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Establishment of Performance Qualification Criteria for HPLC Systems Dedicated to Peptide Mapping

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ABSTRACT

Appropriate qualification procedures for HPLC systems are basic requirements for the validation and use of analytical methods. Peptide mapping is a particularly important tool for the characterization of therapeutic proteins. Because this reversed phase HPLC technique is exceptionally sensitive to experimental variability, it is important to thoroughly characterize the system.
A performance qualification test that best mimics peptide analysis is advantageous for predicting how the system will perform with real samples. We have established performance standards for reversed-phase HPLC in conjunction with both UV and MS detection for systems dedicated to running peptide maps.
The criteria and specifications include retention time reproducibility, injector reproducibility and injector linearity. Mass accuracy was included for MS configurations. This qualification method includes the use of a well-characterized peptide analyte mix, a tested HPLC column specific for peptide analysis, and a detailed protocol. The developed procedure could also be used as a routine system suitability test.

EXPERIMENTAL

HPLC System:	Waters Alliance® Bioseparations Module
Column:	Biosuite™ PA-A C$_{18}$, 3.0 µm, 2.1 x 150 mm
Column temp:	40.0 °C
Flow rate:	0.2 mL/min
Gradient elution:	0–50 %B/30min
Mobile phase:	A: 0.02% TFA
	B: 0.018%TFA/MeCN
UV Detection:	Waters 2487 Dual Wavelength Absorbance Detector, 214 nm
Mass Spectrometer:	Waters ZQ Mass Detector
MS conditions:	
Mode:	ESI +
Capillary:	3.3 kV
Cone:	30 V
Desolvation gas flow:	500 L/hr
Cone gas flow:	50 L/hr
Source temperature:	150 °C
Desolvation temperature:	350 °C
Scan mode	300-2000 (m/z)

Waters MassPREP™ PQ Peptide

Peptide Name	Sequence	Monoisotopic MW
PQ peptide	RGDSPASSKP	1000.5

Figure 1: BioSuite peptide mapping MS system consisting of the 2796 Bioseparations module, MassLynx™ software, Waters 2487 dual wavelength detector, the Waters Micromass® ZQ mass spectrometer , BioSuite PA columns and MassPrep standards

Waters MassPREP™ Peptide Mixture

Peak #	Peptide Name	Sequence	Monoisotopic MW
1	Allantoin (void marker)	$C_4H_6N_4O_3$	158.0
2	RASG-1	RGDSPASSKP	1000.5
3	Angiotensin Fragment 1-7	DRVYIHP	898.5
4	Bradykinin	RPPGFSPFR	1059.6
5	Angiotensin II	DRVYIHPF	1045.5
6	Angiotensin I	DRVYIHPFHL	1295.7
7	Renin Substrate	DRVYIHPFHLLVYS	1757.9
8	Enolase T35	WLTGPQLADLYHSLMK	1872.0
9	Enolase T37	YPIVSIEDPFAEDDWEAWSHFFK	2827.3
10	Melittin	GIGAVLKVLTTGLPALIS-WIKRKRQQ	2845.7

Retention Time Reproducibility

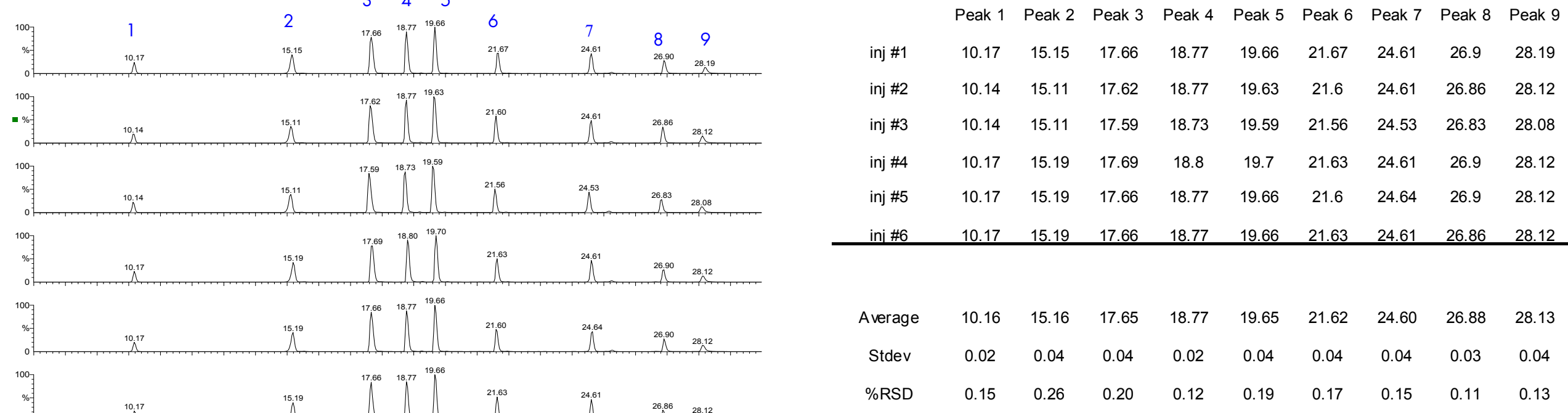|	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9
inj #1	10.17	15.15	17.66	18.77	19.66	21.67	24.61	26.9	28.19
inj #2	10.14	15.11	17.62	18.77	19.63	21.6	24.61	26.86	28.12
inj #3	10.14	15.11	17.59	18.73	19.59	21.56	24.53	26.83	28.08
inj #4	10.17	15.19	17.69	18.8	19.7	21.63	24.61	26.9	28.12
inj #5	10.17	15.19	17.66	18.77	19.66	21.6	24.64	26.9	28.12
inj #6	10.17	15.19	17.66	18.77	19.66	21.63	24.61	26.86	28.12
Average	10.16	15.16	17.65	18.77	19.65	21.62	24.60	26.88	28.13
Stdev	0.02	0.04	0.04	0.02	0.04	0.04	0.04	0.03	0.04
%RSD	0.15	0.26	0.20	0.12	0.19	0.17	0.15	0.11	0.13

Figure 2: Six consecutive injections showing reproducibility of chromatography. Retention time RSD for six injections is below 0.3 % for each peak in the peptide mix .

System Reproducibility

Compound name:PQ Peptide — UV

# Name	Type	Std. Conc	RT	Area	Height	Response	Conc.	Flags
1 PQ Peptide 1	Standard	4.000	4.30	789.085	5609	789.085	4.0	MM
2 PQ Peptide 2	Standard	4.000	4.27	807.345	8463	807.345	4.1	MM
3 PQ Peptide 3	Standard	4.000	4.25	786.712	8611	786.712	4.0	bb
4 PQ Peptide 4	Standard	4.000	4.25	788.441	9016	788.441	4.0	MM
5 PQ Peptide 5	Standard	4.000	4.24	805.705	8987	805.705	4.1	MM
6 PQ Peptide 6	Standard	4.000	4.22	794.477	8497	794.477	4.0	bb

Compound name: PQ Peptide
Response Factor: 198.824
RRF SD: 2.27335, % Relative SD: 1.1434
Response type: External Std, Area
Curve type: RF
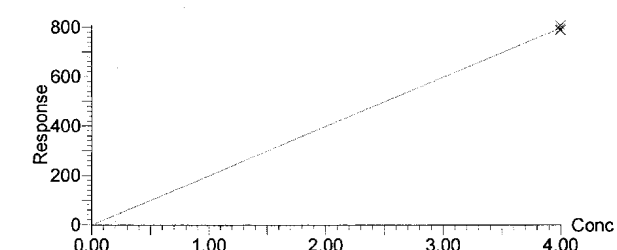
Compound name:PQ Peptide — MS

# Name	Type	Std. Conc	RT	Area	Height	Response	ng	Flags
1 PQ Peptide 1	Standard	4.000	4.33	85127.938	562495	85127.938	3.9	MM
2 PQ Peptide 2	Standard	4.000	4.30	87492.813	829388	87492.813	4.0	MM
3 PQ Peptide 3	Standard	4.000	4.28	86417.664	860083	86417.664	3.9	MM
4 PQ Peptide 4	Standard	4.000	4.27	91949.102	940883	91949.102	4.2	MM
5 PQ Peptide 5	Standard	4.000	4.24	88007.500	898976	88007.500	4.0	MM
6 PQ Peptide 6	Standard	4.000	4.23	88807.852	854174	88807.852	4.0	MM

Compound name: PQ Peptide
Response Factor: 21991.8
RRF SD: 583.646, % Relative SD: 2.65393
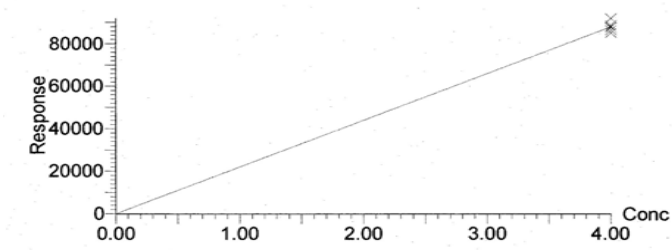Response type: External Std, Area
Curve type: RF

Figure 3: Results acquired during performance qualification testing of injector precision with UV and MS detection.

System Linearity

Compound name:PQ Peptide — UV

# Name	Type	Std. Conc	RT	Area	Height	Response	Conc	Flags
1 PQ Peptide 5uL	Standard	1.000	4.22	374.628	4317	374.628	1.0	MM
2 PQ Peptide 10uL	Standard	2.000	4.22	803.471	8698	803.471	2.0	MM
3 PQ Peptide 15uL	Standard	3.000	4.20	1189.873	13515	1189.873	3.0	MM
4 PQ Peptide 20uL	Standard	4.000	4.18	1598.628	17599	1598.628	4.0	bb

Compound name:PQ Peptide
Correlation coefficient: r = 0.999811, r^2 = 0.999623
Calibration curve: 405.84 * x + -22.9505
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None
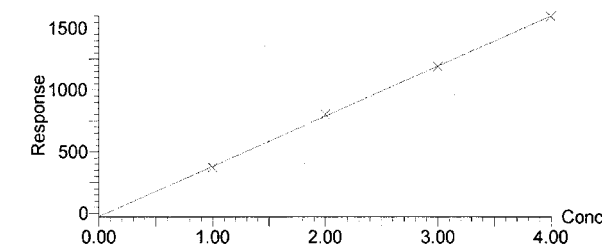
Compound name:PQ Peptide — MS

# Name	Type	Std. Conc	RT	Area	Height	Response	ng	Flags
1 PQ Peptide 5uL	Standard	1.000	4.24	44138.957	463353	44138.957	1.0	bb
2 PQ Peptide 10uL	Standard	2.000	4.24	90170.703	890318	90170.703	2.0	bb
3 PQ Peptide 15uL	Standard	3.000	4.22	138070.359	1414687	138070.359	3.1	bb
4 PQ Peptide 20uL	Standard	4.000	4.21	172733.063	1779690	172733.063	3.9	bb

Compound name:PQ Peptide
Correlation coefficient: r = 0.997683, r^2 = 0.995372
Calibration curve: 43368.2 * x + 2857.78
Response type: External Std, Area
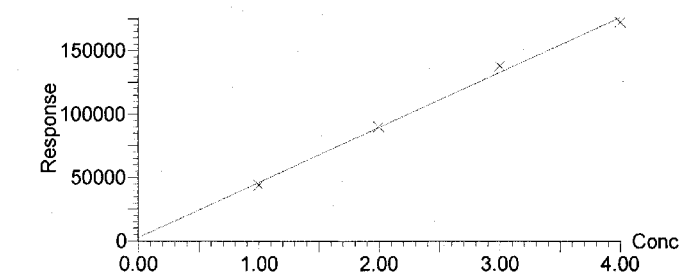Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

Figure 4: Results acquired during performance qualification testing of injector linearity with UV and MS detection.
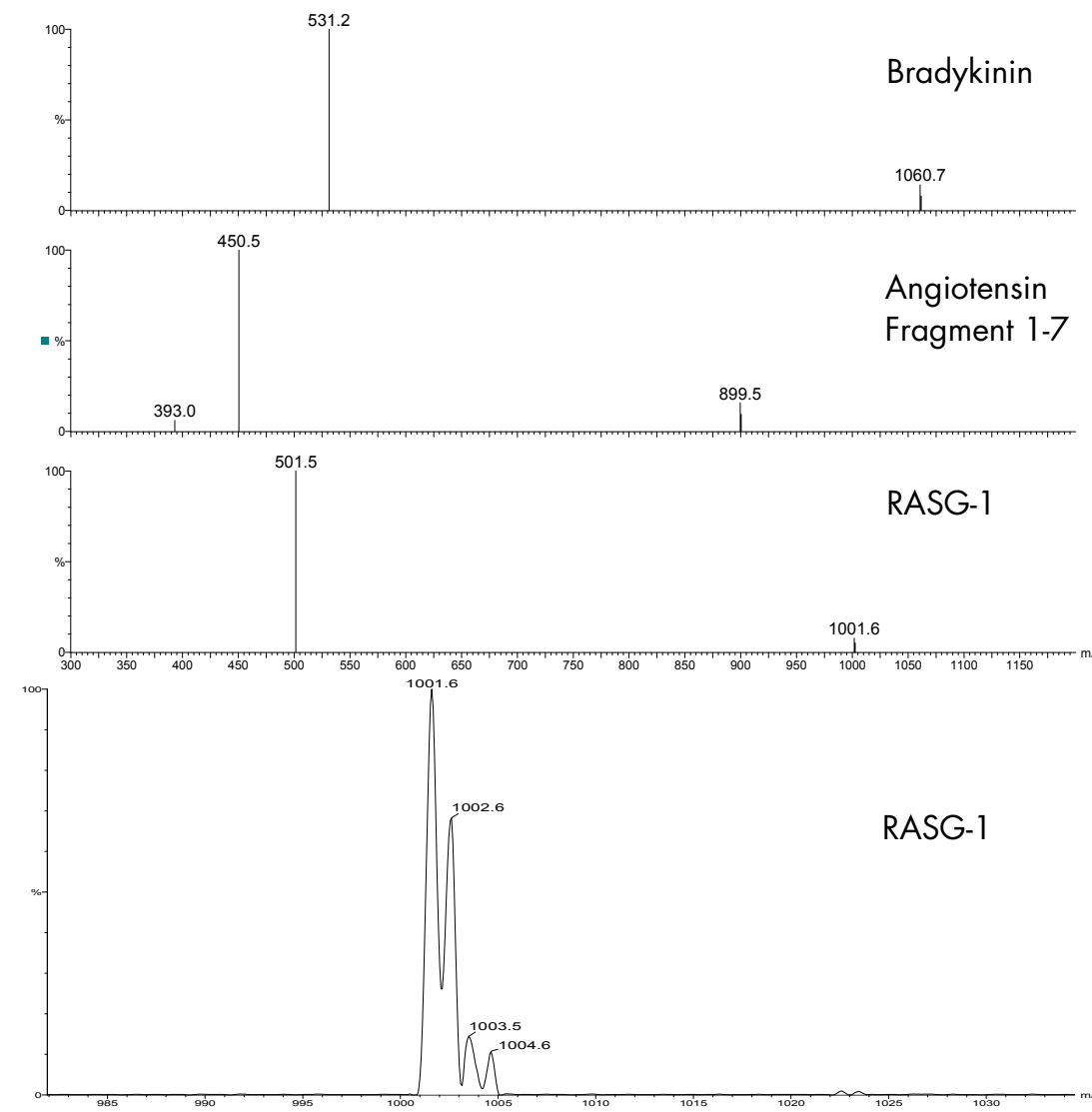
Mass Accuracy

Figure 5: Mass accuracy and resolution of peptides from MassPrep™ peptide standard, mass measurement error is below 100 ppm.

CONCLUSIONS

A systems approach for the qualification of LC-UV/MS instrumentation dedicated for peptide mapping was developed.

The qualification test contains a well-characterized peptide standard mix that elutes over the length of the gradient method.

Injection precision testing using the PQ peptide produced RSD values of less than 2 and 4 percent for UV and MS detection under gradient conditions, respectively.

Injector linearity testing using the PQ peptide yielded R^2 values of greater that 0.999 and 0.990 for UV and MS detection under gradient conditions, respectfully.

Mass accuracy measurement error was below 100 ppm.