DETECTOR CONSIDERATIONS IN DEVELOPING PERFORMANCE QUALIFICATION (PQ) CRITERIA FOR HPLC SYSTEMS

Waters

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INTRODUCTION

Peptide mapping is a particularly important tool for the characterization of therapeutic proteins. When this technique is used in a validated analytical method, it is necessary to characterize the system and to apply appropriate qualification procedures. A performance qualification test based on peptide analysis is required for predicting system performance with real samples. Such qualification protocols, however, only apply to a single instrument configuration. It is common practice, however, to use different detectors for specific purposes at different stages of development. We have, therefore, established performance standards for reversed-phase HPLC in conjunction with both UV and MS detection for systems dedicated to running peptide maps. The qualified optical detectors include tunable UV and photodiode array. The mass spectrometers include single quadrupole, oa-Tof, and tandem quadrupole-oa-Tof. The criteria and specifications include retention time reproducibility and chromatographic selectivity. Mass accuracy was included for MS configurations. Since injector reproducibility and injector linearity can only be judged in conjunction with the selected detector, separate specification criteria were defined were each of the five detectors. The combinations of optical and mass detectors were also tested. This qualification method is based on the separation of a well-characterized peptide analyte mixture on a tested HPLC column specific for peptide analysis. The consistent separation chemistry and test materials ensure that the test may be efficiently adapted to the selected detector. The developed procedure could also be used as a routine system suitability test.

MATERIALS AND METHODS

Test Samples

Waters MassPREP™ Peptide Standard Mixture—dissolved in H_2O with 0.02% TFA and 10% ACN to a concentration of 6.7 pmoles/µL for use with ZQ and UV or to a concentration of 2 pmoles/µL for use with LCT, Q-Tof *micro*™, and UV

Waters BioSuite™ Peptide Standard—dissolved in H_2O with 0.02% TFA to a concentration of 10 pmoles/µL for use with ZQ and UV or to a concentration of 3 pmoles/µL for use with LCT, Q Tof µ, and UV.

Instrumentation

Waters AQUITY UPLC™ System

Detectors

Waters ACQUITY UPLC™ TUVe
Waters ZQ Single Quadrupole Mass Spectrometer
Waters LCT Premier oa-Tof Mass Spectrometer
Waters Q-Tof *micro*™ Hybrid Tandem Mass Spectrometer

Column

Waters AQUITY UPLC™ BEH130 C18 1.7µm, 2.1 x 50mm
Peptide Separation Technology

Mobile Phase

A: 0.02% TFA in water
B: 0.018% TFA in Acetonitrile

Gradient Methods

LC Method for MassPREP Peptide Mixture

Time	Flow Rate (mL/min)	% A	% B
0.00	0.200	100.0	0.0
10.00	0.200	50.0	50.0
11.00	0.200	20.0	80.0
11.10	0.200	100.0	0.0
17.00	0.200	100.0	0.0

LC Method for BioSuite Peptide Standard

Time	Flow Rate (mL/min)	% A	% B
0.00	0.200	100.0	0.0
3.00	0.200	80.0	20.0
3.50	0.200	20.0	80.0
4.00	0.200	100.0	0.0
8.00	0.200	100.0	0.0

Qualification of Chromatographic Elements

Figure 1: Test Chromatogram—UV Detection

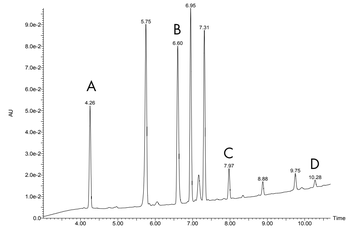
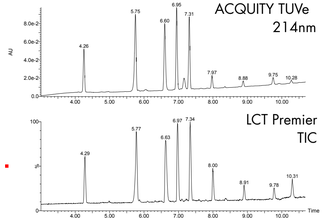


Table 1a and b: Absolute and Relative Retention

	Peak A	Peak B	Peak C	Peak D
	(4.3+/- 0.5)	(6.6+/- 1.0)	(8.0+/- 1.0)	(10.3+/- 1.0)
Inj #1	4.26	6.60	7.97	10.27
In j#2	4.26	6.60	7.97	10.28
Inj #3	4.29	6.60	7.97	10.28
Inj #4	4.24	6.60	7.97	10.29
Inj #5	4.26	6.61	7.97	10.30
Inj #6	4.22	6.61	7.97	10.30
Mean	4.26	6.60	7.97	10.29
Stdev	0.0235	0.0052	0.0000	0.0121
% RSD	0.551	0.078	0.000	0.118

	RT B	RT C	RT D
	RT A	RT B	RT C
Inj #1	1.55	1.21	1.29
In j#2	1.55	1.21	1.29
Inj #3	1.54	1.21	1.29
Inj #4	1.56	1.21	1.29
Inj #5	1.55	1.21	1.29
Inj #6	1.57	1.21	1.29
Mean	1.55	1.21	1.29

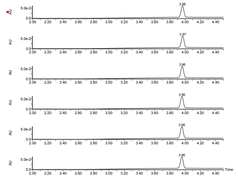
Figure 2: Chromatograms with Alternate Detectors



Qualification of Chromatographic Elements: The chromatogram in Figure 1 shows the separation of the MassPrep™ Peptide Standard Mixture with the defined method. The qualification criteria include the absolute retention times and the repeatability of those retention times over six replicate injections. The specification range shown in the table could be narrower. A second sensitive test of all the factors that affect chromatography is the relative retentions shown in Table 1b. The same test can be used for any of the detectors or combination of detectors that might be used in the system, as shown in Figure 2.

Qualification of System Quantitative Performance

Figure 3a: Reproducibility with TUV



RT	Area	Detectio...	Conc.	%Dev
3.96	2444.8	bb	10.0	0.0
3.96	2452.2	bb	10.0	0.3
3.96	2446.7	bb	10.0	0.1
3.96	2446.5	bb	10.0	0.1
3.97	2438.8	bb	10.0	-0.2
3.96	2438.3	bb	10.0	-0.3

RSD = 0.2%

Figure 3b: Linearity with TUV

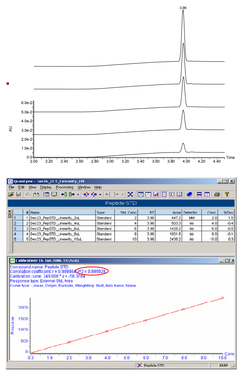
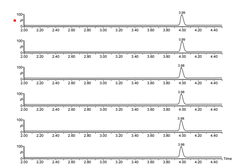


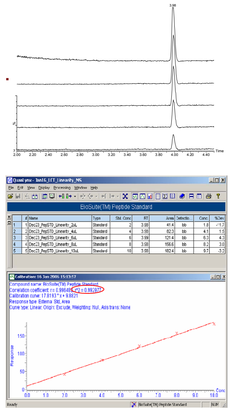
Figure 4a: Reproducibility with LCT



RT	Area	Detectio...	Conc.	%Dev
3.98	173.9	bb	10.0	-0.5
3.98	173.6	bb	9.9	-0.7
3.98	174.1	bb	10.0	-0.4
3.98	174.1	bb	10.0	-0.3
3.99	175.6	bb	10.0	0.5
3.99	177.1	bb	10.1	1.3

RSD 0.8%

Figure 4b: Linearity with LCT



Qualification of System Quantitative Performance: The observed peak areas in a chromatogram are affected by chromatographic performance, by the response of the detector, and by the integration algorithms. Successful tests of the reproducibility of peak areas at constant injection and linearity of response are only possible if all system components are performing properly. Here the same test is used for MS and for UV detection.

MS and MS/MS Qualification Tests

Figure 5a: Mass Accuracy

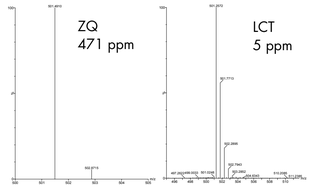


Figure 5b: Mass Resolution

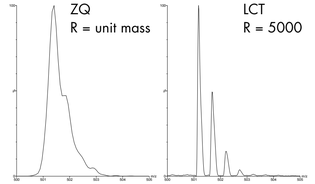
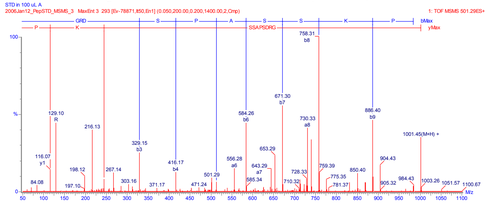


Figure 5c: Product Ion Pattern in Peptide MS/MS



Qualification of MS and MS/MS Functions — The use of a mass spectrometer as the information rich detector in a peptide mapping system expands the range of required qualification tests. As shown above, the same tests of chromatographic and quantitative elements can be used with both UV detectors and mass spectrometers. Further tests are required to ensure that the MS system produces qualitative data suitable for the analytical problem. The tests of mass resolution and mass accuracy are obvious. The data is collected in LC_MS mode, rather than by infusion, to most closely reflect the performance of the detector in a peptide mapping experiment. The same test sample and UPLC™ method can be used for all the detectors. However, different dilutions are required to reflect the different instrument sensitivities. The target specifications are also different as required by the different mass analyzers. When the system includes a Q-Tof *micro*™ , MS/MS function must be tested. The same peptide test can be used here, specifying the known mass and observing the product ions expected for that sequence. The process is simplified because the same sample and chromatographic method can be used for all the mass analyzers. Only differences in expected performance need be defined.

QUALIFICATION REQUIREMENTS

Retention Time Reproducibility — Qualifies chromatographic elements, i.e., Solvent Delivery System, Temperature Control, Column, and Mobile Phase.

Quantitative Reproducibility—Qualifies combination of chromatographic elements, injector, and detector for precision.

Quantitative Linearity—Qualifies combination of chromatographic elements, injector, and detector for usable ranges.

MS Functions—Qualify mass accuracy and resolution for impact on peak identification, as well as, effect on quantitative properties.

MS/MS Function—Qualify selection of peptide in an LC/MS chromatogram and the generation of the correct product ions in the collision cell.

PQ Tests for System Configurations

System Configuration	PQ Tests to Run
UV-based system	Injector Reproducibility and Linearity Tests
	Preliminary Peptide Mapping Run
LC_UV	System Reproducibility Test
UV/ZQ-based system	Injector Reproducibility and Linearity Tests
or ZQ-based system	Preliminary Peptide Mapping Run
	System Reproducibility Test
LC_UV_MS or LC_MS	Mass Accuracy
UV/LCT-based system	Injector Reproducibility and Linearity Tests
or LCT-based system	Preliminary Peptide Mapping Run
	System Reproducibility Test
LC_UV_MS or LC_MS	Mass Accuracy
	Mass Resolution
	Mass Sensitivity
UV/QTof-based system	Injector Reproducibility and Linearity Tests
or QTof-based system	Preliminary Peptide Mapping Run
	System Reproducibility Test
LC_UV_MS/MS or LC_MS/MS	Peptide Identification
	Mass Accuracy
	Mass Resolution
	Mass Sensitivity

SUMMARY

- Procedures have been developed for System-level performance qualification of ACQUITY UPLC™ Peptide Mapping Systems
- The qualification protocols are based on the analysis of peptides under conditions similar to those used for routine peptide mapping
- The same test samples and chromatographic methods can be used for the ACQUITY UPLC™ system with any detector or combination of detectors that would be routinely used in a peptide mapping system
- The only required operating adjustments are in sample concentration for detectors of widely disparate sensitivity
- Suitable specifications can be readily defined for all the instrument combinations using actual peptide data

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