



ThermoFisher
S C I E N T I F I C

Universal Ion Exchange Chromatography Separation Platform

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Biopharma and Pharma Landscape: Top 15 Best Selling Drugs of 2018

Ranking	Drug	Maker	2017 sales	2018 sales	Growth
1	Humira®(adalimumab)	AbbVie	\$19.936 billion	\$19.936 billion	8.2%
2	Eliquis® (apixaban)	BMS and Pfizer	\$7.395 billion	\$9.872 billion	33.5%
3	Revlimid (lenalidomide)	Celgene	\$8.187 billion	\$9.685 billion	18.3%
4	Opdivo® (nivolumab)	BMS	\$5.763 billion	\$7.570 billion	31.4%
5	Keytruda® (pembrolizumab)	Merck	\$3.809 billion	\$7.171 billion	88.3%
6	Enbrel® (etanercept)	Amgen and Pfizer	\$7.885 billion	\$7.126 billion	-9.6%
7	Herceptin® (trastuzumab)	Roche (Genentech)	\$7.013 billion	\$6.981 billion	-0.5%
8	Avastin® (bevacizumab)	Roche (Genentech)	\$6.686 billion	\$6.847 billion	2.4%
9	Rituxan® (rituximab)	Roche (Genentech)	\$7.298 billion	\$6.750 billion	-7.5%
10	Xarelto® (rivaroxaban)	Bayer and JNJ	\$6.234 billion	\$6.589 billion	5.8%
11	Eylea® (afibercept)	Regeneron	\$5.830 billion	\$6.551 billion	12.4%
12	Remicade® (infliximab)	JNJ	\$7.152 billion	\$5.908 billion	-17.4%
13	Prevnar 13 (Pneumococcal 13-valent Conjugate Vaccine)	Pfizer	\$5.601 billion	\$5.802 billion	3.6%
14	Stelara (ustekinumab)	Janssen Biotech	\$4.011 billion	\$5.156 billion	28.5%
15	Lyrica® (pregabaliln)	Pfizer	\$5.065 billion	\$4.970 billion	-1.9%

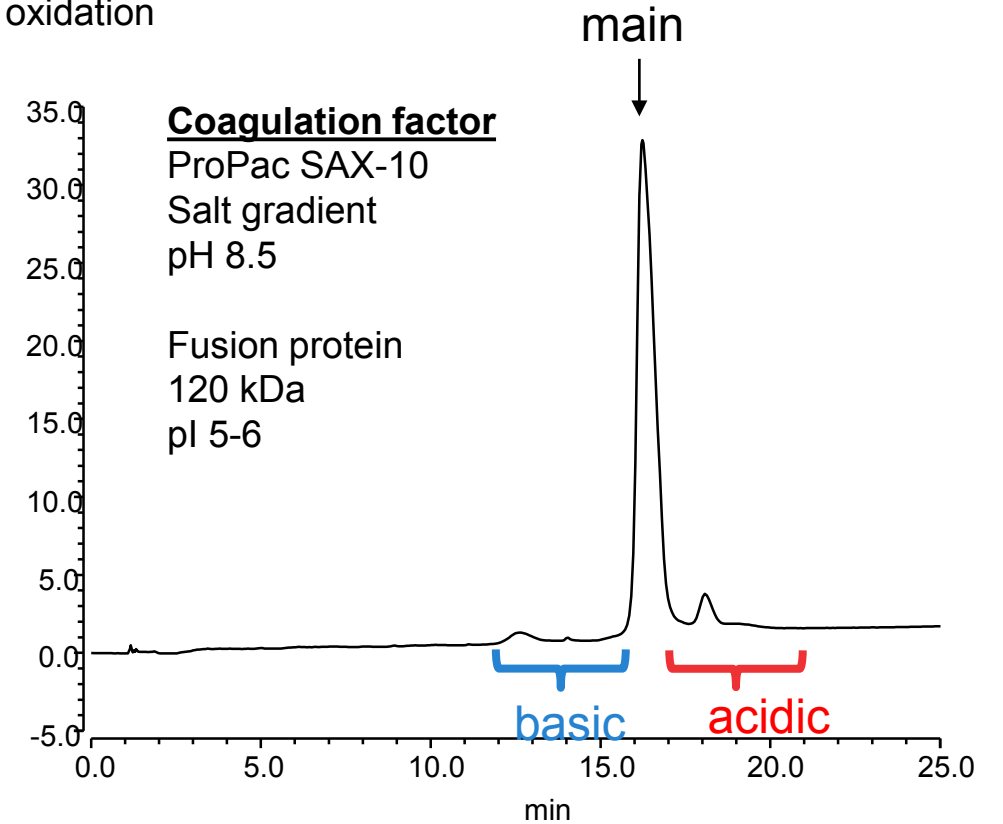
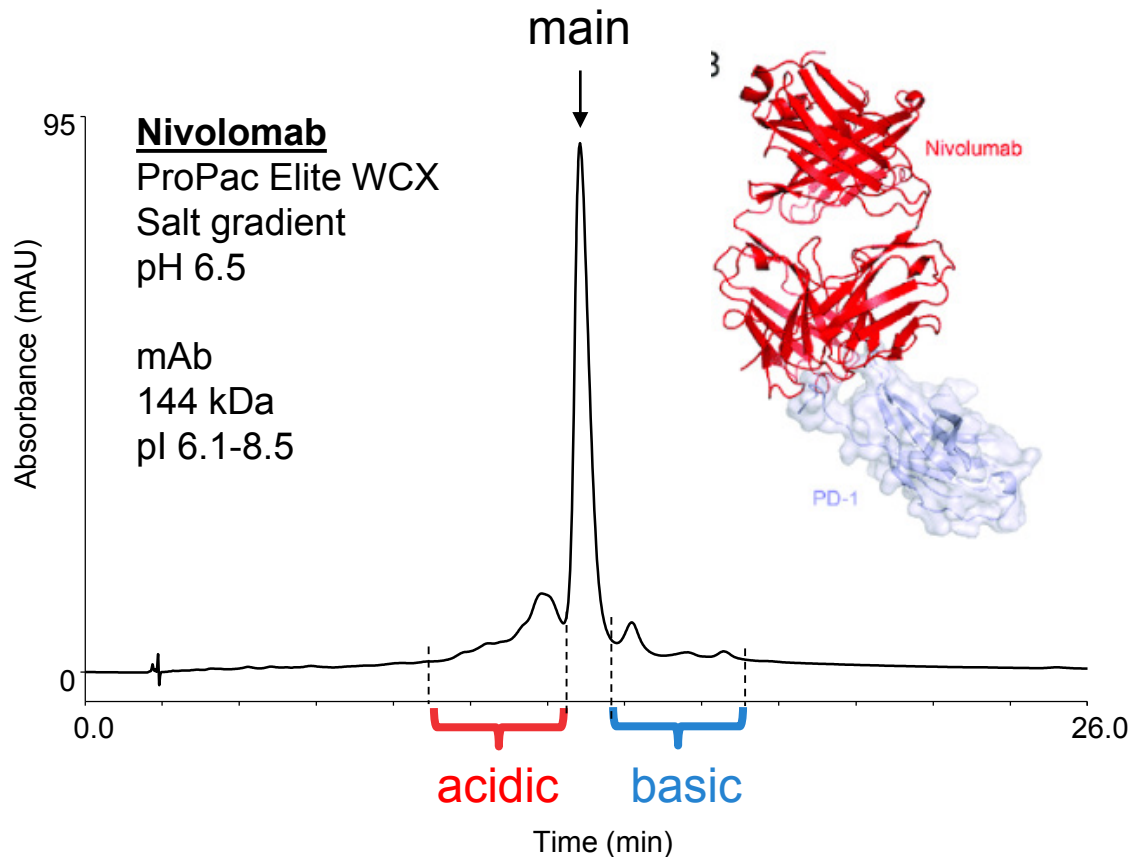
<https://www.genengnews.com/a-lists/top-15-best-selling-drugs-of-2018/>

- **Q6B Specifications:** Test Procedures and Acceptance Criteria for Biotechnological/Biological Products
 - **Purity:** Due to the unique biosynthetic production process and molecular characteristics of biotechnological and biological products, the drug substance can include several molecular entities or variants. When these molecular entities are derived from anticipated post-translational modification, they are part of the desired product. When variants of the desired product are formed during the manufacturing process and/or storage and have properties comparable to the desired product, they are considered **product-related substances** and not impurities (section II.A.1), e.g. **charge variants due to deamidation**.

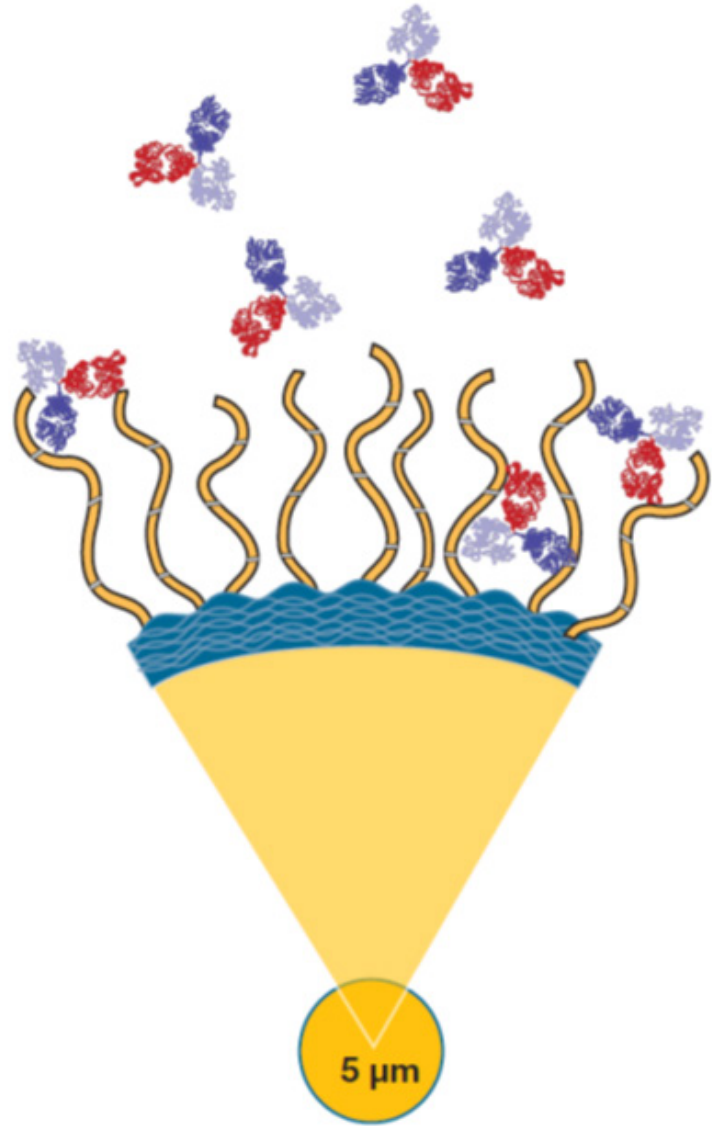
IEC is a Common Method for Protein Charge Variant Analysis

- mAb charge variant analysis (**Cation exchanger**)
 - Acidic: Sialylation, deamidation, C-terminal lysine cleavage
 - Basic: N-terminal cyclization of glutamic acid, methionine oxidation

- Acidic protein charge variant analysis (**Anion exchanger**)
 - Acidic: Sialylation, deamidation, C-terminal lysine cleavage
 - Basic: N-terminal cyclization of glutamic acid, methionine oxidation



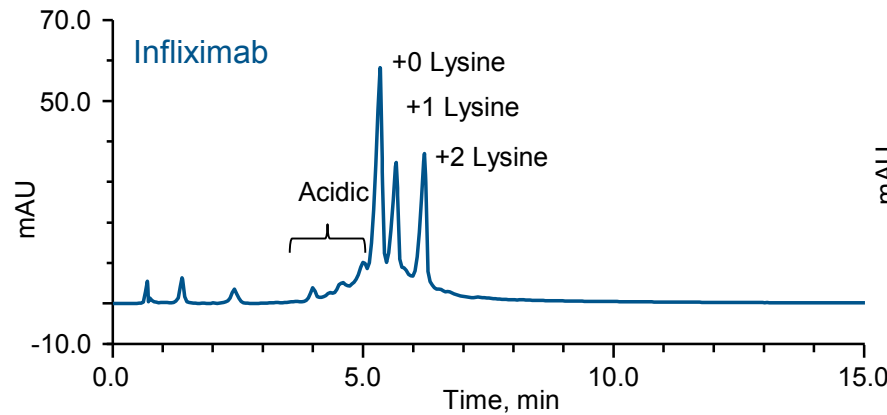
Two Types of Ion-Exchange Methods



- Interactions of protein cationic sites with anionic sites of stationary phase determine separation
- Chromatographic parameters influence separation

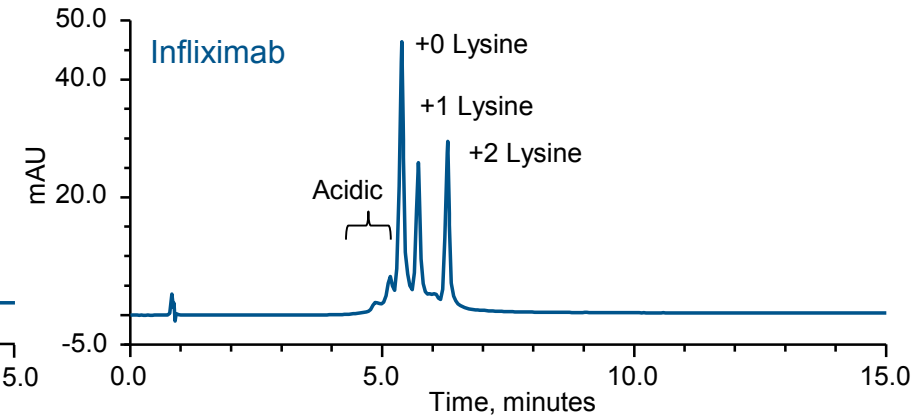
Salt gradient

- Mobile phase **pH**
- Salt gradient slope



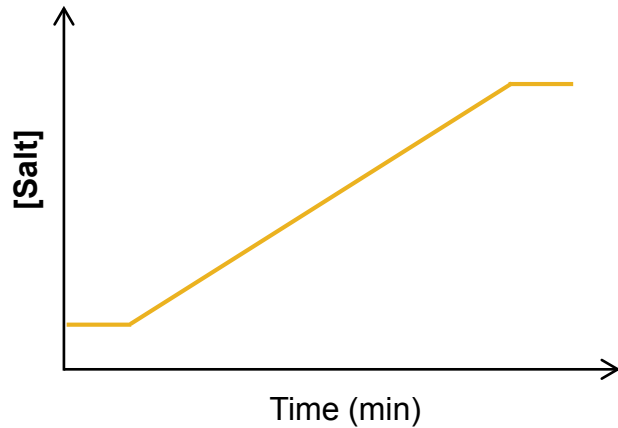
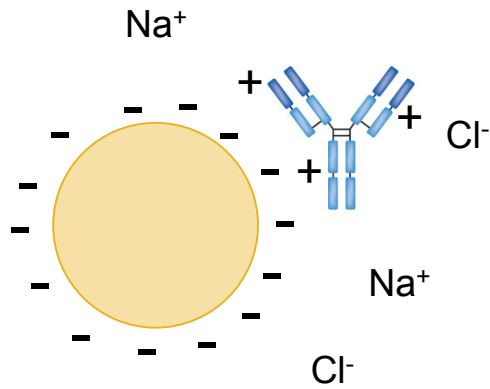
pH gradient

- **Electrolyte** concentration
- pH gradient slope

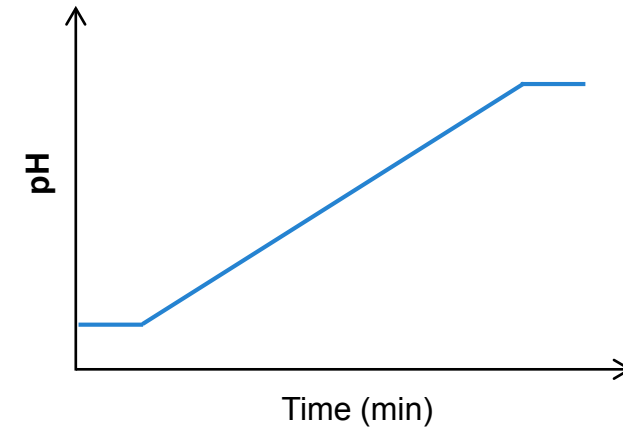
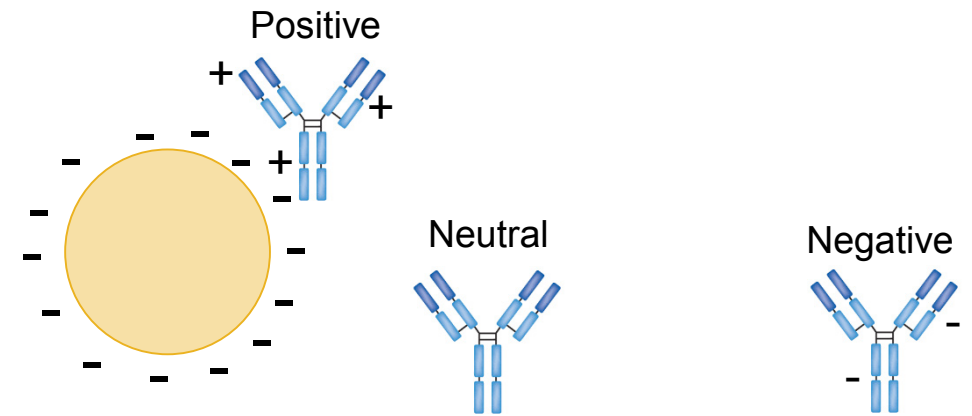


Salt Gradient Method and pH Gradient Method

Salt gradient method



pH gradient method



Two Methods for Protein/mAb Separation on IEX Columns

Salt gradient

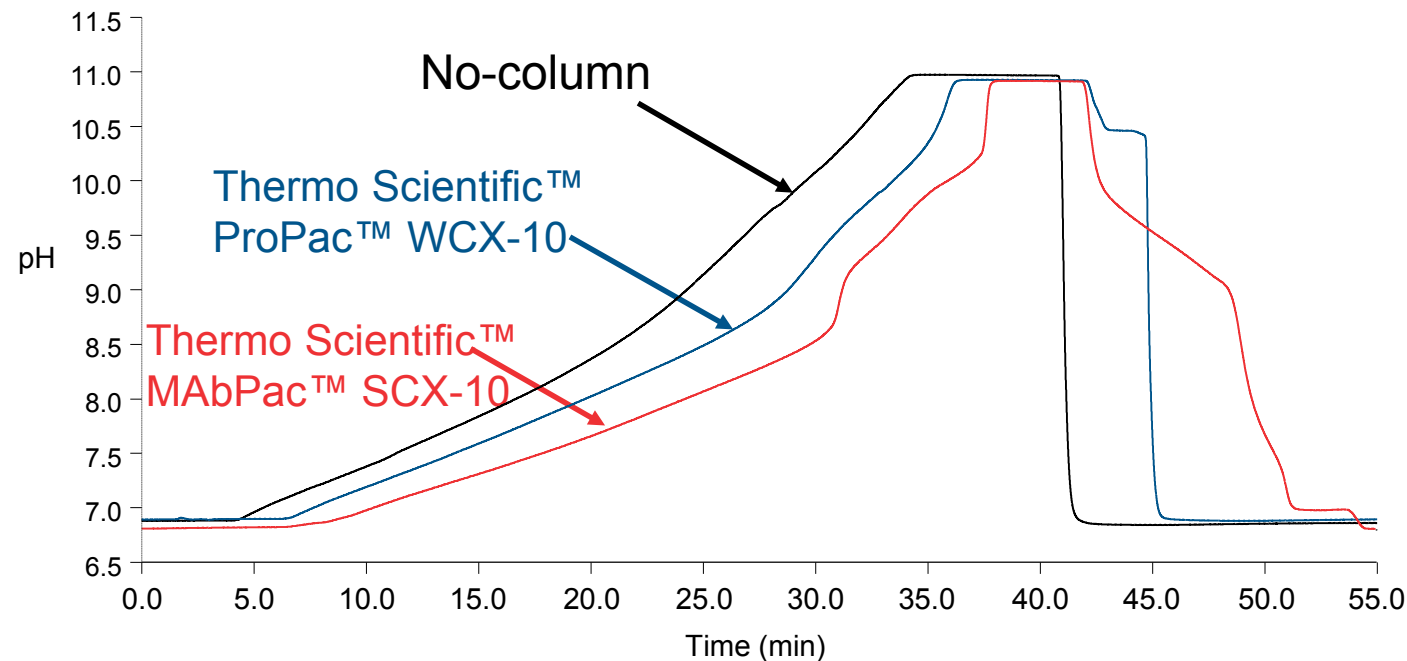
- Weaken ionic interactions between analyte and stationary phase by increasing the salt concentration
- Salt disrupts protein-solid phase interactions
- Most widely used method
- Relatively simple to make the buffer
- Buffer design (pH, salt concentration) can optimize the separation

pH gradient

- Change the pH of the eluent at constant ionic strength
- The change in protein charge disrupts protein-solid phase interactions
- Possible to predict elution profile with pI value
- Lower salt concentration in collected fractions
- Thermo Scientific™ CX-1 pH Gradient Buffers provide a linear pH gradient

Non-linear pH Gradient Method

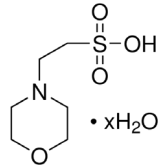
- [Piperazine](#) , pKa 5.33, 9.73 (6 mM): Cation (Binds to stationary phase), bivalent
- [Imidazole](#), pKa 6.95 (11 mM): Hazardous material
- [Tris](#), pKa 8.1 (9.6 mM): Cation (Binds to stationary phase)



Rea, J.C., et al. Validation of a pH gradient-based ion-exchange chromatography Method for high-resolution monoclonal antibody charge variant separations, *J. Pharm. Biomed. Anal.* **2011**, Jan 25;54(2), 317-23.

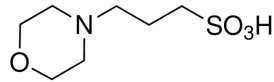
Buffer Development Strategy

- Replace cationic buffer components with zwitterionic buffer species (Good's Buffers)
- These buffer species contain one amine group and one sulfonic acid group. They do not bind to the stationary phase in the pH range of 6-10.
- They are not repelled by the stationary phase so they can buffer the stationary phase.

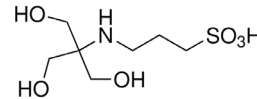


MES
6.1

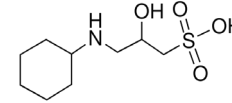
pKa



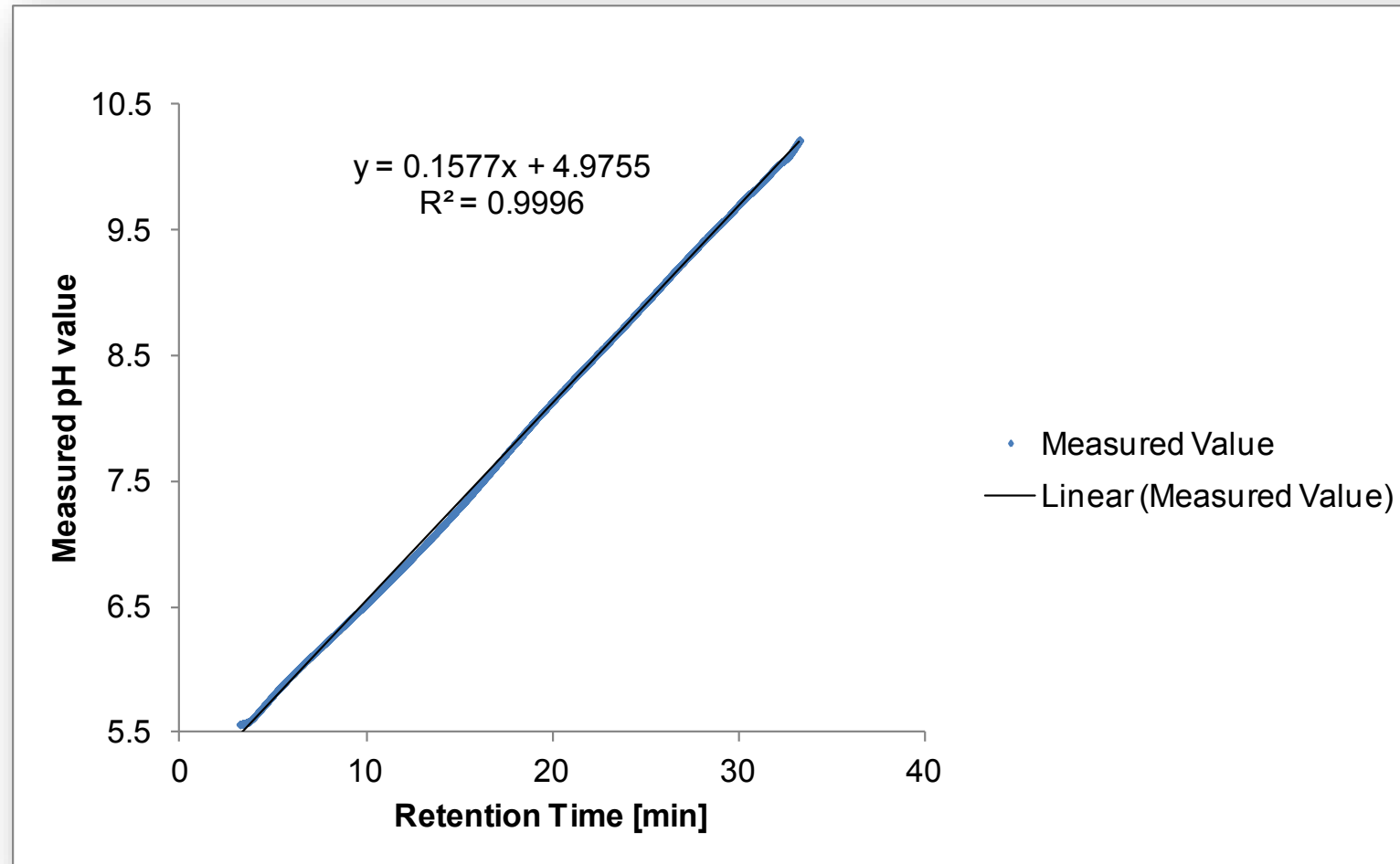
MOPS
7.2



TAPS
8.4

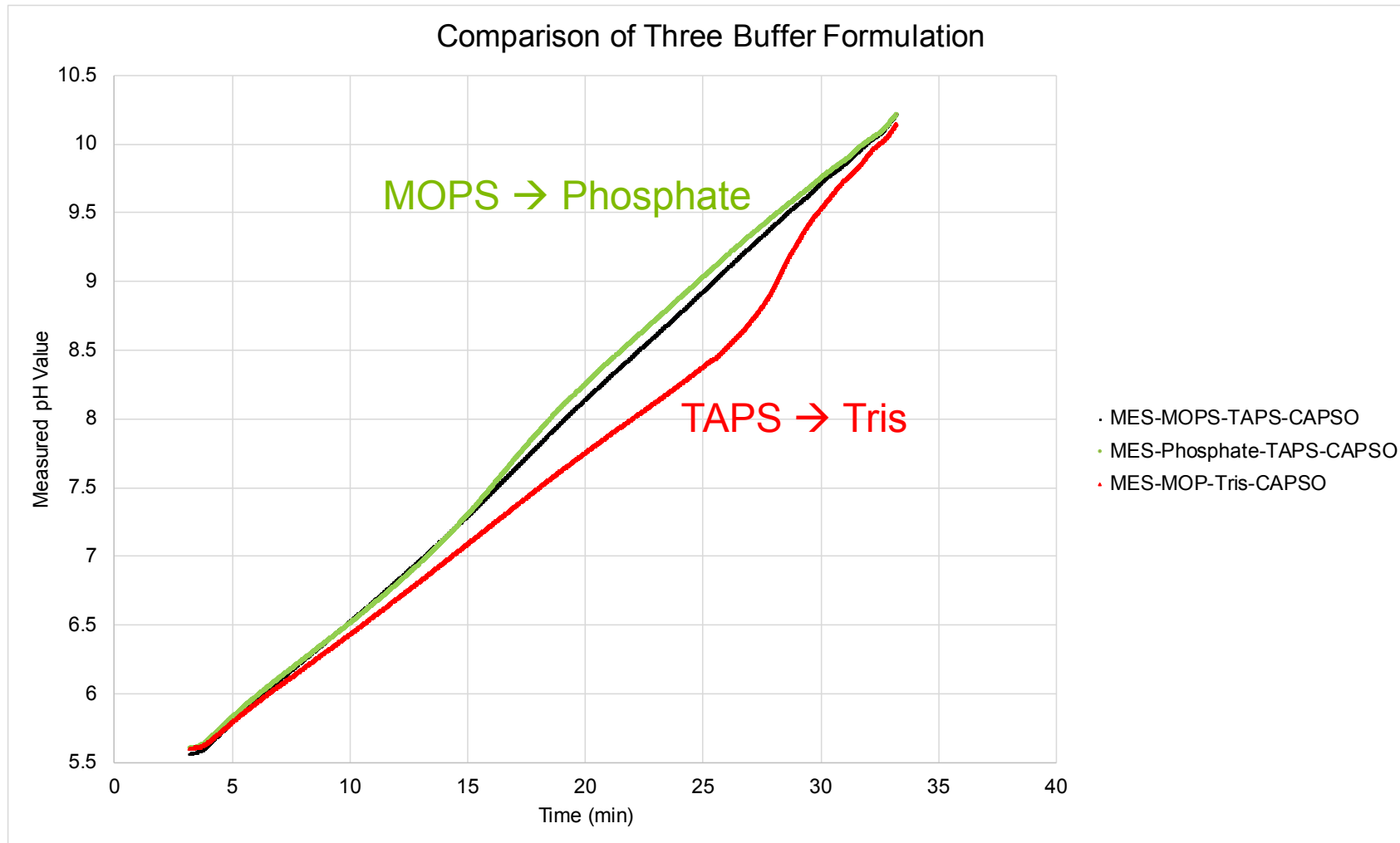


CAPSO
9.6



US8921113 B2: Buffer kit and method of generating a linear pH gradient

Effect of Buffer Salt Repelled or Adsorbed to the Stationary phase



Thermo Scientific™ CX-1 pH Gradient Buffer



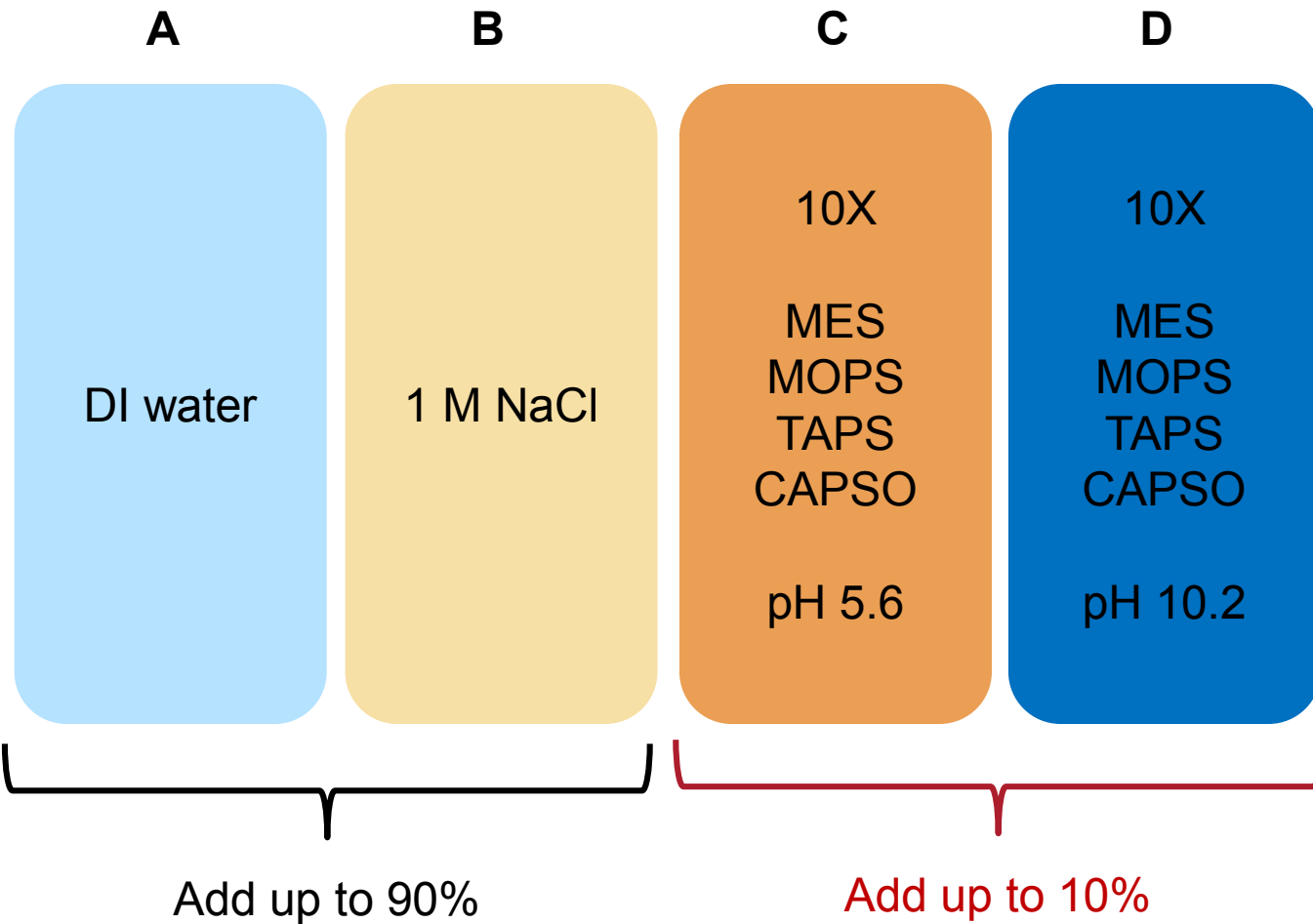
	Buffer A	Buffer B
pH	5.6	10.2
Form	Liquid	Liquid
Concentrate	10X	10X
Shipping	Room temp.	Room temp.
Storage temp.	4 ~ 8 °C	4 ~ 8 °C

pH gradient applications

- Dilute buffers 10-fold with DI water
- A linear pH gradient (pH 5.6 – 10.2) is generated by running a linear pump gradient from 100% Buffer A to 100% Buffer B
- Platform, fast, and high-resolution!

Question: In addition to pH gradient applications, could we perform both CEX and AEX with these buffers, running salt gradient at different pHs?

Universal IEC Platform Set Up: Chromatography Conditions



- Column
 - ProPac WCX-10 for cation exchange
 - ProPac SAX-10 for anion exchange
- Format: 4x150 mm
- Flow rate: 1 mL/min
- Temperature: 30 °C
- **Salt gradient: 0 to 200 mM NaCl (0 to 20%B) gradient in 15 minutes for ProPac WCX-10**
- **pH gradient: 10%C, 0%D (pH 5.6) → 0%C, 10%D (pH 10.2) in 15 minutes**
- **Salt gradient: 20 to 500 mM NaCl (2% to 50%B) gradient in 20 minutes for ProPac SAX-10**

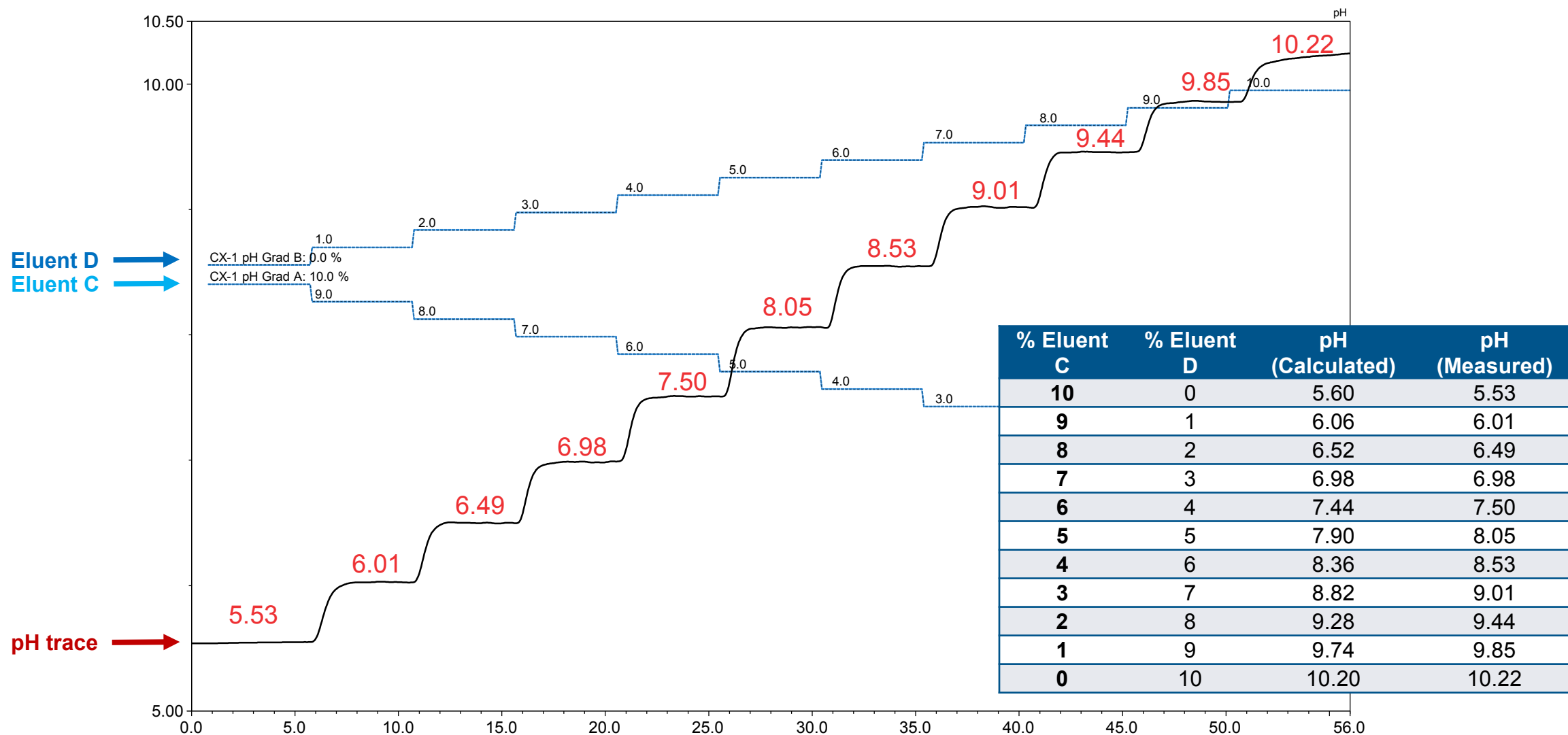
Salt gradient

Program No.	% Eluent C	% Eluent D	Nominal pH
I	10	0	5.60
II	9	1	6.06
III	8	2	6.52
IV	7	3	6.98
V	6	4	7.44
VI	5	5	7.9
VII	4	6	8.36
VIII	3	7	8.82
IX	2	8	9.28
X	1	9	9.74
XI	0	10	10.2

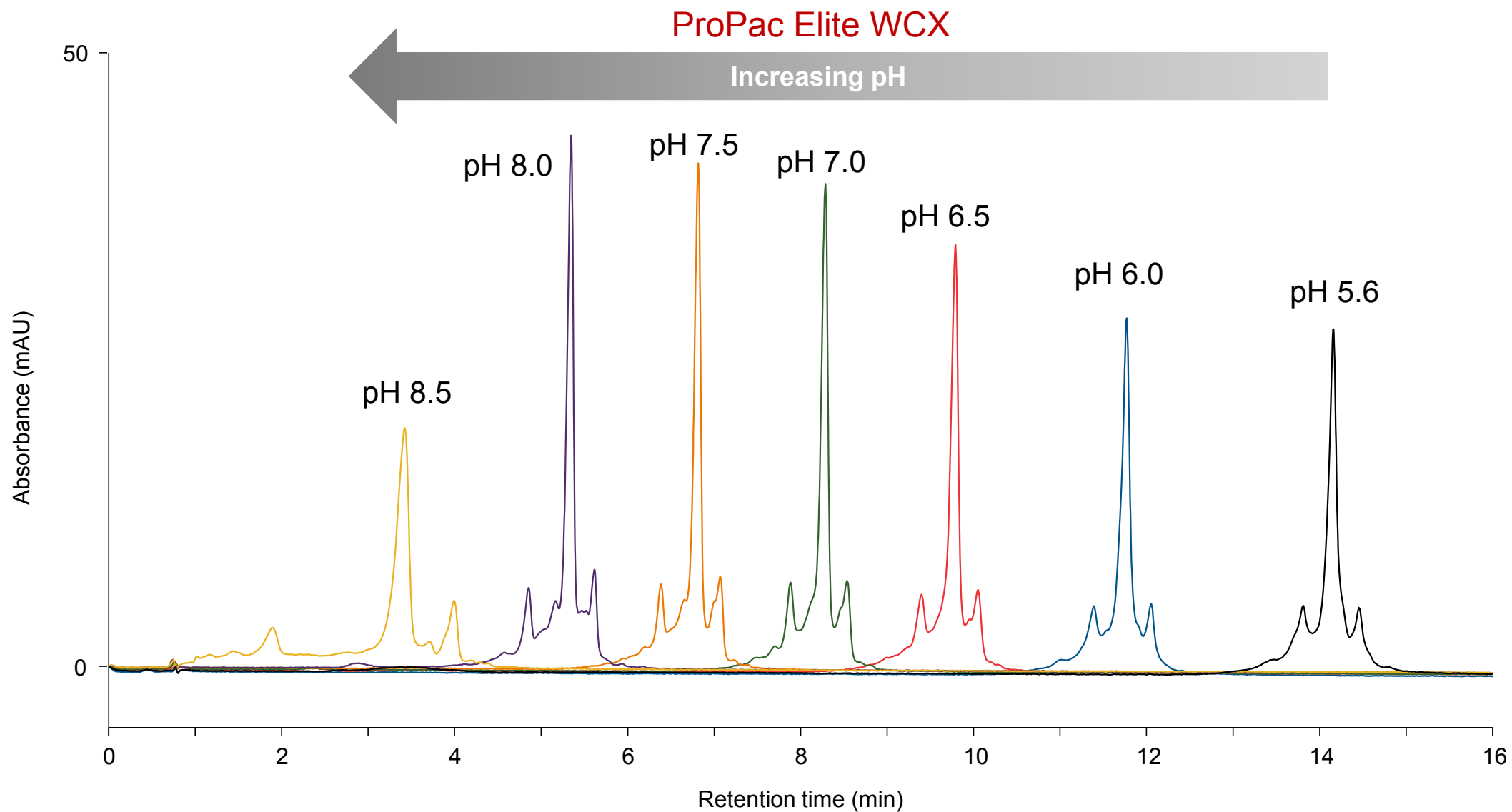
pH gradient

Program No.	% Eluent A	% Eluent B	[NaCl] (mM)
I	89	1	10
II	87	3	30
III	84	6	60
V	81	9	90

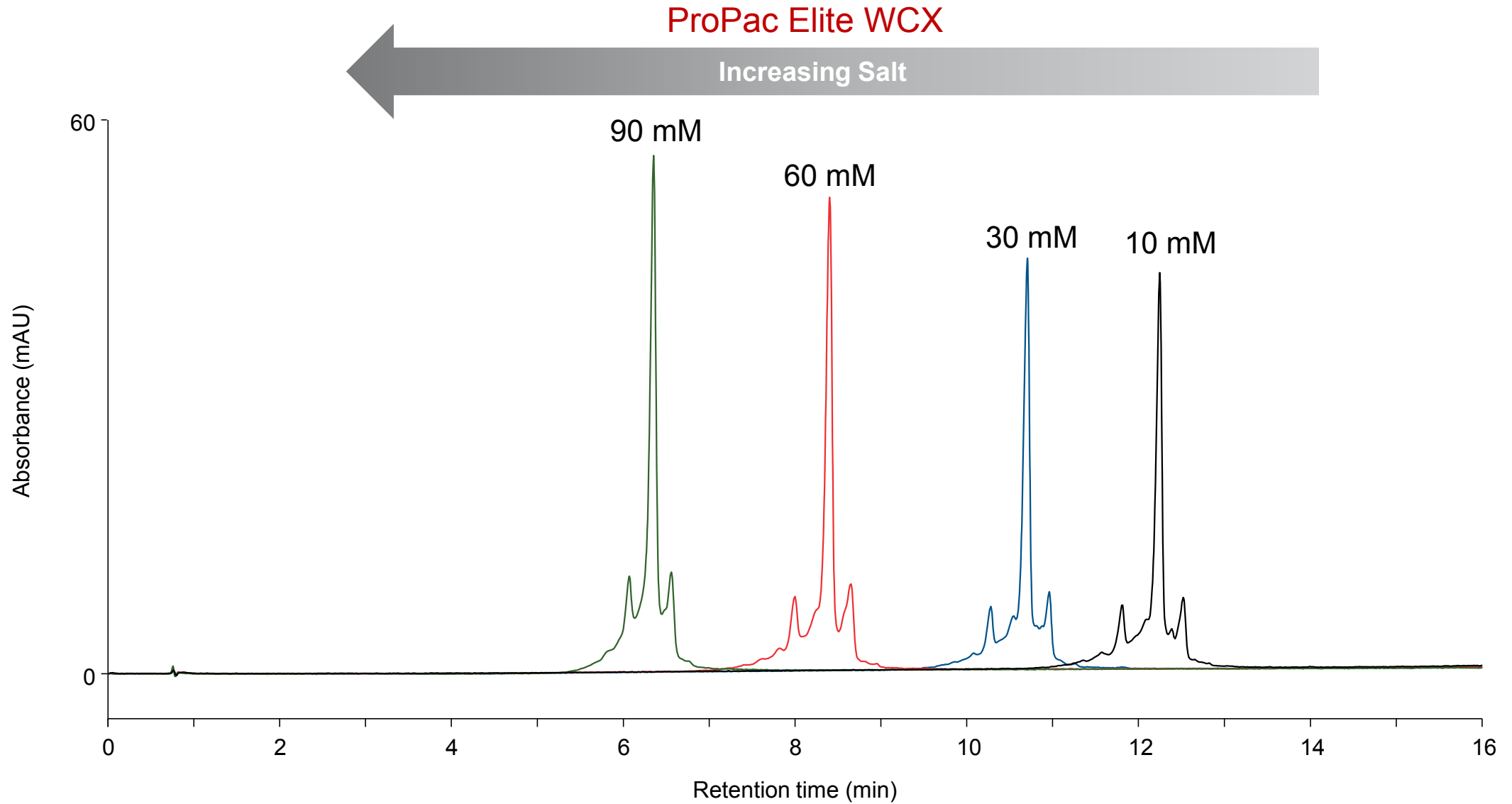
Regulating the Mobile Phase pH by Proportioning Eluents C and D



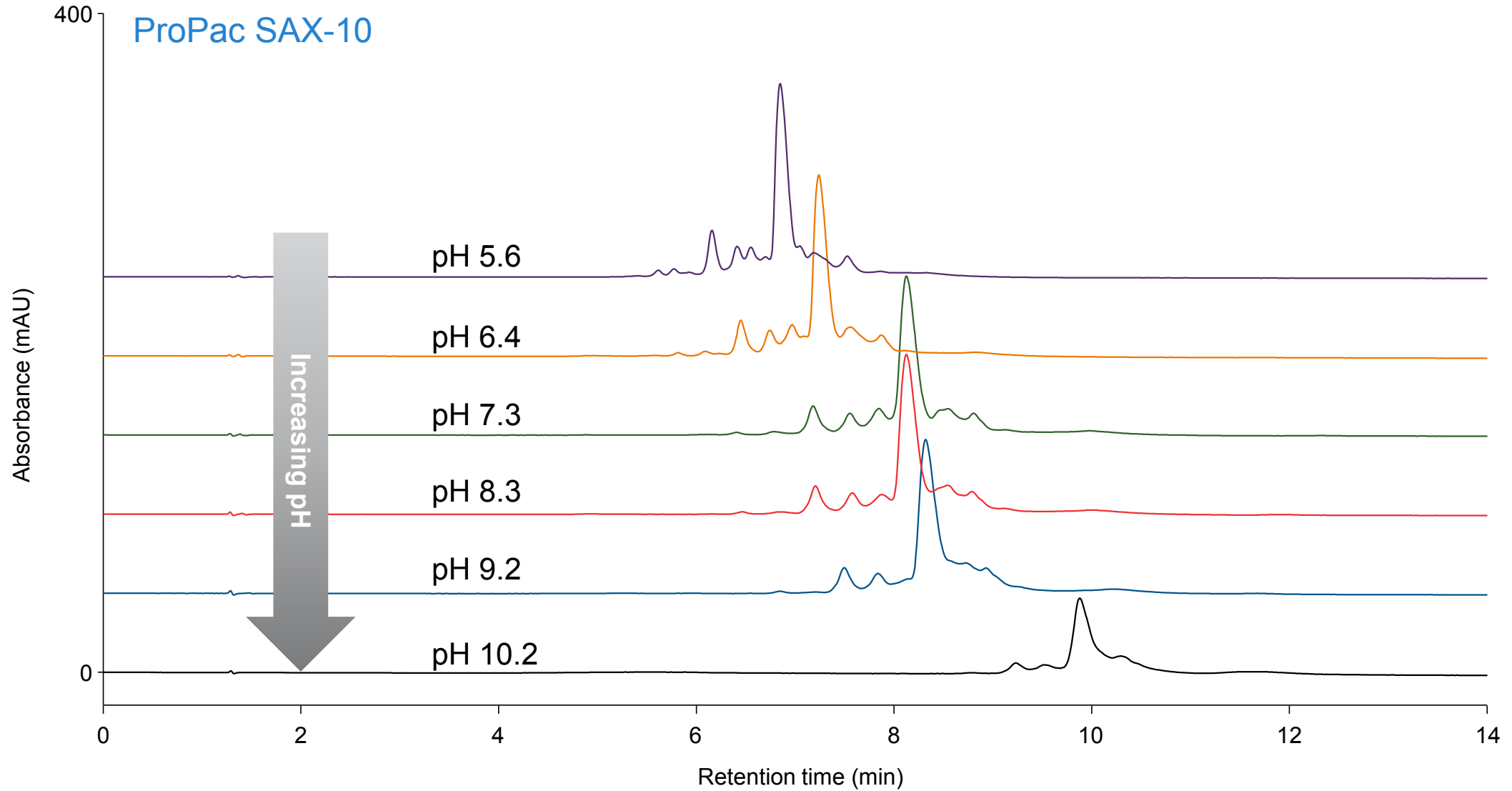
Salt Gradient Separation at Multiple pH Conditions on Cation Exchange Column



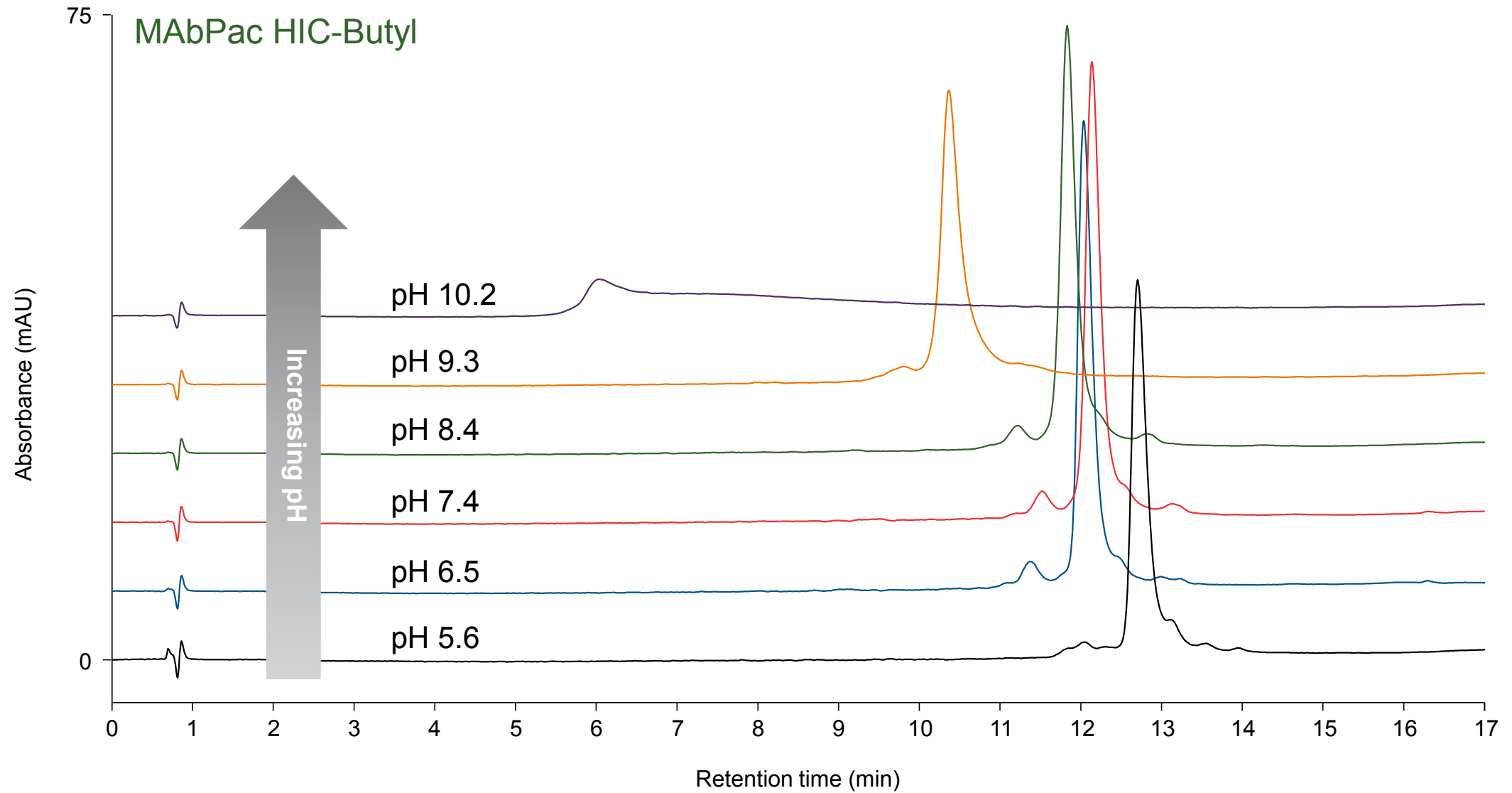
pH Gradient Separation at Multiple Salt Concentration on Cation Exchange Columns



Salt Gradient Separation at Multiple pH conditions on Anion Exchange Column

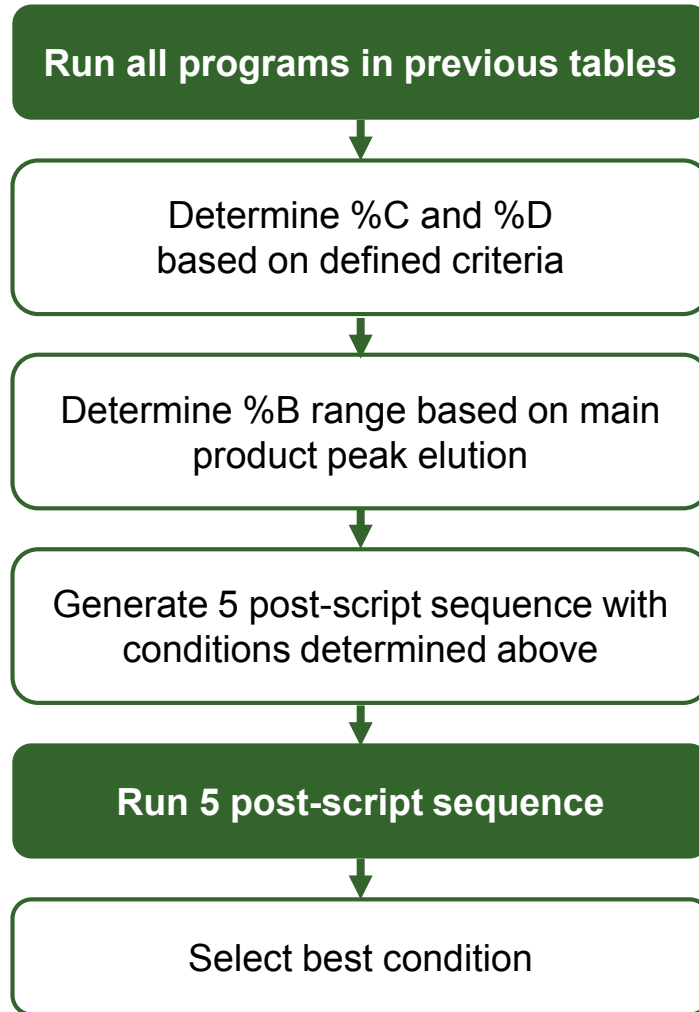


Salt Gradient Separation at Multiple pH conditions on HIC Column



Workflow Automation

Work flow



Condition selection criteria

Number of peaks

Peak capacity

$$n' = 1 + \frac{t_g}{\text{AveragePWHH}_M \times 1.7}$$

Peak to valley ratio

$$\text{Peak to valley (acidic peak)} = \frac{h_{\text{apex}}}{h_{\text{start}}}$$

$$\text{Peak to valley (basic peak)} = \frac{h_{\text{apex}}}{h_{\text{end}}}$$

$$\sqrt{\frac{h_{\text{apex}}}{h_{\text{start}}} \times \frac{h_{\text{apex}}}{h_{\text{end}}}}$$

