

응용 자료

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief outlines good practices for the routine maintenance and use of UltraPerformance LC Systems in conjunction with high ionic strength, 100% aqueous mobile phases, typical eluents used in size-exclusion and ion-exchange chromatography.

Benefits

- Proper Set-Up and Maintenance of an ACQUITY UPLC System Allows for Robust and Reliable SEC and IEX Separations.
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Introduction

Given the complexity of biotherapeutics, full characterization typically requires a variety of orthogonal methods. While many chromatographic techniques are conducted under reversed-phase conditions, others are conducted under native separation conditions, requiring high ionic strength, 100% aqueous eluents. For high performance liquid chromatography systems, these conditions can be problematic: in the absence of bactericides, lack of proper maintenance can lead to bacterial contamination within hours. The presence of high salt concentrations increases the potential of particulates in the mobile phases. The presence of bacteria and particulates in the LC system can affect chromatography quality and column lifetime.

The components of the chromatographic system are equally important. If the chromatographic system is not inert or bio-compatible, metal-protein adducts or undesired protein interactions can occur. Long-term use of high ionic strength, 100% aqueous mobile phases can also lead to rust formation if the chromatographic system contains steel components in the wetted path. However, with proper set-up and care of a chromatographic system, robust and reproducible chromatography can be achieved with minimal down time.^{1,2}

Results and Discussion

The care and use of a size-exclusion and/or ion-exchange chromatographic system requires many of the same standard practices as any other system. However, there are some additional protocols that are required for high salt, aqueous mobile phases. While the practices outlined in this document are described for ACQUITY UPLC Systems, the principles apply to any chromatographic system. Overall system recommendations include:

- If using a steel system, modify according to manufacturer's recommendations. For use with a Waters UPLC System, detailed guidelines are available.
- Clean laboratory glassware properly.
- If chronic loss of prime, check valve problems, reproducibility of retention time, pressure or quantitative reproducibility are observed, clean the system following a standard protocol.³
- If possible, use mobile phases containing a bacteriostat (i.e., 0.02% sodium azide) to prevent microbial growth.
- Retention time or pressure fluctuation can be indicative of problems with the pump.
- Area or peak variability without retention time variation can be indicative of an injector problem.

Additional recommendations are listed below by component. These considerations are for microbial growth, system suitability and/or protein stability.

Solvent Delivery System:

The buffers used in SEC and IEX can favor microbial growth leading to contamination of the column and system (Figure 1). Recommendations include:

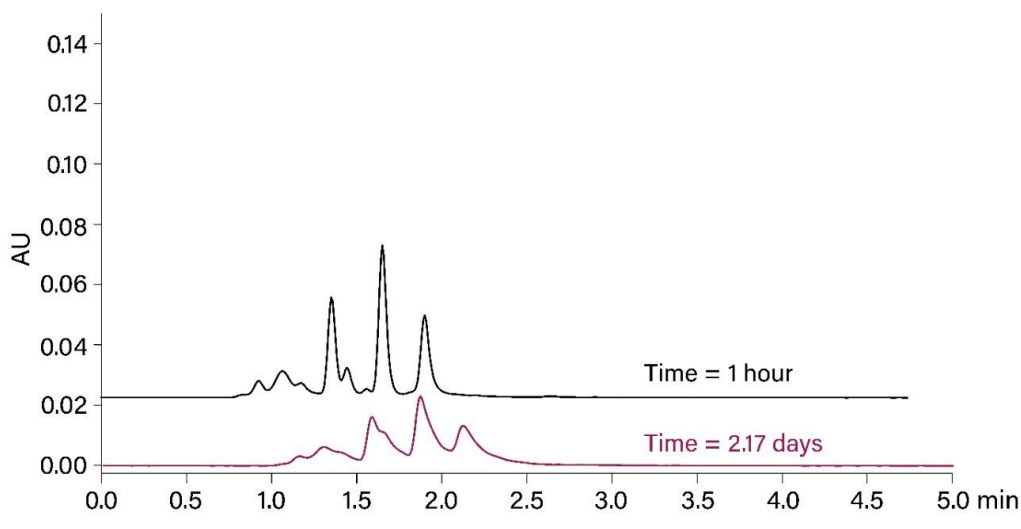
- Always filter aqueous mobile phase through compatible 0.22 µm or smaller membrane filters. The use of

sterile filters and containers is also recommended.

- Use only high purity water (18.2 MΩ cm). Bottled water should be opened the day of use.
- Never 'top-off' mobile-phase bottles. Always change bottles when replacing mobile phase.
- High-ionic-strength eluents (> 100 mM) should be replaced every two weeks.
- Low-ionic-strength eluents (< 100 mM) should be replaced every 2-3 days.
- Water (100%) bottles should be replaced daily.
- Higher salt concentrations, which inhibit microbial growth, would reduce the frequency of solvent replacement. While it is not always practical to change mobile phases, a quaternary solvent mixing system can accomplish the same effect by combining high concentration buffers (> 100 mM) to produce SEC and IEX mobile phases.⁵
- All eluent bottles should be visually inspected daily for microbial growth and/or particulates. Microbial growth can be a film on the bottle surface or may be observed by swirling the bottle.
- If microbial growth has occurred in the eluent bottle, replace the mobile-phase filter or flush it with a 70% isopropanol solution. Microbes can contaminate mobile-phase filters.
- Use compatible mobile-phase filters, such as titanium. Clean mobile-phase filters weekly to reduce microbial contamination. Sonicate or flush with 70% isopropanol solution.
- Recommended seal wash cycle time of 0.10 minutes (6 seconds).
- Seal wash recommendation of 90/10 water/methanol.
- The solvent manager should never be left idle in either high salt mobile phases or 100% water.

For short-term storage, maintain 0.1 mL/min of drawing an equal percentage of mobile phase from all lines in use.

If system will be idle for more than 2 days, prime each line for 10 minutes with high purity water. Thoroughly flush system. Repeat steps with 90/10 water/methanol.



*Figure 1. Effect of microbial growth on a SEC chromatogram of protein mix.
Contamination confirmed by analytical analysis of column frit.*

Sample Manager:

SEC and IEX conditions often require high-ionic-strength solutions in the sample manager wash lines (wash/purge, strong and weak needle). However, these eluents may have a detrimental effect on the sample syringe and/or needle. For variable flow through needle systems, remove salt deposit on a regular basis to minimize maintenance and repairs. Other recommendations include:

- Ensure the sample is soluble in the mobile-phase and sample manager washes.
- Follow the manufacturer's recommendations for wash solvents. For example, washes containing less than 500 mM salt(s) are recommended for the ACQUITY UPLC H-Class Bio System.
- If visible salt deposits appear, clean the surfaces. If salt deposits reappear, check connections and system for problems.⁴
- If the sample manager is idle for more than 2 days, purge needle and/or wash lines with high purity water (minimum of 20 cycles or 200 seconds). Repeat steps with 90/10 water/methanol. The sample manager should never be left idle for longer than 2 days in lines containing high salt washes (>100 mM).

UV Detectors:

Waters recommends titanium or stainless steel optical flow cells when performing SEC or IEX under aqueous

conditions. The standard ACQUITY optical flow cell contains Teflon AF in the fluidic path. Some proteins, under native conditions, may interact with the flow cell surface, resulting in peak tailing and sloped baseline (Figure 2). Recommendations for detectors include:

- Use titanium or stainless steel flow cells to reduce proteinsurface interactions. Other flow cell material (i.e., Teflon) may cause peak tailing.
- Never leave the detector idle in high salt eluents. Flush thoroughly with water (60 minutes at 0.2 mL/min) followed by 90/10 water/methanol or higher organic eluent.

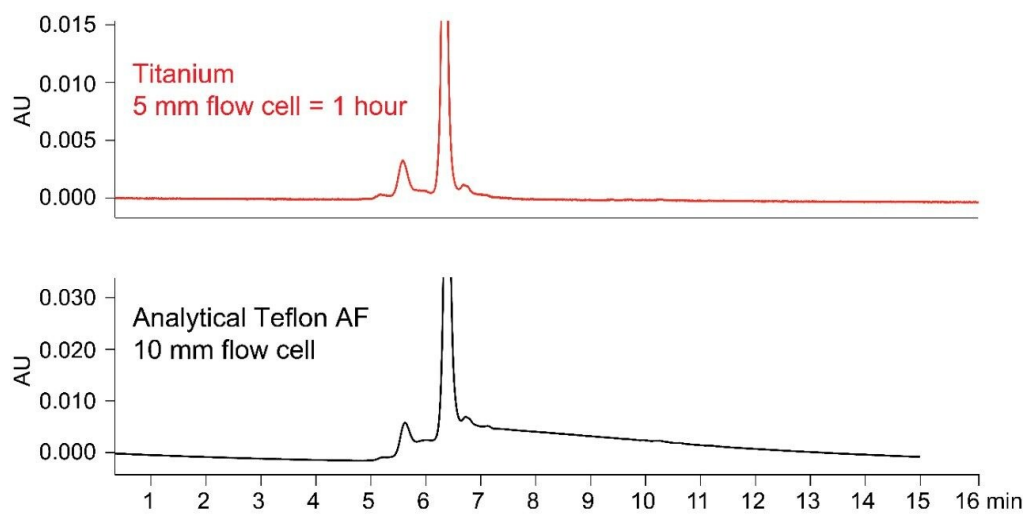


Figure 2. SEC-PDA chromatogram of bovine serum albumin (BSA) (5 mg/mL in water) shows the effect of flow cell material on peak shape. BSA monomer exhibits extensive peak tailing.

Column Storage:

To maintain long column lifetimes and minimize the risk of microbial contamination, the following recommendations should be followed:

- Columns should never be stored in high salt, aqueous mobile phase or 100% water.
- Before switching to recommended storage conditions, flush columns with 10-20 column volumes of water.
- Flush and store columns following the manufacturer's recommendations. Typical recommendations are 10-

20% methanol or with a bactericide (i.e., 0.1% sodium azide).

- Consider the use of guard columns to extend column lifetimes. Regular replacement may be required. Frequency of guard column replacement may be dependent on sample cleanliness.
- Size-exclusion columns can typically be stored at 4-8 °C to reduce microbial growth. Ion-exchange columns are usually stored at room temperature. Check the manufacturer's recommendations for details.

Conclusion

SEC and IEX chromatography are performed under native conditions, requiring high-ionic strength, 100% aqueous eluents. To minimize protein-surface interactions these conditions may require the use of a bio-compatible chromatographic system specifically designed for these applications. Precautions must be taken to prevent and minimize bacterial contamination. Signs of such contamination, which can occur within hours include: deteriorating peak shape, resolution and column lifetime. Unfortunately, once the column has been contaminated, regeneration is difficult. To decrease the frequency of system repairs and contamination, a series of steps have been outlined for maintenance and care of a chromatographic system and columns used for the analysis of biomolecules. These recommendations include maintenance for the solvent manager, sample manager, detector and column. Using these procedures in combination with good laboratory practices ensures a robust, reproducible system for ultra-performance-size-exclusion and ion-exchange chromatography.

References

1. *"Size-Exclusion and Ion-Exchange Chromatography of Proteins using the ACQUITY UPLC System"*, Waters User Manual [2010], Rev A, Part Number 715002147.
2. *"Size-Exclusion and Ion-Exchange Chromatography of Proteins using the ACQUITY UPLC H-Class System"*, Waters User Manual [2010], Rev A, Part Number 715002909.
3. *"Controlling Contamination in LC/MS and HPLC/MS Systems"*, Waters User Manual, Part Number 715001307.
4. *"ACQUITY UPLC H Class Sample Manager Flow-Through Needle Operator's Overview and Maintenance"*

Information", Waters User Manual [2010], Rev B, Support Number USRM10144216.

5. Waters Corporation (Producer) (2011). Auto•Blend Plus Technology Tutorial [Video]. Retrieved from <http://www.waters.com/waters/nav.htm?cid=134623262>

6. Hong P., Fountain K.J., Wheat T. E., Morrison D. *"IEX Method Development of a Monoclonal Antibody and Its Charge Variants"*, Waters Application Note [2011], Part Number 720003836EN.

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