.23	4.80	6.29	4.81	4.90	4.82
CAH1_VLDALQAIK	20.58	41.02	96.75	201.47	998.66	13.19	6.02	12.89	6.93	10.74
CAH2_AVQQPDGLAVLGIFLK	16.93	33.76	87.94	154.83	852.65	45.22	28.31	27.13	19.93	10.87
CAH2_SADFTNFDPR	19.58	41.44	107.41	221.13	1125.46	19.80	19.19	18.15	10.02	12.65
theor. fmol	4.00	8.00	20.00	40.00	200.00					
NADPH_EGHLSPDIVAEQK	3.66	7.15	19.41	35.38	173.10	27.02	20.71	20.90	13.50	11.60
NADPH_ALIVLAHSER	1.85	3.76	9.25	19.35	93.68	22.78	15.78	15.45	11.50	7.86
CRP_ESDTSYVSLK	3.92	7.87	18.43	36.81	185.79	22.05	28.90	9.89	6.88	7.42
CRP_GYSIFSYATK	2.16	4.47	13.32	28.76	149.52	47.30	27.58	13.06	14.66	9.00
theor. fmol	0.80	1.60	4.00	8.00	40.00					
PPIA_FEDENFILK	0.50	0.93	2.23	4.33	22.23	18.92	18.67	22.08	12.87	15.91
PPIA_VSFELFADK	0.72	1.02	2.59	4.48	22.27	48.45	37.92	28.81	14.34	14.65
CATA_GAGAFGYFEVTHDITK	1.15	0.53	1.47	2.56	8.29	75.82	48.13	67.03	45.56	24.25
CATA_FSTVAGESGSADTVR	0.55	0.92	2.49	4.69	22.13	113.34	41.87	33.56	18.53	11.48
CATA_NLSVEDAAR	0.27	0.63	2.33	4.96	27.22	79.90	61.85	41.77	30.83	12.96



Representative boxplots of the distribution of absolute concentration measurements for a peptide of each of the concentration tiers. The graphs for the most concentrated sample (E) and the most diluted (A) are shown. Measurements are grouped by type of measurement/instrument: TQ: SRM on triple quadrupole; OT, QTOF: pseudoSRM in orbitrap and Q-TOF instruments, respectively. In general, no major differences in terms of median value or dispersion were observed between the three groups.

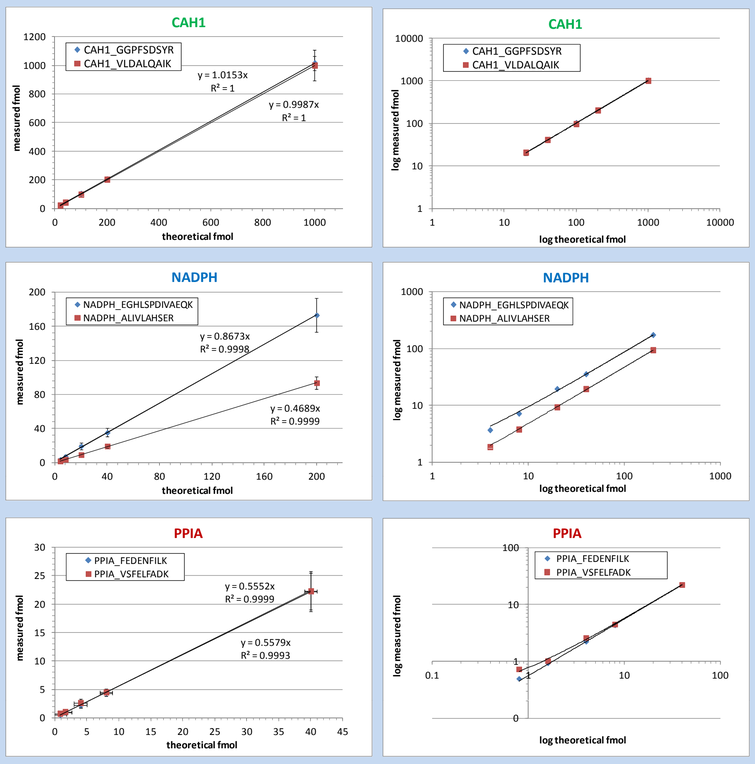
INTRA- LABORATORY VARIABILITY





Solid bars represent the measured % coefficients of variation (%CV) between the three replicate analysis performed, averaged for all datasets. Each bar is the %CV value for the measurement of the indicated peptide. Error bars indicate the standard deviation of the values for all labs. Bars are colored according to the concentration tiers in the samples. Empty bars represent the value for Inter-Laboratory %CV for the corresponding peptide measurement. The graphs for the most concentrated sample (E) and the most diluted (A) are shown.

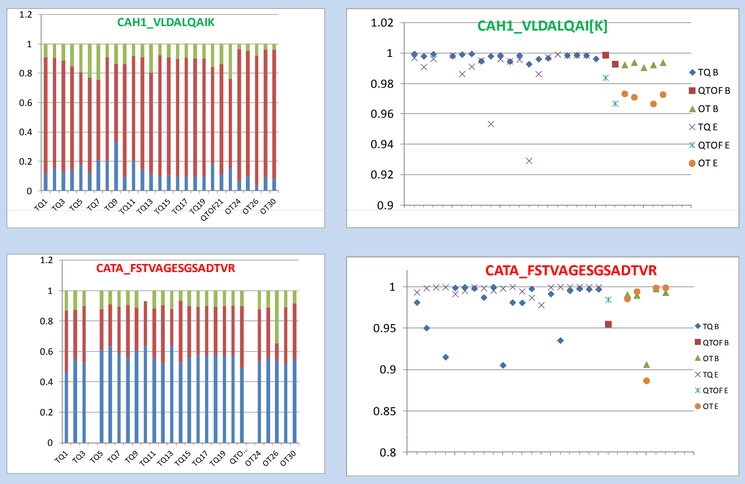
LINEARITY OF THE RESPONSE

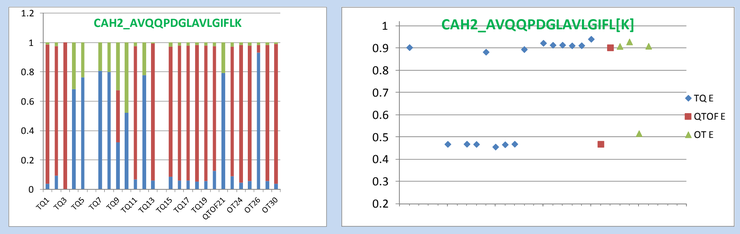


Plots of measured amounts (average of 30 datasets) in fmol versus theoretical amounts, in each of the five samples, for two different peptides of a protein of each concentration tier. Error bars represent the standard deviation of the measurements across labs. Graphs on the right show the linearity in log-log scale. A good linearity of the response was observed for all peptides in the measured ranges.

A deviation from the expected theoretical amounts was observed in some cases. In the case of NADPH peptides (middle graph), both peptides give a different quantification, being one of them around half of the theoretical amount. This could be the result of an uncomplete digestion of the protein during sample preparation. In the case of PPIA (bottom graph), both peptides give the same quantification, but the value is about half of the theoretical amount. This could potentially be due to unaccuracy in the quantification of the protein standard.

REPRODUCIBILITY OF TRANSITION PATTERN





Bar plots (left panels) represent the contribution of each of the three monitored transitions to the total MS signal, for three example peptides, for each instrument/laboratory. To compare the patterns, the dotproducts of the normalized vectors defined by the three contributions and the one corresponding to the average of all laboratories have been calculated. The plots on the right panels show the dotproduct values for each lab, grouped by type of instrument.

The two peptide graphs shown on the left side are representative of the behaviour of most of the signals, showing no major differences between instruments, irrespective of the signal level (dotproducts for samples B and E shown for comparison).

The plots shown on top right are an example of a peptide for which different labs selected different patterns, probably due to the presence of interfering signals. Two different patterns are clearly seen both in he bar or dotproduct plots, not depending on the instrument type. Quantification for this peptide shows accordingly a higher variability between laboratories.

CONCLUSIONS

- The results demonstrate a good degree of reproducibility of targeted quantification measurements by SRM at different laboratories, irrespective of the method of analysis and the spectrometer used.
- The average Inter-Laboratory %CV of the measured absolute protein amounts ranges from less than 10%CV for Tier 1 proteins, to 40-60% for the proteins at the lowest concentrations.
- The pattern of relative intensities of the transitions monitored is fairly consistent among different instruments and fragmentation modes, underscoring the utility of spectral databases for the design of quantification methods.
- The results obtained at each laboratory allow the assessment of the limitations in sensitivity and limits of quantification under the diverse analytical conditions used