

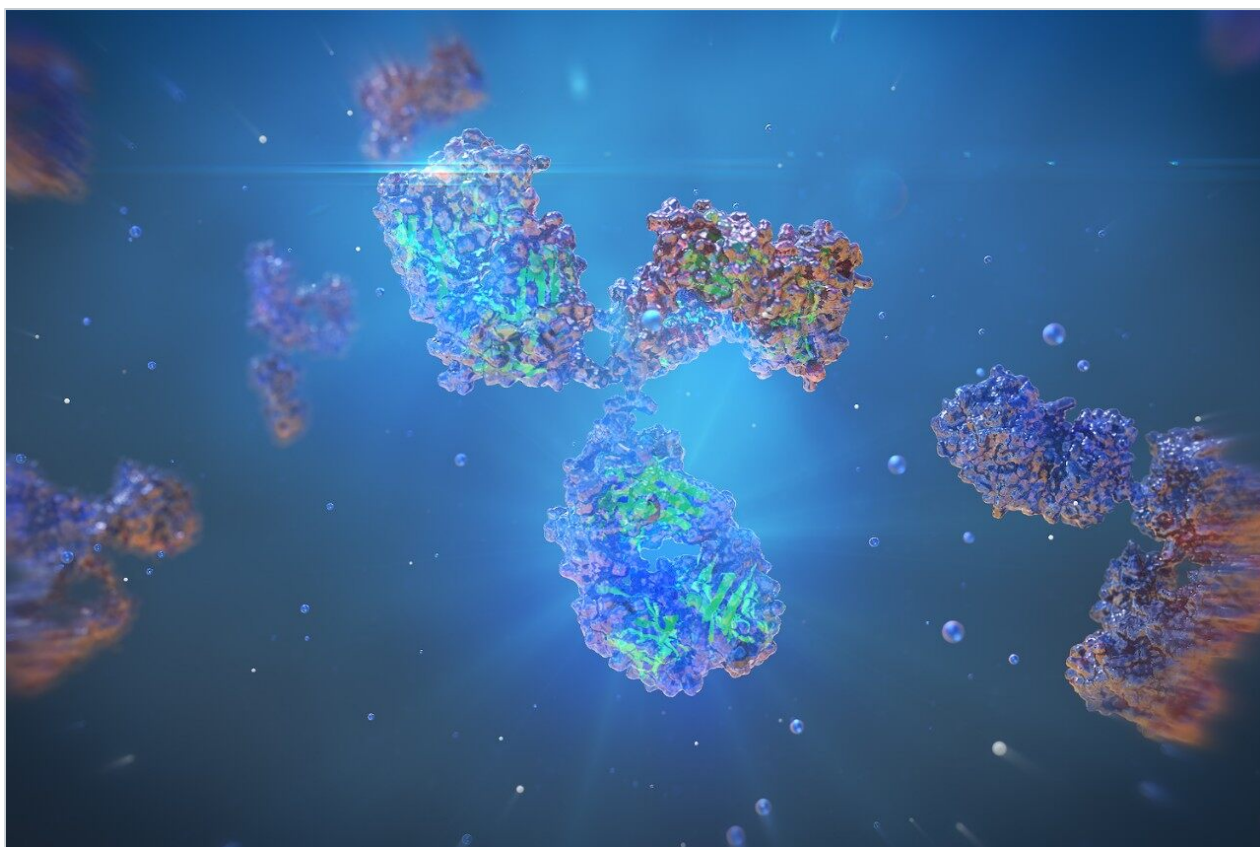
응용 자료

## IEX Method Transfer: Replicating a Method for Monoclonal Antibody Analysis on an ACQUITY Arc System

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Brooke M. Koshel, Sean M. McCarthy

Waters Corporation



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## Abstract

This application note demonstrates that an established ion-exchange method for the separation of a monoclonal antibody can be easily replicated and can yield repeatable results. The ACQUITY Arc System offers users the ability to emulate legacy HPLC methods or update methods to UHPLC separations through the use of Arc Multi-flow path technology.

### Benefits

- Seamless method transfer to an ACQUITY Arc System without changing method parameters
- HPLC or UHPLC separations can be easily replicated with Arc Multi-flow path technology

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## Introduction

Many of the top grossing biopharmaceuticals today are protein-based drugs. Unlike the synthesis of small molecule drugs which is a controlled chemical process, biologics are produced from live cell cultures, making them more prone to variation. A host of techniques are often required throughout the life cycle process to ensure product quality and safety. Ion-exchange chromatography (IEX) is a common technique used to monitor charge variants, which can reflect changes made in various stages of production such as fermentation, purification, and formulation.

The ACQUITY Arc System, part of the quaternary based LC portfolio, is equipped to address the challenge of maintaining consistent results throughout the life cycle of product testing. Arc Multi-flow path technology allows the ACQUITY Arc System to easily replicate legacy HPLC methods (Path 1) or improve existing methods by transitioning to UHPLC-based separations (Path 2). The focus of this application note is to demonstrate that an established ion-exchange method for the separation of a monoclonal antibody can be easily replicated and can yield repeatable results.

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## Experimental

## LC conditions

LC systems:	ACQUITY Arc System with 2489 UV/Vis detector, flow path 1 Agilent 1100 Series HPLC System with quaternary pump and DAD detector
Absorption wavelength:	280 nm
Sampling rate:	20 Hz
Column:	Dionex ProPac WCX-10 10 $\mu$ m,4.0 mm x 250 mm
Column temp.:	30 °C
Mobile phase A:	0.02 M MES, pH 6.0
Mobile phase B:	0.02 M MES, 0.4 M sodium chloride, pH 6.0
Sample:	1 mg/mL rituximab in 0.15 M sodium chloride, 0.025 M sodium citrate, 0.06% (v/v) polysorbate 80, pH 6.5
Sample temp.:	5 °C
Injection volume:	40 $\mu$ L

## Gradient table:

Time (min)	Flow rate (mL/min)	%A	%B	%C	%D
Initial	0.700	100	0	0	0

Time (min)	Flow rate (mL/min)	%A	%B	%C	%D
0.01	0.700	100	0	0	0
5.00	0.700	100	0	0	0
10.00	0.700	83	17	0	0
95.00	0.700	55	45	0	0
100.00	0.700	0	100	0	0
102.00	0.700	0	100	0	0
102.01	0.700	100	0	0	0
115.00	0.700	100	0	0	0

## Data management

Empower 3 CDS Software, SR2

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## Results and Discussion

### The ACQUITY Arc System replicates established IEX-HPLC method and offers high reproducibility

Charge variants are often monitored throughout the life cycle of a drug product to monitor process control. As such, system-to-system variability must be determined to ensure product quality, regardless of the instrument used for testing. Ideally, each system used should provide comparable results that fall within the accepted guidelines for that product. To investigate the ability of a cation-exchange method to migrate from a legacy instrument to a modern LC platform, rituximab, a monoclonal antibody, was analyzed on an Agilent 1100 Series HPLC System and compared to results from the ACQUITY Arc System. The method used is from

the USP Medicines Compendium,<sup>1</sup> and although the method has since been discontinued, it serves as a representative method used in industry.

The method described above was executed on both the Agilent 1100 Series HPLC System and the ACQUITY Arc System without modification (Figure 1). As shown, the chromatographic performance between the two systems is nearly identical. When looking at the insets in Figure 1, it can be seen that selectivity and resolution are preserved across platforms. Treatment of the data to determine relative retention time of the main peak and additional acidic and basic variants is shown in Table 1. Although differences in retention time exist, it is more important to note that the relative retention times of all major peaks are negligible.

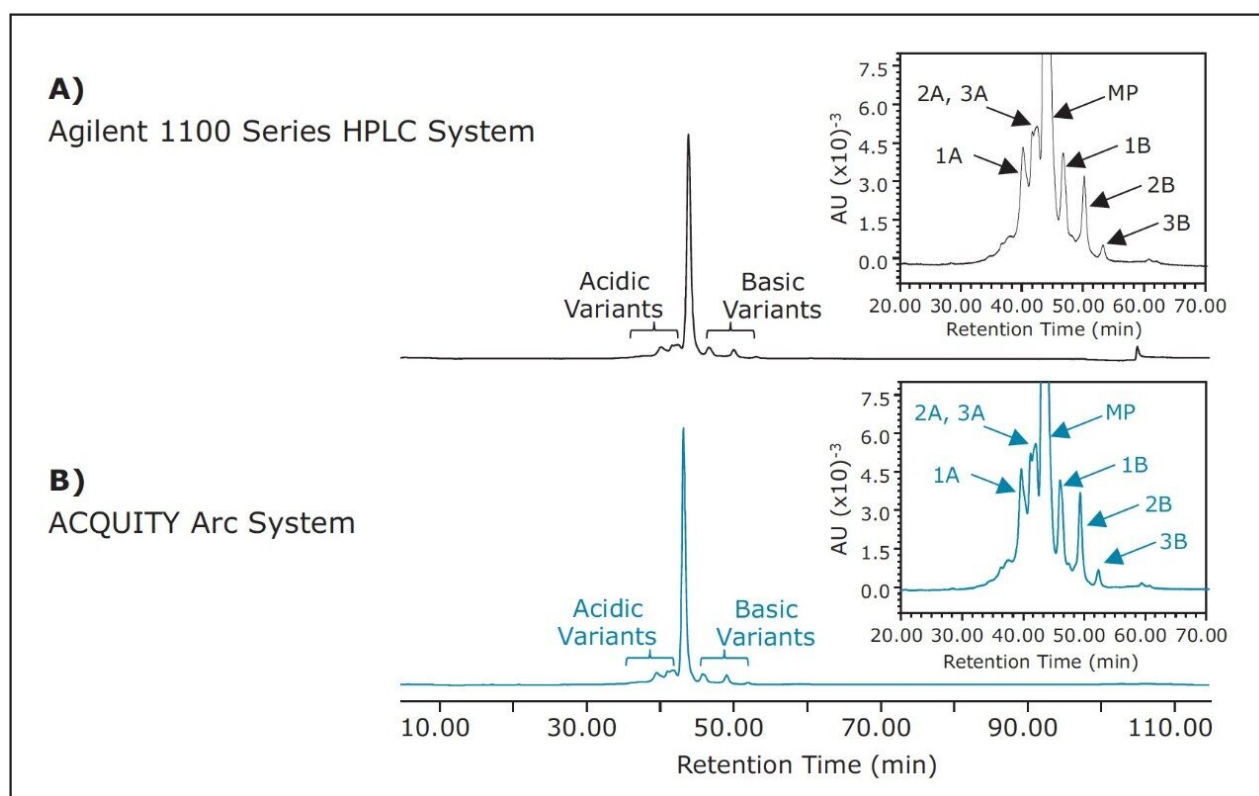


Figure 1. Comparison of IEX data acquired on an A) Agilent 1100 Series HPLC System and B) ACQUITY Arc System. Inset shows separation of acidic and basic variants. The main peak of rituximab is designated MP. Additional acidic (1A, 2A, 3A) and basic (1B, 2B, 3B) residues are also labeled for reference to retention times reported in Table 1.

Peak	Agilent 1100 Series HPLC System		ACQUITY Arc System		$\Delta$
	Retention time (min)	Relative retention time	Retention time (min)	Relative retention time	
1A	40.06	0.91	39.52	0.92	-0.003
2A	41.59	0.95	40.96	0.95	-0.001
3A	42.37	0.97	41.78	0.97	-0.002
MP	43.82	1.00	43.10	1.00	0.000
1B	46.60	1.06	45.82	1.06	0.001
2B	50.01	1.14	49.08	1.14	0.003
3B	53.06	1.21	51.95	1.21	0.006

*Table 1. Retention time comparison of an IEX analysis on an Agilent 1100 Series HPLC System and the ACQUITY Arc System. Peak designations refer to acidic and basic components as assigned in Figure 1. Delta values refer to differences in relative retention time between the two systems. All results are average values from five injections.*

Resolution is an example of one parameter that can be calculated to determine if system suitability criteria is met. Per the USP Medicines Compendium,<sup>1</sup> resolution between the main peak of rituximab (MP) and the lysine variant (1B) should be no less than 1.5. Over five injections, the Agilent 1100 Series HPLC System reports an average resolution of  $1.95 \pm 0.02$  compared to  $2.09 \pm 0.02$  reported on the ACQUITY Arc System. Both systems fall within regulations.

To ensure product consistency, reproducibility must not only be demonstrated from system to system, but within an instrument as well. Figure 2 shows an overlay of five rituximab injections on the ACQUITY Arc System run under the same method conditions as described above. Each chromatogram is nearly indistinguishable from the next throughout the length of the run. From the inset, an image of a zoomed in chromatogram further confirms this agreement.

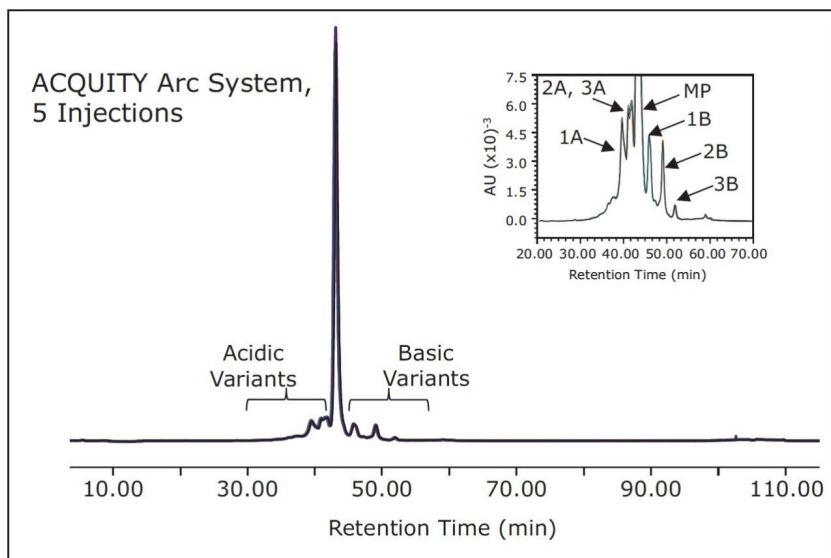


Figure 2. Overlay of five IEX chromatograms acquired on the ACQUITY Arc System. Acidic variants are identified as all peaks eluting prior to the main peak, while basic variants include all peaks eluting after the main peak. Inset identifies three acidic variants (1A, 2A, 3A) and three basic variants (1B, 2B, 3B) which are included in the sum of total acidic variants and total basic variants respectively, as reported in Table 2.

Table 2 provides quantitative analysis of the data displayed in Figure 2 by reporting peak area percent of the main peak, acidic variants, and basic variants and comparing these percentages to five injections made on the Agilent 1100 Series HPLC System. Overall, the values reported between the two systems are comparable. The difference in peak areas between the systems for the main peak, acidic variants, and basic variants are 0.53%, 0.38%, and 0.90%, respectively.

System	Relative peak area (%)								
	Acidic variants			Main peak			Basic variants		
	$\bar{x}$	$\sigma$	%RSD	$\bar{x}$	$\sigma$	%RSD	$\bar{x}$	$\sigma$	%RSD
Agilent 1100 Series HPLC System	19.49	0.21	1.10	69.67	0.28	0.41	10.84	0.13	1.22
ACQUITY Arc System	19.87	0.10	0.50	70.20	0.12	0.17	9.93	0.04	0.38
$\Delta$	0.38			0.53			-0.90		

*Table 2. Quantitative comparison of IEX data collected on an Agilent 1100 Series HPLC System and the ACQUITY Arc System. Acidic and basic variants include all species eluting earlier or later than the main peak respectively. Delta values are calculated for the difference in peak area between the two systems. All results are average values from five injections.*

## Conclusion

The ACQUITY Arc System offers users the ability to emulate legacy HPLC methods or update methods to UHPLC separations through the use of Arc Multi-flow path technology. This application note demonstrated the ease of transferring an IEX method from an Agilent 1100 Series HPLC System to the ACQUITY Arc System (Path 1). When keeping all method parameters the same, resulting chromatograms had relative retention times that were virtually indistinguishable from one another. Further inspection of five replicate injections on the ACQUITY Arc System shows a high degree of reproducibility.

## References

1. U.S. Pharmacopeial Convention <<http://www.usp.org/global/medicines-compendium>> (accessed March 2015).

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