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Method Development Tools for More Efficient Screening of Biopharmaceutical Method Conditions Using the ACQUITY Arc Bio System Part 2 of 2: Screening SEC and IEX Conditions in a Single Experiment

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APPLICATION BENEFITS

- Column switching capabilities enables screening of multiple column chemistries and method conditions for higher throughput analyses
- Automated solvent blending with Auto•Blend™ Plus technology for rapid screening of pH and ionic strength for SEC and IEX separations
- Iron-free flow path and bio-inert construction reduces the potential for LC system corrosion

WATERS SOLUTIONS

ACQUITY™ Arc™ Bio System

Empower™ Chromatography Data Software

Auto•Blend Plus

KEYWORDS

ACQUITY Arc, Empower, method development, monoclonal antibody, mAb, SEC, IEX, Auto•Blend Plus

INTRODUCTION

Methods that demonstrate robustness and repeatability are critical in biopharmaceutical analyses. Although platform methods can offer a generalized "starting point" for analysis, screening multiple method parameters is often a necessary part of the method development process to gain a deeper understanding of target analytes and their physicochemical properties when present in increasingly complex samples. The Waters™ ACQUITY Arc Bio System is a modern LC platform that employs functionality to help scientists work more efficiently and effectively for developing higher quality products through enhanced instrumentation and methods.

This two-part application note series demonstrates how features of the ACQUITY Arc Bio System, such as column-switching capabilities and buffer preparation technology, can help streamline method development. In part 1, four RPLC columns were screened in series to evaluate differences in peak capacity and selectivity of targeted peptides across a diverse group of chemistries. In part 2, a common buffer system is used to screen mobile phase pH and ionic strength for size exclusion chromatography (SEC) and ion exchange chromatography (IEX) methods in a single chromatographic run. Auto•Blend Plus is a tool incorporated within Empower Software that uses four mobile phase stock solutions (acid, base, salt, and water) to program changes in mobile phase composition. This allows for adjustments to be made to pH and salt content without the need for manual buffer preparation. By using one set of buffers, a single experiment can be used to screen mobile phase conditions for both SEC and IEX by switching between two columns. This allows for a high-level screening protocol to be carried out in a timelier manner so that methods can be further optimized using a narrower and well-defined set of experimental conditions.

EXPERIMENTAL

Sample description

Formulated trastuzumab at 21 mg/mL was diluted to 10 mg/mL in water. Sample load was based on column care and use recommendations and was confirmed to be within the dynamic range of the optical detector (data not shown).

LC conditions

LC system:	ACQUITY Arc Bio System with CM-A
Detector:	2489 UV/Visible (UV/Vis) Detector
Columns:	XBridge™ Protein BEH SEC, 200 Å, 2.5 µm, 7.8 mm × 150 mm (SEC); BioResolve™ SCX mAb, 3 µm, 4.6 mm × 50 mm (IEX)
Wavelength:	280 nm
Injection volume:	10 µL
Column temp.:	Ambient (SEC); 30 °C (IEX)
Flow rate:	0.500 mL/min (SEC) 0.750 mL/min (IEX)
Mobile phase A:	100 mM sodium phosphate monobasic
Mobile phase B:	100 mM sodium phosphate dibasic
Mobile phase C:	1 M sodium chloride
Mobile phase D:	Water
SEC method:	Auto•Blend Plus was used to deliver 20 mM phosphate; pH and salt concentrations are indicated in figures (15-min isocratic run)
IEX method:	Auto•Blend Plus was used to deliver 20 mM phosphate and a 25–125-mM linear salt gradient over 10 min; pH is indicated in figures (30-min run)

**Please note that phosphate containing buffers should be evaluated with the intended analyte when using the BioResolve SCX mAb Column as performance may vary.*

Data management

Empower 3 Chromatography Data Software SR2, FR4

RESULTS AND DISCUSSION

An ACQUITY Arc Bio System was configured with a CM-A column compartment module, which houses the valves for column selection between the SEC and IEX columns or up to four 50-mm-long columns (Figure 1). The quaternary pump enables the use of Auto•Blend Plus, which is a buffer preparation technology capable of blending mobile phase compositions at various pH and salt concentrations using concentrated stock solutions.[1] From Figure 2, flow is directed from the injection valve through one of the columns to the optical detector. Column selection is indicated by the user in the instrument method or selected in the Empower console so that valve switching is carried out automatically by the software.

Figure 1. The ACQUITY Arc Bio System. The system is configured with a CM-A column compartment module that houses the valves for column selection between two columns. The quaternary pump enables the use of Auto•Blend Plus, which adjusts mobile phase pH and salt composition through the use of concentrated stock solutions.

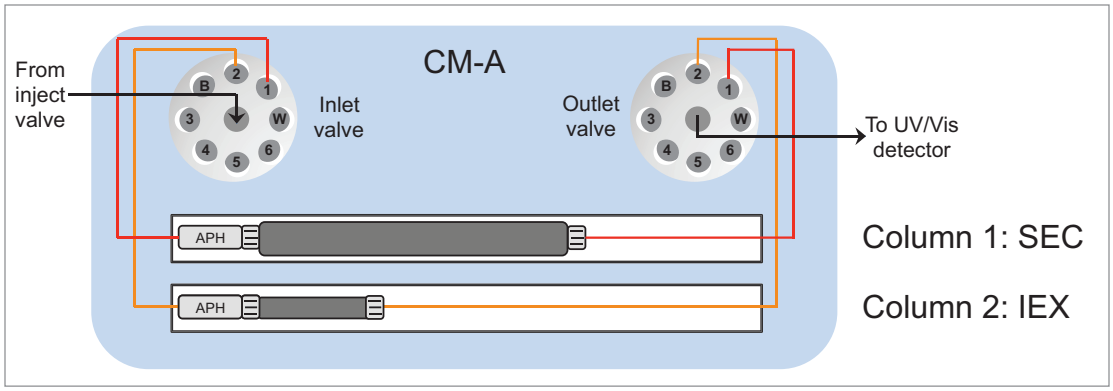

Figure 2. Column manager configuration for two-column switching. Flow is directed from the injection valve through one of the columns to the optical detector. Column selection is indicated by the user in the instrument method, so that valve switching is carried out automatically by Empower Software. Colored tubing represents an independent flow path through the user-selected column, while black tubing represents a shared flow path. All inlet and outlet tubing uses the same dimensions.

Both SEC and IEX parameters can be screened within a single sample set using a common buffer system, where Auto•Blend Plus eliminates the need to prepare each set of mobile phase conditions independently. To demonstrate this functionality, pH and ionic strength were screened using stock solutions to determine their impact on SEC of a monoclonal antibody (trastuzumab). After the final SEC run, the column was flushed with water for one hour (which is intended for short-term storage only) before switching to the second column position for screening IEX at various pH conditions. Because methods are intended for rapidly screening many method conditions for higher throughput analysis, short column lengths were used. Should greater resolution be required, longer columns can be used to further develop methods once method parameters have been narrowed down from the screening protocol.

Mobile phase ionic strength and pH are important considerations when developing SEC methods. Both parameters should ensure that there are no secondary interactions between the stationary phase of the column and the analyte. In general, a higher salt concentration can help improve peak shape and reduce peak tailing, but because high salt concentrations can potentially corrode stainless steel LC systems or shorten column lifetime, excessive salt concentrations should be avoided where possible.

Figures 3 and 4 show the effects of pH and ionic strength on SEC of trastuzumab. Auto•Blend Plus was used to deliver 20 mM phosphate and variable pH and salt concentrations as indicated in the respective figures. From the insets, aggregate, or high molecular weight species (HMWS), recovery is poor at low pH and ionic strength. When pH and ionic strength are increased, the monomer peak becomes visibly narrower and peak tailing is reduced. A fragment, or low molecular weight species (LMWS), is also observed but not evaluated in this work due to being present at such a low relative percentage. It should be noted that if the intended application is meant to evaluate all major product impurities, including an additional LMWS that co-elutes with the monomer peak under the current conditions, a longer column length will be required to achieve the necessary resolution. Figure 5 shows the HMWS peak area percent, USP tailing, and USP resolution between the HMWS and monomer peak under all conditions tested. Data was collected from pH 6.2 to 7.4 (0.2 pH increments) and 50 to 350 mM NaCl (50-mM increments). (Figures 3 and 4 are meant to show trending behavior and do not show all data points for simplicity purposes.) When determining final method conditions, it is important to establish aggregate recovery while minimizing tailing and enhancing resolution. From Figure 5, changes in aggregate recovery, tailing, and resolution are less notable from approximately pH 7.0 to 7.4 and 250 to 350 mM NaCl. From these conditions, the user can further optimize the method. In addition to adjusting instrument method parameters, evaluating additional pore sizes and column lengths are also good practice, especially in cases where analyte properties are unknown.

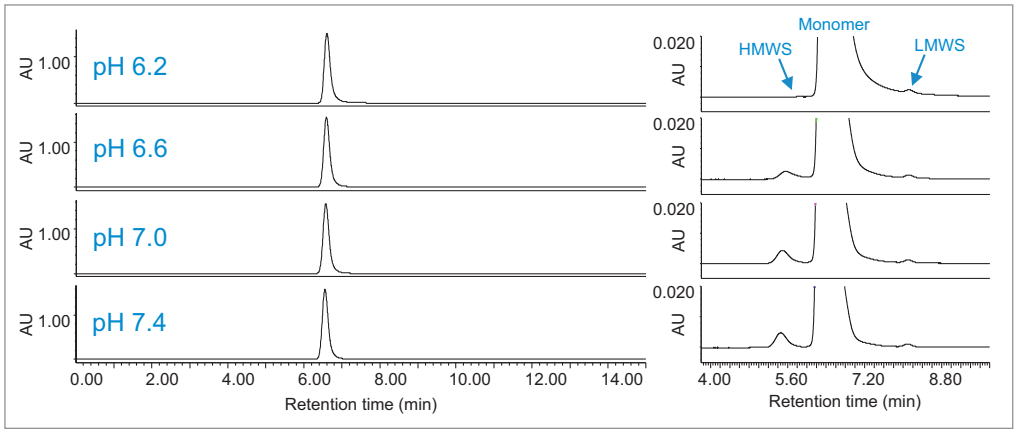

Figure 3. Effect of pH on SEC of trastuzumab. Auto•Blend Plus was used to manipulate small changes in pH, while holding the salt concentration constant at 200 mM NaCl across injections.

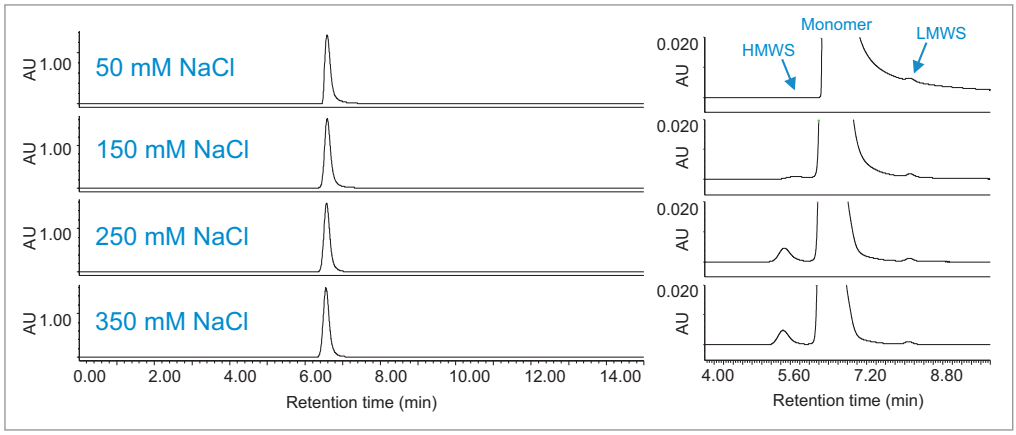

Figure 4. Effect of salt concentration on SEC of trastuzumab. Auto•Blend Plus was used to manipulate changes in ionic strength, while holding pH constant at 6.8 across injections.

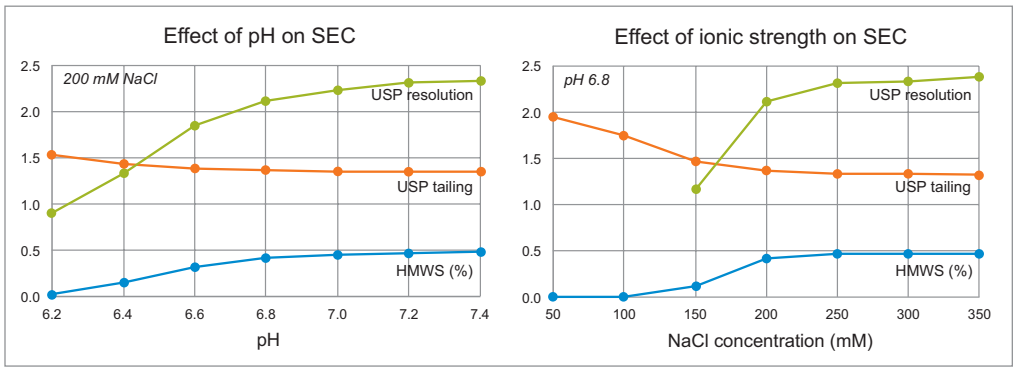

Figure 5. Evaluating the effect of pH and ionic strength on SEC of trastuzumab. Auto•Blend Plus was used to manipulate changes in pH and ionic strength to screen multiple method conditions in a single sample set. Peak area percent of the HMWS, USP tailing of the monomer peak, and USP resolution between the HMWS and monomer peak are reported. Because of the low aggregate recovery at 50 mM and 100 mM NaCl, the software could not calculate resolution for these data points. Final method conditions should verify aggregate recovery, while minimizing tailing and enhancing resolution.

Although there are several parameters involved in optimizing IEX separations, it is important to understand the impact that pH has on selectivity. As previously mentioned, the column switching capability of the ACQUITY Arc Bio System allows users the flexibility to evaluate different techniques when using common mobile phase systems in conjunction with Auto•Blend Plus. Using the same mobile phase system, valve positions were switched to direct flow to the IEX column (Figure 2). Auto•Blend Plus was used to deliver 20 mM phosphate and a linear salt gradient from 25–125 mM NaCl over 10 minutes while holding pH constant throughout the run. Figure 6 shows the effect of pH on charge variant analysis of trastuzumab from pH 6.2 to 7.4 at 0.4 pH unit increments. (Data was collected for every 0.2 pH unit change and is further described in Table 1.)

pH	Peak area, acidic variants (%)	Peak area, main peak (%)	Peak area, basic variants (%)	USP resolution (Acidic–MP)	End p/v (MP)
6.0	23.71	62.48	13.81	1.4	5.7
6.2	23.59	61.59	14.81	1.6	6.1
6.4	23.20	61.37	15.43	1.9	6.2
6.6	23.41	61.12	15.47	2.2	5.9
6.8	23.87	60.73	15.40	2.4	5.6
7.0	23.95	60.74	15.32	2.8	5.3
7.2	25.88	59.50	14.62	2.2	6.2
7.4	23.39	64.70	11.90	–	6.1

Table 1. Evaluating the effect of pH on charge variant analysis of trastuzumab. Auto•Blend Plus was used to manipulate small changes in pH to screen multiple method conditions in a single sample set. A salt gradient from 25–125 mM NaCl over 10 minutes was used, while holding the indicated pH constant over the length of the run. Peak area, resolution, and the ratio of peak height to valley height (p/v) are reported. Reported results in combination with chromatographic results should be used to determine the pH range in which the method demonstrates robustness (~pH 6.2 to pH 6.6).

As expected, increasing the pH results in less retention, as the higher pH reduces the positive charge on the analyte and weakens the interaction with the stationary phase. From visual inspection of the data, similar chromatographic profiles are seen at lower pH. Ideally, an IEX method would be further developed in this lower pH region to ensure analyte stability. Because small changes in pH impact the chromatography at higher pH, the final method should account for small variations in pH due to different mobile phase preparations.

Further treatment of the IEX data evaluates peak area percentage of acidic and basic variants and the main peak, USP resolution, and peak height to valley height (p/v) (Table 1). This data, in combination with chromatographic results, suggest that further method development should be interrogated in the range of ~pH 6.2 to 6.6, where the method appears to be more robust. In addition to using a longer column, the salt concentration can also be narrowed to improve resolution, if desired, once an appropriate pH is determined. Salt concentration was not evaluated with the initial conditions screened as a user would have to evaluate the pH data to determine how to manipulate the salt gradient for best results.

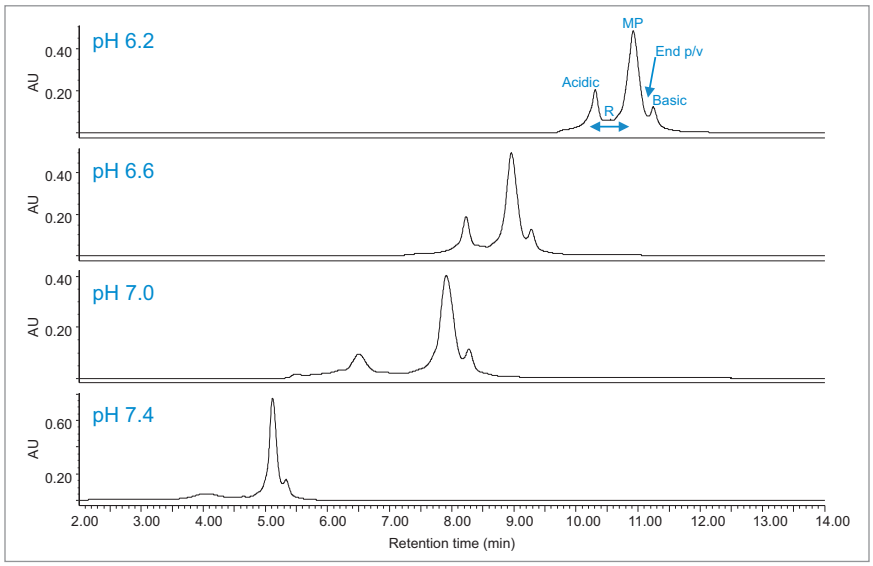

Figure 6. Effect of pH on charge variant analysis of trastuzumab. Auto•Blend Plus was used to manipulate small changes in pH, while programming a salt gradient from 25–125 mM NaCl over 10 minutes, while holding the indicated pH constant over the length of the run. (Table 1 reports peak area percentage for the main peak (MP), sum of the acidic variants, and sum of the basic variants. Resolution (R) between the acidic residues and main peak and the ratio of peak height to valley height (End p/v) for the main peak are also reported.)

CONCLUSIONS

In this work, column switching capabilities and buffer preparation technology were used to develop high-level screening protocols. Auto•Blend Plus was used to blend various mobile phase compositions from concentrated stock solutions so that method conditions could be readily screened without the need for preparation of separate mobile phases at each set of conditions. This allowed for a single sample set to be generated to screen pH and ionic strength for both SEC and IEX methods with minimal user intervention. The bio-inert flow path of the ACQUITY Arc Bio System is especially advantageous for applications such as these, which otherwise leave an LC system susceptible to corrosion due to the buffer systems used for analysis. These features of the ACQUITY Arc Bio System allow for a simplified and higher throughput approach to early method development, which can help guide further method optimization.

Reference

1. Please visit https://www.waters.com/autoblendplus for more information on how to use Auto•Blend Plus technology.

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