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Transferring Methods for Monoclonal Antibody Analysis from an Agilent 1260 Infinity Quaternary LC System to an ACQUITY Arc System

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Abstract

This application note demonstrates method equivalency between an Agilent 1260 Infinity Quaternary LC System and an ACQUITY Arc System for transfer of isocratic and gradient methods for monoclonal antibody analysis.

The ACQUITY Arc System combined with Gradient SmartStart Technology minimizes any differences in dwell volume between two systems, resulting in comparable relative retention times. These results demonstrate the ability of the ACQUITY Arc System for emulating HPLC/UHPLC methods, and its ability to streamline the method transfer process.

Benefits

- Straightforward transfer of a sizeexclusion chromatography (SEC) platform method for assessment of size variants using isocratic conditions from an Agilent 1260 Infinity Quaternary LC System to an ACQUITY Arc System
- Efficient transfer of a gradient method for peptide map profiling from an Agilent 1260 Infinity Quaternary LC

Introduction

Biopharmaceutical companies often transfer analytical methods within their own organization as well as to external contract organizations that may or may not use the same instrumentation for analysis. It is important that method equivalency is demonstrated across laboratories, regardless of the analyst or instrument used, in order to minimize lost productivity on method revalidation. The ACQUITY Arc System (Figure 1) allows scientists to switch seamlessly between two flow paths in order to emulate LC systems with different dwell volumes using a single platform. The purpose of this application note is to demonstrate method equivalency between an Agilent 1260 Infinity Quaternary LC System and an ACQUITY Arc System for transfer of isocratic and gradient methods for monoclonal antibody analysis.

Figure 1. The ACQUITY Arc System. Seamless and efficient transfer of methods is enabled by Multi-flow path technology, which allows HPLC and UHPLC separations to be run on a single platform.

Experimental

SEC sample description

Rituximab was used at its supplied concentration of 10 mg/mL without further dilution.

Peptide map sample description

Trypsin (Promega, Madison, WI) was used to prepare a digest of reduced and alkylated rituximab. Digested samples were injected neat at a final concentration of 0.5 mg/mL.

LC conditions

LC systems:	ACQUITY Arc System with 2489 UV/Vis Detector, Path 1 Agilent 1260 Infinity Quaternary LC System with HiP ALS and DAD Detector

SEC conditions

Absorption wavelength:	280 nm
Sampling rate:	20 Hz
Column:	Tosoh TSKgel G3000SWxl, 250 Å, 5 µm, 7.8 mm x 300 mm
Column temp.:	Ambient
Mobile phase:	0.2 M potassium phosphate, 0.25 M potassium chloride, pH 6.2
Sample temp.:	5 °C
Injection volume:	20 µL
Flow rate:	0.500 mL/min
Method length:	30 min

Peptide map conditions

Absorption wavelength:	215 nm
Sampling rate:	20 Hz
Column:	XSelect CSH C_{18} , 130 Å, 3.5 µm, 3 mm x 100 mm
Column temp.:	60 °C
Mobile phase A:	Water with 0.1% (v/v) formic acid
Mobile phase B:	Acetonitrile with 0.1% (v/v) formic acid
Sample temp.:	5 °C
Injection volume:	10 µL

Gradient:

Time(min)	Flow rate(ml/min)	%A	%B	%C	%D
Initial	0.300	95	5	0	0
5.00	0.300	95	5	0	0
45.00	0.300	50	50	0	0
47.50	0.300	5	95	0	0

Time(min)	Flow rate(ml/min)	%A	%B	%C	%D
52.50	0.300	5	95	0	0
52.60	0.300	95	5	0	0
60.00	0.300	95	5	0	0

Data management

Empower 3 chromatography data software (CDS), service release 2 (SR2)

Results and Discussion

SEC Method Transfer

SEC is a common analytical tool used in the product life cycle of monoclonal antibodies and other protein-based drugs to assess the presence of aggregates, which can potentially impact product safety and efficacy. A platform SEC method from USP General Chapter <129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies was used to assess method transfer from an Agilent 1260 Infinity Quaternary LC System to an ACQUITY Arc System.[1] Rituximab, a monoclonal antibody which targets the CD20 protein, was used as an analyte at its supplied concentration of 10 mg/mL. The method described above was first run on an Agilent 1260 Infinity Quaternary LC System. The same method was then run on an ACQUITY Arc System without changing any method parameters. The resulting chromatography can be seen in Figure 2. Visual inspection of the data shows comparable chromatographic profiles with similar retention times and resolution between the two systems.

To further assess the data shown in Figure 2, the optical channels from five replicate injections on each system were batch processed using Empower to determine retention time and peak area. To keep in line with Chapter <129>, retention time and peak area percentage for the high molecular weight species (HMWS), main peak, and

low molecular weight species (LMWS) are reported in Table 1. The retention times of the four peaks indicated in the inset of Figure 2 are comparable between the two systems, but more importantly, peak area is conserved from one system to the next. Peak area of the HMWS includes all peaks eluting before the main peak, including the polymer and dimer peaks, which are added together and reported as a single value.

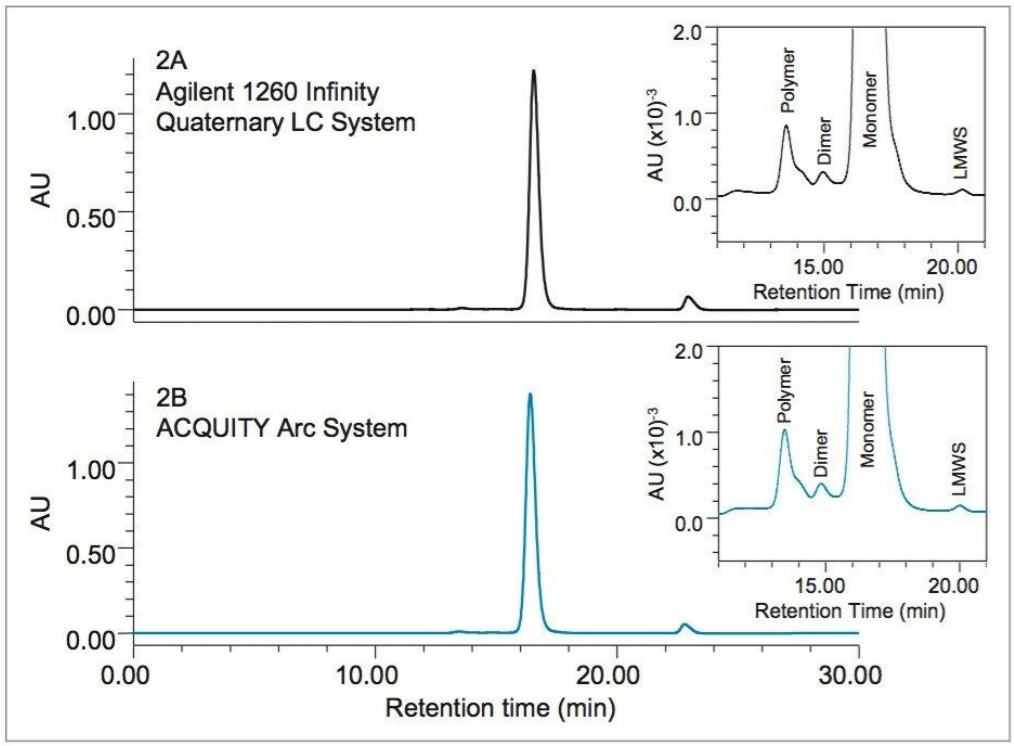

Figure 2. SEC of rituximab. 2A) Optical data acquired on an Agilent 1260 Infinity Quaternary LC System. 2B) Optical data showing the same separation (identical method conditions) transferred to an ACQUITY Arc System.

System	Retention time (min)				Peak area (%)		
	Polymer	Dimer	Monomer	LMWS	HMWS	Monomer	LMWS
Agilent 1260 Infinity Quaternary LC System	13.58	14.95	16.56	20.16	1.41	98.52	0.07
ACQUITY Arc System	13.46	14.83	16.41	20.00	1.47	98.46	0.07
Δ	0.12	0.12	0.15	0.16	0.07	0.06	0.00

Table 1. Comparison of SEC data acquired from an Agilent 1260 Infinity Quaternary LC System and an ACQUITY Arc System. Note that Peak area (%) of the HMWS refers to all peaks eluting before the main peak, including polymer and dimer peaks. All results are average values from five injections.

Chapter <129> relies on a system suitability reference standard to set minimum quality requirements, with specific criteria of an individual drug designated in the individual product monograph. Table 2 compares %RSD for retention time and peak area for the peaks identified in Figure 2. Performance requirements in this case are defined according to USP General Chapter <621> Chromatography.[2] Both retention time and peak area are below the 2.0% RSD requirement for five injections on both systems.

System	%RSD retention time				%RSD peak area			USP resolution	USP tailing
	Polymer	Dimer	Monomer	LMWS	HMWS	Monomer	LMWS		
Agilent 1260 Infinity Quaternary LC System	0.03	0.11	0.02	0.12	0.29	0.00	1.60	1.6	1.2
ACQUITY Arc System	0.02	0.03	0.01	0.06	1.33	0.02	1.64	1.5	1.2

Table 2. Quantitative comparison of SEC data acquired from an Agilent 1260 Infinity Quaternary LC System and an ACQUITY Arc System. All results are average values from five injections.

In addition to using SEC to aid in product purity determination, suitability requirements may also address resolution, peak tailing, or additional method parameters that further demonstrate that the drug product can be separated from impurities and reliably quantified. As shown in Table 2, values for resolution between the monomer peak and dimer peak, and peak tailing show good agreement between systems. This further demonstrates method equivalency in transfer of a SEC method from an Agilent 1260 Infinity Quaternary LC System to an ACQUITY Arc System.

Peptide map method transfer

Peptide profiling is frequently used in the manufacturing process of biotherapeutics in the pharmaceutical industry from early stage research and development to late stage QC environments. Developing methods can be challenging due to sample complexity, which often requires that methods be developed for each individual drug product. These methods can then be transferred to various laboratories, which rely on a seamless transition to avoid downtime and lost productivity. To assess method transfer of a peptide map method, a general platform method was used because a validated method was not available. Following the method conditions described above, a peptide map of rituximab was collected using an Agilent 1260 Infinity Quaternary LC System. This same method was then run on an ACQUITY Arc System configured with an active preheater (CH-30A) without making any changes to the method conditions. The resulting chromatograms showed a gradient offset equivalent to a difference of 60 µL between the two systems (data not shown). Gradient SmartStart Technology can be used with the ACQUITY Arc System to account for this difference by applying an isocratic hold before each injection, which is done without making changes to the gradient table.3 The resulting chromatograms from each of these runs can be seen in Figure 3. Chromatographic profiles are similar, and relative retention times are comparable.

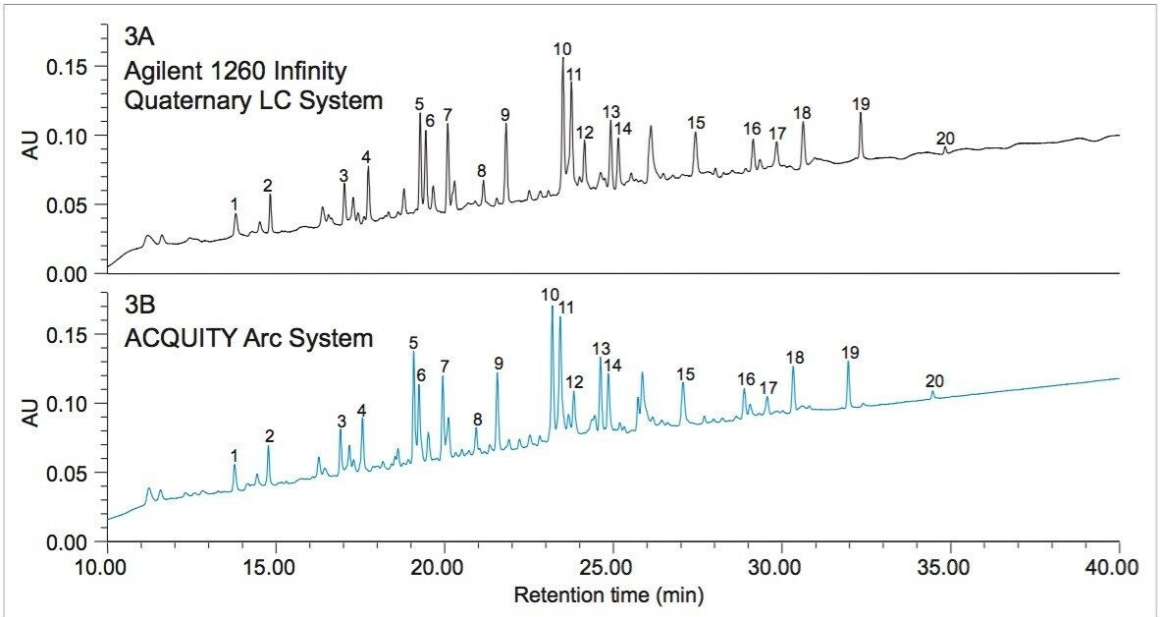

Figure 3. Peptide map of rituximab. 3A) Optical data acquired on an Agilent 1260 Infinity Quaternary LC System. 3B) Optical data showing the same separation (identical method conditions) transferred to an ACQUITY Arc System.

Like any chromatographic method used for assessing purity and identity of the desired product, determining the performance of the method relies on parallel assessment of the sample with reference material. For the purpose of this work, demonstrating method equivalency of the peptide map is done without reference material and assumes that characterization would have already taken place under optimized method conditions. Table 3 reports the difference in relative retention time between the two systems over the course of triplicate injections for 20 peaks. The negligible differences observed in relative retention time between systems demonstrate that the ACQUITY Arc System is capable of accurately transferring a peptide map method from an Agilent 1260 Infinity Quaternary LC System in a straightforward manner.

Peak	Agilent 1260 Infinity Quaternary LC System			ACQUITY Arc System			Δ RRT
	Retention time (min)	%RSD retention time	Relative retention time (RRT)	Retention time (min)	%RSD retention time	Relative retention time (RRT)	
1	13.79	0.10	1.00	13.84	0.07	1.00	0.000
2	14.82	0.09	1.07	14.80	0.03	1.07	0.005
3	17.02	0.05	1.23	16.95	0.04	1.22	0.010
4	17.71	0.12	1.28	17.60	0.02	1.27	0.013
5	19.26	0.04	1.40	19.11	0.01	1.38	0.016
6	19.43	0.02	1.41	19.27	0.01	1.39	0.017
7	20.09	0.03	1.46	19.97	0.01	1.44	0.014
8	21.14	0.16	1.53	20.97	0.01	1.51	0.019
9	21.80	0.10	1.58	21.59	0.01	1.56	0.021
10	23.47	0.15	1.70	23.22	0.02	1.68	0.024
11	23.72	0.11	1.72	23.45	0.01	1.69	0.026
12	24.11	0.13	1.75	23.85	0.01	1.72	0.025
13	24.90	0.06	1.81	24.64	0.01	1.78	0.026
14	25.12	0.07	1.82	24.88	0.01	1.80	0.025
15	27.41	0.10	1.99	27.10	0.01	1.96	0.030
16	29.14	0.10	2.11	28.90	0.01	2.09	0.025
17	29.82	0.14	2.16	29.58	0.02	2.14	0.026
18	30.61	0.11	2.22	30.35	0.01	2.19	0.027
19	32.34	0.08	2.35	31.99	0.01	2.31	0.034
20	34.84	0.19	2.53	34.48	0.02	2.49	0.035

Table 3. Comparison of the retention times of 20 peaks identified in Figure 3 from an Agilent 1260 Infinity Quaternary LC System and an ACQUITY Arc System. Gradient SmartStart Technology was used to account for differences in dwell volume between the two systems. Retention times are average values from three injections. The Δ values shown are calculated from the difference in relative retention time between the two systems.

Conclusion

Because biopharmaceutical companies often transfer methods to various in-house laboratories as well as to outside contract organizations, it becomes important to demonstrate successful method transfer to a modern LC system such as the ACQUITY Arc System. Method equivalency was demonstrated between the Agilent 1260 Infinity Quaternary LC System and the ACQUITY Arc System for both an isocratic and a gradient method for monoclonal antibody analysis. A SEC method from USP Chapter <129> was used to show that retention time, peak area percent, resolution, and peak tailing were comparable between the two systems. Because peptide mapping methods need to be developed for each individual protein, a general platform peptide map method was used in comparing performance between the two systems. The ACQUITY Arc System combined with Gradient SmartStart Technology minimizes any differences in dwell volume between two systems, resulting in comparable relative retention times. These results demonstrate the ability of the ACQUITY Arc System for emulating HPLC/UHPLC methods, and its ability to streamline the method transfer process.

References

1. U.S. Pharmacopeial Convention, General Chapter <129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies, USP39-NF34. Official from May 1, 2016.
2. U.S. Pharmacopeial Convention, General Chapter <621> Chromatography, USP38-NF33. Official from August 1, 2015.
3. ACQUITY Arc System Brochure. Waters Brochure. 2015; 720005393en.

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