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Nota de aplicación

Accelerating Charge Variant Analysis of Biotherapeutics with the BioAccord™ System

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Abstract

Ion Exchange Chromatography (IEX) is a valuable technique given its ability to resolve product/process related charge variants as part of the development and manufacturing process of biopharmaceuticals. However, the value of the technique to provide in-depth information is limited when using optical detection alone. In this study, Waters high purity IonHance™ CX-MS pH concentrates were used in conjunction with the BioAccord Liquid Chromatography-Mass Spectrometry (LC-MS) System to develop a MS-compatible IEX-based method. Separations were carried out using the BioResolve™ SCX mAb Column with data acquisition performed on a BioAccord System controlled by and with data processed with the waters_connect™ Informatics Platform. Results from this study demonstrate that mass spectral data can be acquired and automatically deconvoluted when using IonHance CX-MS pH concentrates to resolve charge variants associated with biotherapeutics. Furthermore, comparative studies carried out an ACQUITY™ Premier System configured with an optical detector indicate that the BioResolve SCX mAb Column delivers comparable performance using either pH or “salt” gradients allowing for increased flexibility in the support of development and manufacturing activity associated with the production of biopharmaceuticals.

Benefits

- IonHance CX-MS concentrates facilitate MS identification of charge variants
- BioResolve SCX mAb columns pH/salt compatibility increases flexibility in deployment
- ACQUITY Premier System delivers consistent performance for improved assay results

Introduction

IEX is a technique driven by charge interaction between the analyte and sorbent or stationary phase. For proteins, surface charges associated with amino acid functional groups and their modifications can cooperatively interact with active adsorption sites that exhibit the opposite charge making IEX a valuable technique to resolve product/process related charge variants associated with biopharmaceuticals.[1,2] IEX analyte elution is often a salt driven mechanism where mobility of analytes is achieved by increasing the ionic strength (salt) to inhibit binding of the analyte to the active site of the stationary phase. However, a drawback to salt-driven methods is that they are largely MS incompatible due to the ion suppression brought on by the use of high-ionic strength buffers or non-volatile salts. In this respect, salt-driven methods are limited to optical detection that limits the amount of useful information they can deliver. An alternative to this is to use pH to elute analytes where the ionic strength is held constant and the charge of the protein and/or binding site is manipulated by altering the pH of the mobile phase. These approaches have more appeal for their compatibility with MS detection that can provide more insight into charge variants. However, finding appropriate buffers that offer acceptable resolution and MS detector response can be challenging.

Recently, Waters has expanded its portfolio with the introduction of products specifically engineered to address challenges associated in the analysis of Biopharmaceuticals (Figure 1A). As part of these offerings, the IonHance CX-MS and BioResolve CX pH concentrates represent an innovative step forward for the analysis of biotherapeutics. These specially designed buffers are shipped as ready-to-use 10x pre-formulated concentrates. Once diluted, the concentrates are engineered to deliver linear pH responses over a wide range (Figure 1B) suitable for IEX–based analyses. The focus of this study is to demonstrate the value of such products in the analysis of charge variants related to mAb biotherapeutics and how they can be deployed to speed up the development and manufacturing of drug products.[3,4]

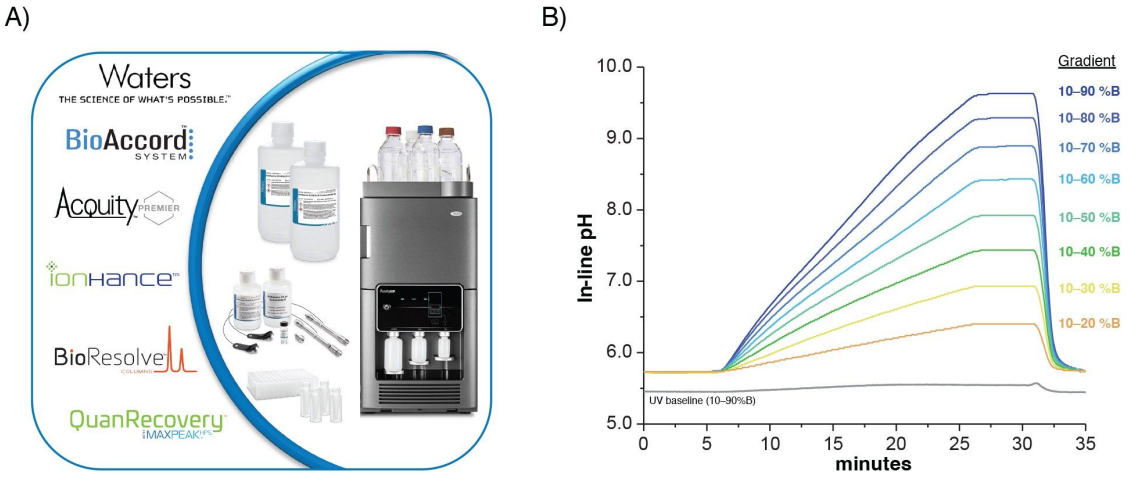

Figure 1. Accelerating Biopharmaceutical Analysis.

A) Waters transformational approach to the analysis of biopharmaceuticals has led to product innovations including the BioAccord System, BioResolve product line, and ACQUITY Premier Technology.

B) In-line pH measurements of Waters CX pH Concentrates were observed to have a linear and proportional response based on gradient.

Experimental

Sodium chloride, MES monohydrate, and MES salt was purchased from Fisher Chemical. The Infliximab drug product Remicade™ and its approved biosimilar Renflexis™ was purchased from Amerisource Bergen and prepared at the dosage concentration (10 mg/mL) using sterile water as per manufacturer's instructions and injected neat.

BioAccord LC-MS System Conditions

LC system:	ACQUITY Premier System (BSM-variant)

Detection:	ACQUITY TUV, FC=Ti 5 mm, λ=280, 214 nm
Vials:	QuanRecovery™ MaxPeak™ vials, (p/n: 186009186)
Column(s):	BioResolve SCX mAb Column, 3 µm, 2.1 mm X 100 mm (p/n: 186009056)
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	2 µL
Flow rate:	0.100 mL/min
Mobile phase A:	IonHance CX-MS pH Concentrate A (p/n: 186009280)
Mobile phase B:	IonHance CX-MS pH Concentrate B (p/n: 186009281)
RDa settings:	
Ionization mode:	ESI+
Acquisition mode:	Full Scan
Acquisition range:	High (400 – 7000 *m/z*)
Scan rate:	1 Hz
Capillary voltage:	1.5 kV

Cone voltage:	125 V
Desolvation temp.:	350 °C
Intelligent data capture:	Enabled
Chromatography software:	UNIFI™ Application v2.1.2.4 within waters_connect

Gradient (IonHance CX-MS concentrates)

Time	Flow (mL/min)	%A	%B	Curve
Initial	0.100	54.3	45.7	Initial
45.00	0.100	40.0	60.0	6
46.00	0.100	2.0	98.0	6
49.00	0.100	2.0	98.0	6
50.00	0.100	54.3	45.7	6
70.00	0.100	54.3	45.7	6

ACQUITY Premier LC/UV Conditions

LC system:	ACQUITY Premier System (QSM-variant)
Detection:	ACQUITY TUV, FC=Ti 5 mm, λ=214 nm, 280 nm
Vials:	QuanRecovery MaxPeak vials, (p/n: 186009186)
Column(s):	BioResolve SCX mAb Column, 3 µm, 2.1 mm X 100 mm (p/n: 186009056)
Column temp.:	40 °C

Sample temp.:	10 °C
Injection volume:	1 µL
Flow rate:	0.100 mL/min
Mobile phase A:	BioResolve CX pH Concentrate A (p/n:186009063) or 20 mM MES buffer, pH 6.7
Mobile phase B:	BioResolve CX pH Concentrate B (p/n:186009064) or 20 mM MES buffer with 200 mM NaCl, pH 6.7
Mobile phase C:	H_2O
Mobile phase D:	H_2O
Chromatography software:	Empower™ 3, FR4

Gradient (BioResolve CX pH concentrates)

Time	Flow (mL/min)	%A	%B	%C	%D	Curve
Initial	0.100	81.5	18.5	0.0	0.0	Initial
45.00	0.100	61.0	39.0	0.0	0.0	6
46.00	0.100	10.0	90.0	0.0	0.0	6
49.00	0.100	10.0	90.0	0.0	0.0	6
50.00	0.100	81.5	18.5	0.0	0.0	6
70.00	0.100	81.5	18.5	0.0	0.0	6

Gradient (20 mM MES Buffer, pH 6.7)

Time	Flow (mL/min)	%A	%B	%C	%D	Curve
Initial	0.100	91.0	9.0	0.0	0.0	Initial
45.00	0.100	72.0	28.0	0.0	0.0	6
46.00	0.100	0.0	100.0	0.0	0.0	6
49.00	0.100	0.0	100.0	0.0	0.0	6
50.00	0.100	91.0	9.0	0.0	0.0	6
70.00	0.100	91.0	9.0	0.0	0.0	6

Results and Discussion

UV-based IEX charge variant analyses are frequently deployed in late-stage development to assist in process control, or as monitoring assays in manufacturing environments, as well as screening assays in stability and formulation studies. When using UV-based assays, pre-existing knowledge of charge variants and/or empirical experimentation, is required to make useful inferences from results. An example of this is shown in the UV chromatogram of Figure 2A for the mAb-based therapy Remicade where one or both c-terminal lysine residues (peaks 1–3) are truncated in the drug product. While IEX chromatography can resolve these species due to the charge imparted by the lysine amino acid residue, an analyst would not necessarily know which peak represents which truncation species without prior knowledge or direct experimentation. In contrast, an MS-based approach to this analysis would be able to provide direct mass information of truncated species bypassing the need for additional experiments.

To demonstrate this, the IEX separation shown in Figure 2A was performed on a BioAccord LC-MS System using the IonHance CX-MS pH concentrates with charge variants separated on a BioResolve SCX mAb Column using a 0.3 %B/min gradient. Dual detection of charge variants was performed in a serial fashion using optical (TUV) and MS (RDa) detection. Deconvolution of MS spectral data of the three dominant peaks is shown in Figure 2B. Looking at the deconvoluted spectrum, a mass shift of +128 Da was observed in the spectrum for each peak indicating the addition of a lysine residue. This allows for direct identification of the truncated species by mass where peaks 1-3 represent the presence of zero, one, and two lysine residues, respectively. The benefits of MS-based IEX analysis is not restricted to only identifying lysine truncation species. As shown in Figure 2C, mass information can be used to discern differences between samples or drug products. In this example, using the

UNIFI Application, a binary comparison was made between the innovator Remicade and its biosimilar Renflexis using the same separation conditions. As shown in Figure 2C, comparison of deconvoluted spectra indicate profile deviations (dashed circle). Further investigation of mass data indicate these differences can be attributed to varying glycosylation patterns between samples. These examples demonstrate the value MS brings in the rapid assessment of charge variants as part of process development and how products such as Waters IonHance CX-MS concentrates can be used to facilitate such workflows.

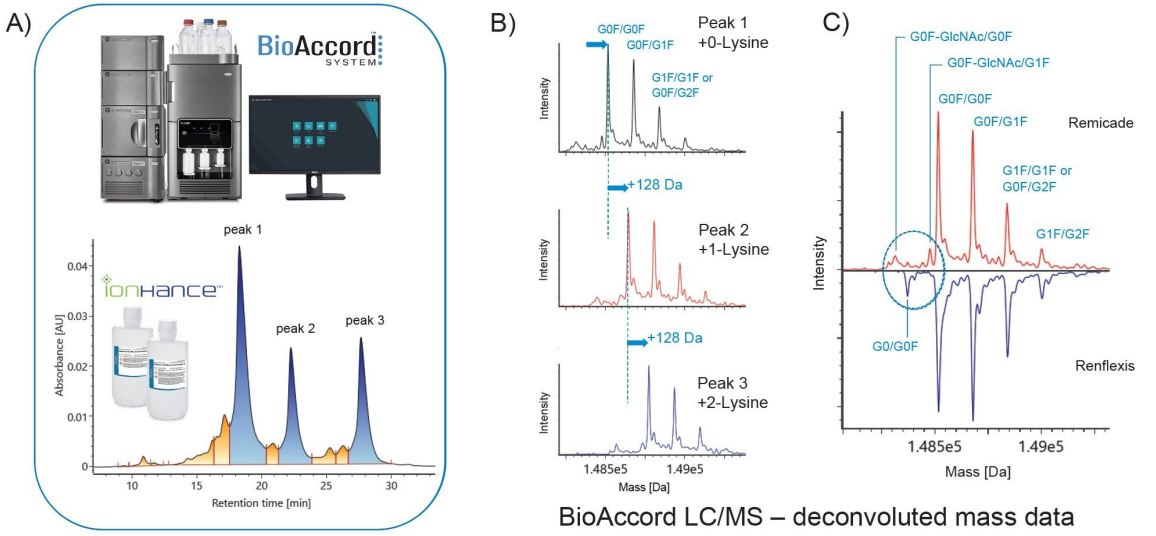

Figure 2. IEX-MS Analysis of Charge Variants.

A) UV Chromatogram of Remicade acquired on the BioAccord System with separation performed on the BioResolve SCX mAb Column using IonHance CX-MS pH Concentrates with the lysine truncation species labeled peak 1–3.

B) A mass shift of +128 Da was observed in the deconvoluted mass data acquired for peaks 1–3.

C) Binary comparison of the deconvoluted mass data of the innovator Remicade and biosimilar Renflexis. Mass differences were identified as differences in glycosylation levels of the intact mAbs.

As part of this study, the BioResolve SCX mAb Column was evaluated in terms of its ability to support downstream activity where traditional or legacy methods using high-ionic strength buffers may be deployed. To accomplish this, a comparative study was performed on an ACQUITY Premier System configured with an optical detector (UV) representative of an LC configuration deployed in a manufacturing environment (Figure 3A). As a UV-based method, Waters BioResolve CX pH concentrates were used in lieu of the IonHance MS pH

concentrates. While both concentrates are engineered to deliver linear pH gradients, differences reside in the purity of reagents used and their effective pH-range which is formulated for MS (IonHance CX-MS) or UV (BioResolve CX) detection. Taking this into account, a modified gradient of 0.5 %B/min was used for the IEX separation using the BioResolve CX pH concentrates. As shown in Figure 3B, the BioResolve CX concentrates were able to deliver a pH gradient equally capable of separating the charge variants of Remicade with the same fidelity of the IonHance MS-CX concentrates (Figure 2A). Furthermore, the high reproducibility of the results (% RSD < 1.0) indicate the BioResolve CX concentrates are stable and capable of robust performance suitable for manufacturing environments.

Lastly, a method using 2-(N-Morpholino)ethanesulfonic acid (MES) was derived from the BioResolve CX pH Concentrate results to demonstrate the BioResolve SCX mAb columns ability to support legacy salt-based methods. As shown in Figure 3C, the BioResolve SCX mAb Column was able to perform with comparable results in terms of charge variant profile and reproducibility (%RSD < 1.0) when using a salt gradient, demonstrating the columns ability to perform robustly with more traditional separation methods. These results demonstrate the BioResolve SCX mAb Column in conjunction with Waters IonHance CX-MS or BioResolve CX pH concentrates offer a flexible IEX platform that can be deployed across labs to speed up the optimization of methods for the effective development and manufacturing of biotherapeutic drug products.

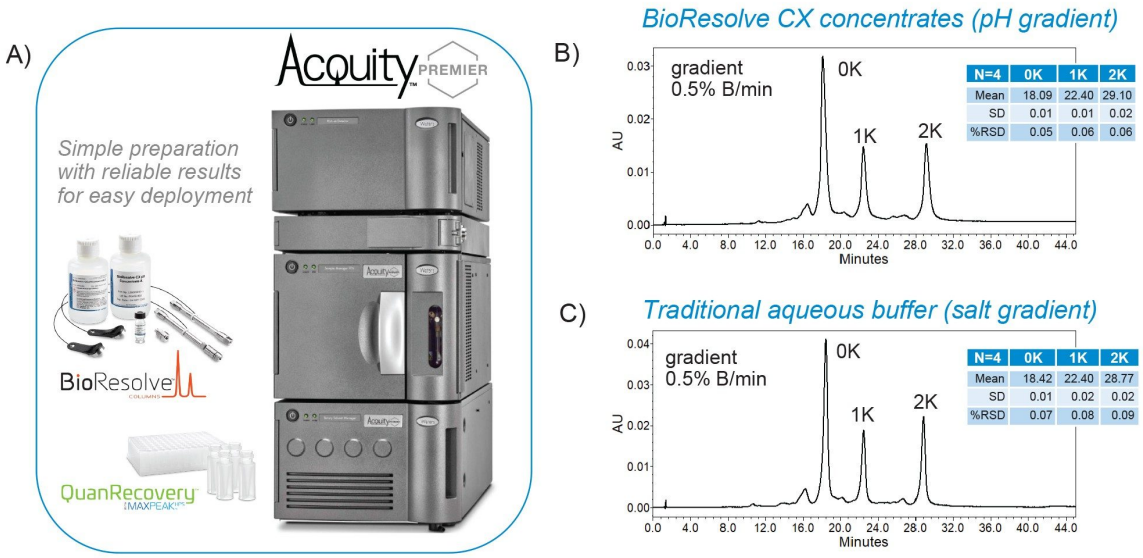

Figure 3. IEX-UV Comparative Study.

A) The ACQUITY Premier System configured with a UV detector was used to acquire IEX-UV data with separations performed on the BioResolve SCX mAb Column.

B) IEX profiles using BioResolve CX pH Concentrates were comparable to IEX-MS profiles (Figure 2A).

C) The BioResolve SCX mAB column was able to deliver comparable results in terms of profile and reproducibility when using high-ionic strength gradients (MES buffer and 200 mM NaCl) versus pH gradients.

Conclusion

IEX is a valuable technique given its ability to resolve product/process related charge variants as part of the development and manufacturing process of biopharmaceuticals. To fully realize the benefit of this technique, Waters has expanded its portfolio with the innovative BioResolve SCX mAb Column and CX pH concentrates. Through this study, it was demonstrated when used in conjunction, the BioResolve SCX mAb Column and IonHance CX-MS pH concentrates afford users the ability to interrogate their drug products with MS for direct identification of charge variants in IEX-based analyses. Furthermore, the BioResolve CX pH concentrates, which

are tailored towards UV-based analyses, offer users the ability to migrate these methods downstream for increased flexibility in the support of development and manufacturing activity associated with the production of biopharmaceuticals.

References

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