Development of Standards for Cation Exchange Chromatography Column Qualification

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Introduction

Standards for qualification of chromatographic columns were identified as a high priority during USP-sponsored roundtables with stakeholders to identify challenges in biologics development that could be alleviated with standards. Cation Exchange Chromatography (CEX) analysis is commonly used to characterize the charge heterogeneity of therapeutic proteins by determining acidic and basic charge variants. Salt gradient and pH gradient are two widely used methods of CEX analysis. The aims of this proof-of-concept study were to establish both pH gradient and salt gradient CEX methods and utilize them to evaluate three USP monoclonal antibody standards in development (USP mAb001, USP mAb002 & USP mAb003) on columns from three different vendors. Resulting profiles and peak resolution were evaluated to select a candidate for further development as a standard for CEX column qualification.

Materials and Methods

Materials:

Table 1: USP mAbs for CEX column qualification

#	Name	Subclass	pI*	Formulation Buffer
1	USP mAb001	IgG1	~9.3	9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, pH 6.5
2	USP mAb002	IgG1	~7.8	0.58% Monobasic sodium phosphate monohydrate, 0.12% Dibasic sodium phosphate anhydrous, 6% α,α-trehalose dehydrate, 0.04% Polysorbate 20, pH 6.2
3	USP mAb003	IgG1	~7.8	20 mM Histidine HCl, 120 mM Sucrose, 0.02% Polysorbate 20, pH 6.0

* pI value from in-house cIEF data

Table 2: Columns used for evaluation of USP mAbs for CEX column qualification standards

	Column 1	Column 2	Column 3
Column Name	BioResolve SCX mAb	BioPro SF	BioMab NP5
Column Vendor	Water	Agilent	YMC
Column Type	SCX	SCX	WCX
Particle morphology	Non porous	Non porous	Non porous
Matrix	Hydrophilic polymer	Hydrophilic polymer	Hydrophilic polymer
Particle size (µm)	3	5	5
Functional group ligand	Sulfonic acid (SO_3^-)	Sulfonic acid (SO_3^-)	COOH
Denmension	4.6 x 100 mm	4.6 x 100 mm	4.6 x 250 mm
Column Material	Stainless steel	PEEK	PEEK

Methods:

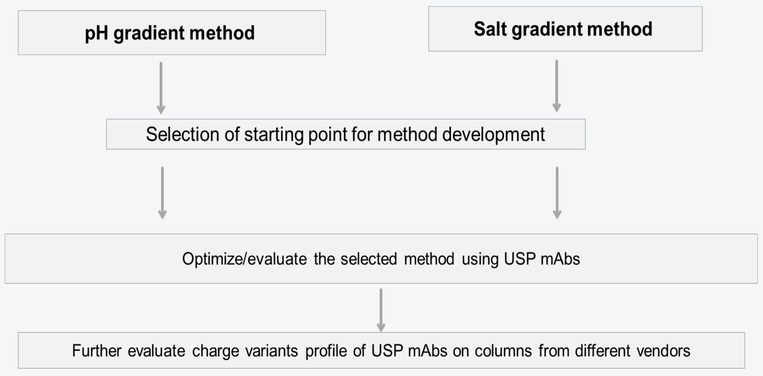

Figure 1. Workflow for evaluation of USP mAbs for CEX column qualification standards

Table 3: HPLC conditions by pH gradient and salt gradient methods

HPLC Conditions	pH Gradient Method	Salt Gradient Method
Mobile Phase A	BioResolve CX pH Buffer A (pH 5.2 -6.0)	20 mM phosphate, pH 6.7; 20 mM MES, pH 6.1; 20 mM MES, pH 6.7
Mobile Phase B	BioResolve CX pH Buffer B (pH 9.5 -10.2)	20 mM phosphate, pH 6.7 + 0.5 M NaCl; 20 mM MES, pH 6.1 + 0.5 M NaCl; 20 mM MES, pH 6.7 + 0.5 M NaCl
Salt Gradient Slope	0.219 pH unit/min	15-115 mM or 50-115 mM NaCl over 30 min
Detection (UV)	280 nm	
Loading Amount (µg)	~30	

Results

Part I: by pH Gradient Method

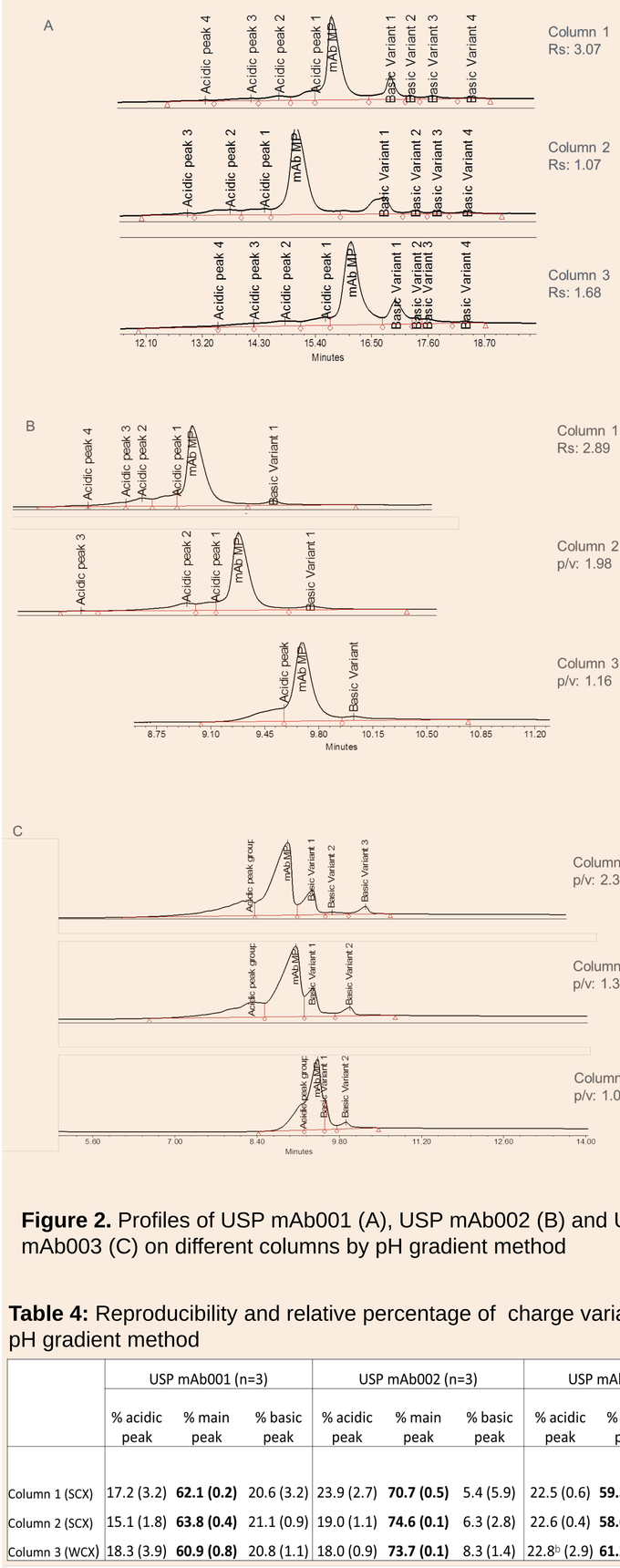

Figure 2. Profiles of USP mAb001 (A), USP mAb002 (B) and USP mAb003 (C) on different columns by pH gradient method

Table 4: Reproducibility and relative percentage of charge variants by pH gradient method

	USP mAb001 (n=3)			USP mAb002 (n=3)			USP mAb003[a] (n=3)		
	% acidic peak	% main peak	% basic peak	% acidic peak	% main peak	% basic peak	% acidic peak	% main peak	% basic peak
Column 1 (SCX)	17.2 (3.2)	**62.1 (0.2)**	20.6 (3.2)	23.9 (2.7)	**70.7 (0.5)**	5.4 (5.9)	22.5 (0.6)	**59.3 (0.3)**	18.2 (0.3)
Column 2 (SCX)	15.1 (1.8)	**63.8 (0.4)**	21.1 (0.9)	19.0 (1.1)	**74.6 (0.1)**	6.3 (2.8)	22.6 (0.4)	**58.6 (0.4)**	18.8 (1.3)
Column 3 (WCX)	18.3 (3.9)	**60.9 (0.8)**	20.8 (1.1)	18.0 (0.9)	**73.7 (0.1)**	8.3 (1.4)	22.8[b] (2.9)	**61.2 (0.1)**	16.0[b] (3.7)

Data are shown as mean of n =3 and CV% in brackets
[a] Peak fronting was observed
[b] Lower Rs

Results – cont'd

Part II: by Salt Gradient Method

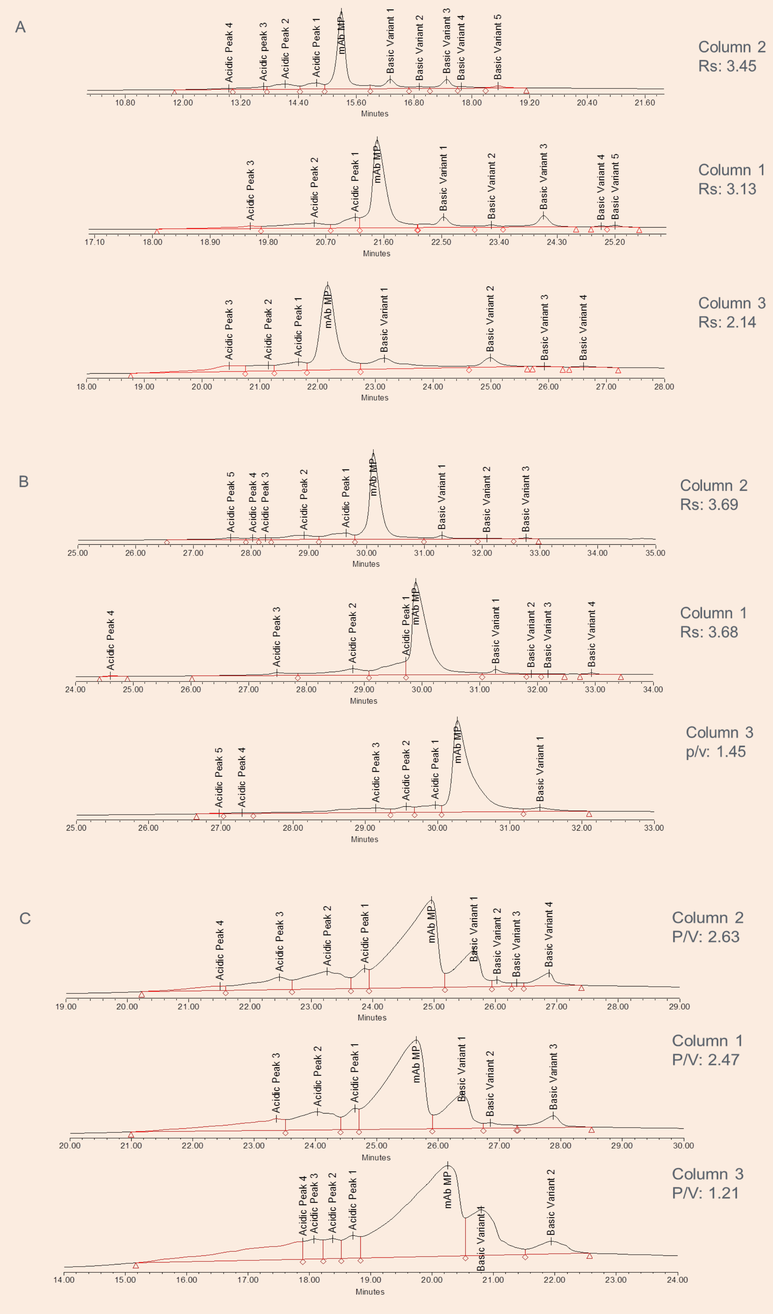

Figure 3. Profiles of USP mAb001 (A), USP mAb002 (B) and USP mAb003 (C) on different columns by salt gradient method

Table 5: Reproducibility and relative percentage of charge variants by salt gradient methods

	USP mAb001 (n=3)			USP mAb002 (n=3)			USP mAb003[a] (n=3)		
	% acidic peak	% main peak	% basic peak	% acidic peak	% main peak	% basic peak	% acidic peak	% main peak	% basic peak
Column 2 (SCX)	22.5 (0.1)	**52.3 (0.2)**	25.0 (0.5)	25.2 (0.1)	**69.3 (0.1)**	5.2 (1.6)	26.2 (0.5)	**53.9 (0.3)**	20.0 (0.3)
Column 1 (SCX)	22.5 (3.2)	**59.1 (1.1)**	19.2 (0.3)	27.9 (0.6)	**67.9 (0.3)**	4.2 (0.7)	26.2 (0.9)	**54.0 (0.3)**	19.8 (0.5)
Column 3 (WCX)	22.2 (0.2)	**55.6 (0.2)**	22.2 (0.5)	22.8 (0.6)	**73.4 (0.2)**	3.8 (1.1)	24.5 (1.1)	**56.1 (0.3)**	19.4 (0.7)

Data are shown as mean of n =3 and CV% in brackets
[a] Peak fronting was observed

Conclusions

- Generic pH gradient and salt gradient CEX methods were established and used to evaluate the charge variant profiles for three USP mAbs on columns from 3 different vendors, including two SCX columns and one WCX column
  - Better resolution of charge variants on SCX columns was observed, suggesting further optimization may be needed for CEX columns
  - Peak fronting was observed for USP mAb003 on all three columns
  - The percentages of Main, Acidic and Basic peaks were similar across columns for each mAb
- USP mAb001 was selected as the top candidate for further development. This mAb was prioritized because:
  - Four acidic and multiple basic charge variants could be resolved by both pH gradient and salt gradient CEX chromatography
  - It yielded a consistent charge variant profile across the 3 columns tested
- Next steps include identification of individual peaks and testing on additional columns

Note: USP mAbs standards will be released in Spring/Summer 2020

References

1. Qi Wang, et al. (2019) Development of pH Gradient Mobile Phase Concentrates for Robust, High Resolution mAb Charge Variant Analysis (Waters application note).
2. David A. Michels, et al. (2015) Separation Methods and Orthogonal Techniques: State-of-the-Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization Volume 2. 237-284.

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