

DoE-Assisted Method Development for the Analysis of Monoclonal Antibodies Charge Variants by Cation-Exchange Chromatography

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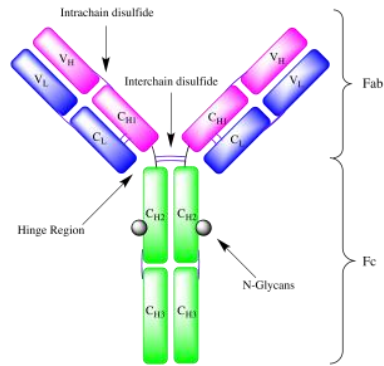
AstraZeneca



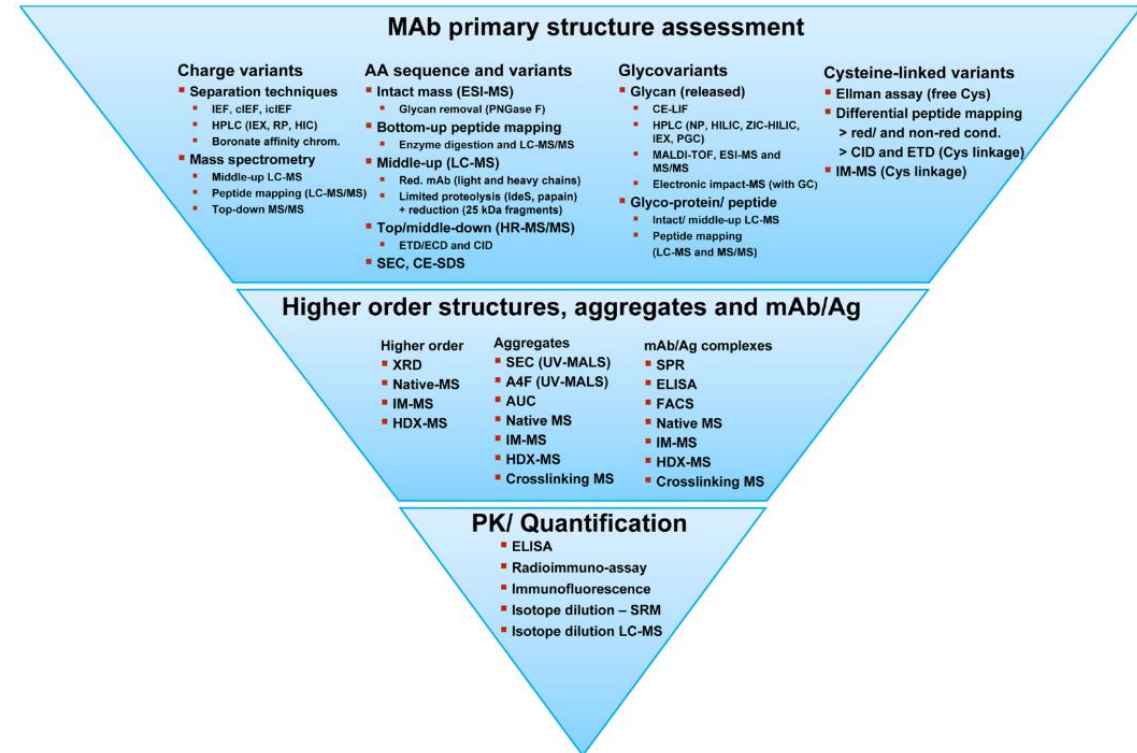
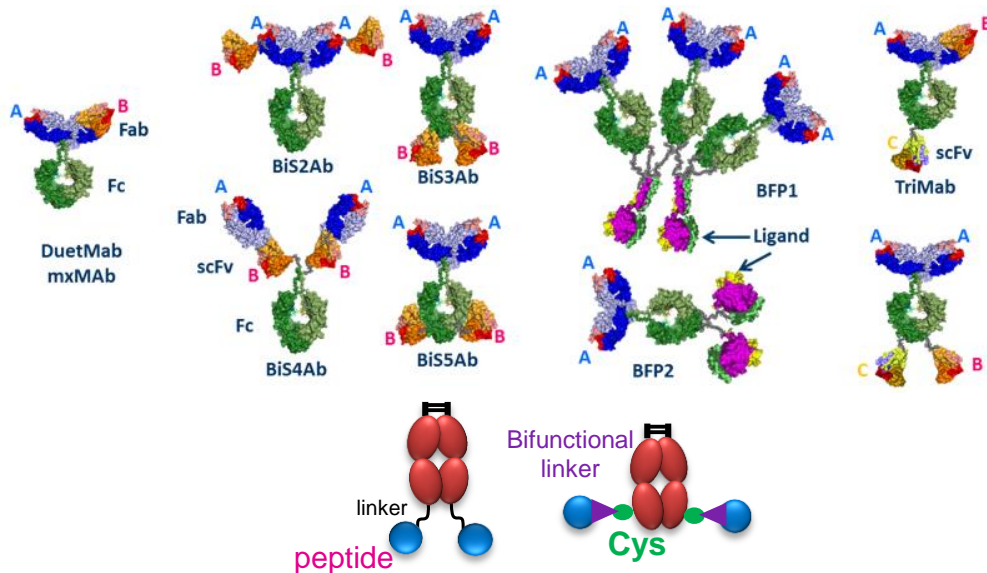
- 1** **Therapeutic protein analysis**
- 2** **Introduction to Design-of-Experiment (DoE)**
- 3** **Application of DoE to analytical method development**
- 4** **Chemometrics tools for data analysis**
- 5** **Case studies and Key considerations**



Analytical characterisation of therapeutic proteins



Monoclonal Antibody (mAb)



The inherent structural complexity of proteins constitutes an analytical challenge. Analytical methods are necessary to support product development, manufacturing and commercialisation.

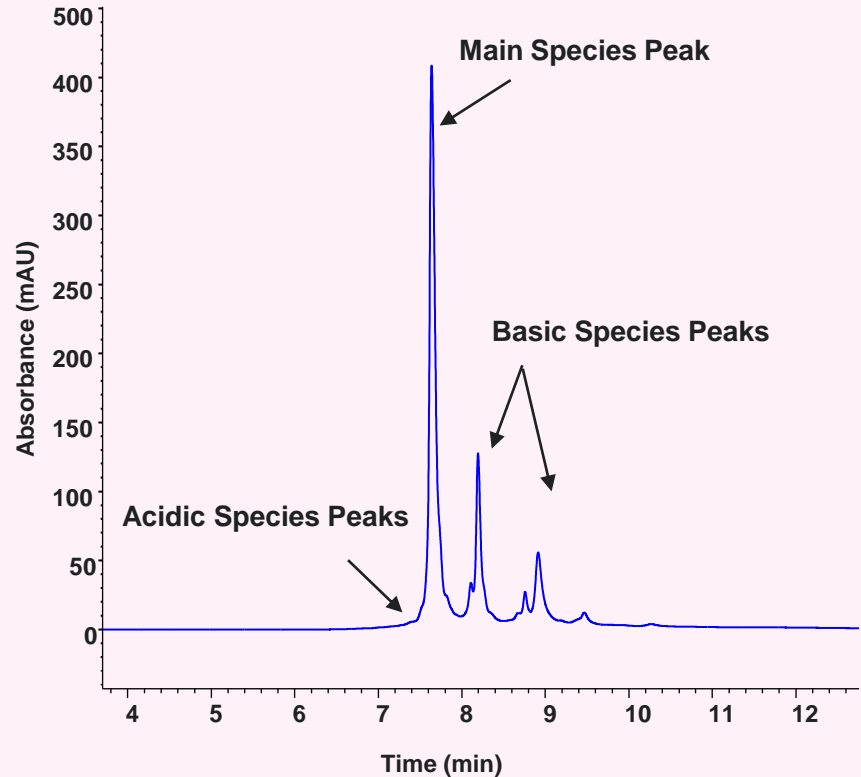


Why DoE?

- **One Factor At a Time (OFAT) approach:**
 - Variation of one parameter at a time maintaining the other constant:
 - Large experimental runs;
 - No information on factors interactions;
 - Lack of information leads to additional experiments during method validation;
 - Lengthy experimentation may retard the overall process pertaining to drug development.
- **DoE:**
 - Variation of multiple parameters at a time:
 - Reduction of experimental runs;
 - Comprehensive investigation of the factors interactions leading to better understanding;
 - Development of mathematical models that permit assessment of relevance and statistical significance facilitating method validation;
 - **Faster, Cheaper and Smarter experiments → Stronger and Better analytical methods**

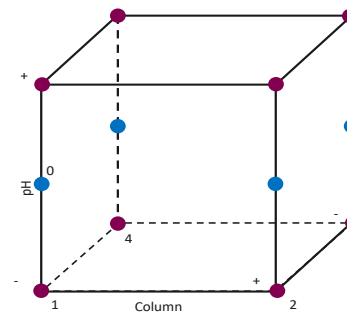


A Split-DoE Approach for Cation Exchange Chromatography Method Development



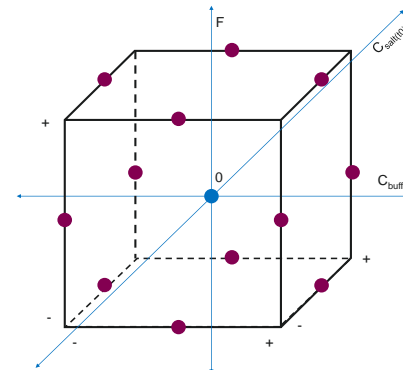
Charge variants separation of a mAb by cation exchange chromatography (CEX)

Main Effects Screening Design



- Column
- Mobile phase pH

Central Composite Design

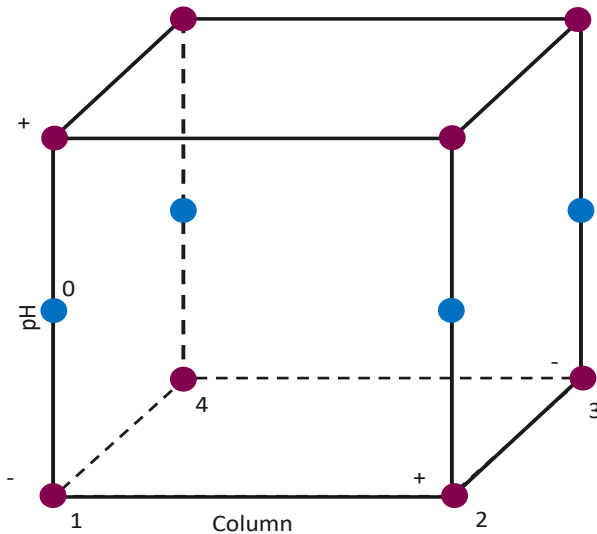


- Flow rate
- Gradient steepness
 - Mobile phase salt concentration at t_0
 - Mobile phase buffer system concentration



A Split-DoE Approach for Cation Exchange Chromatography Method Development

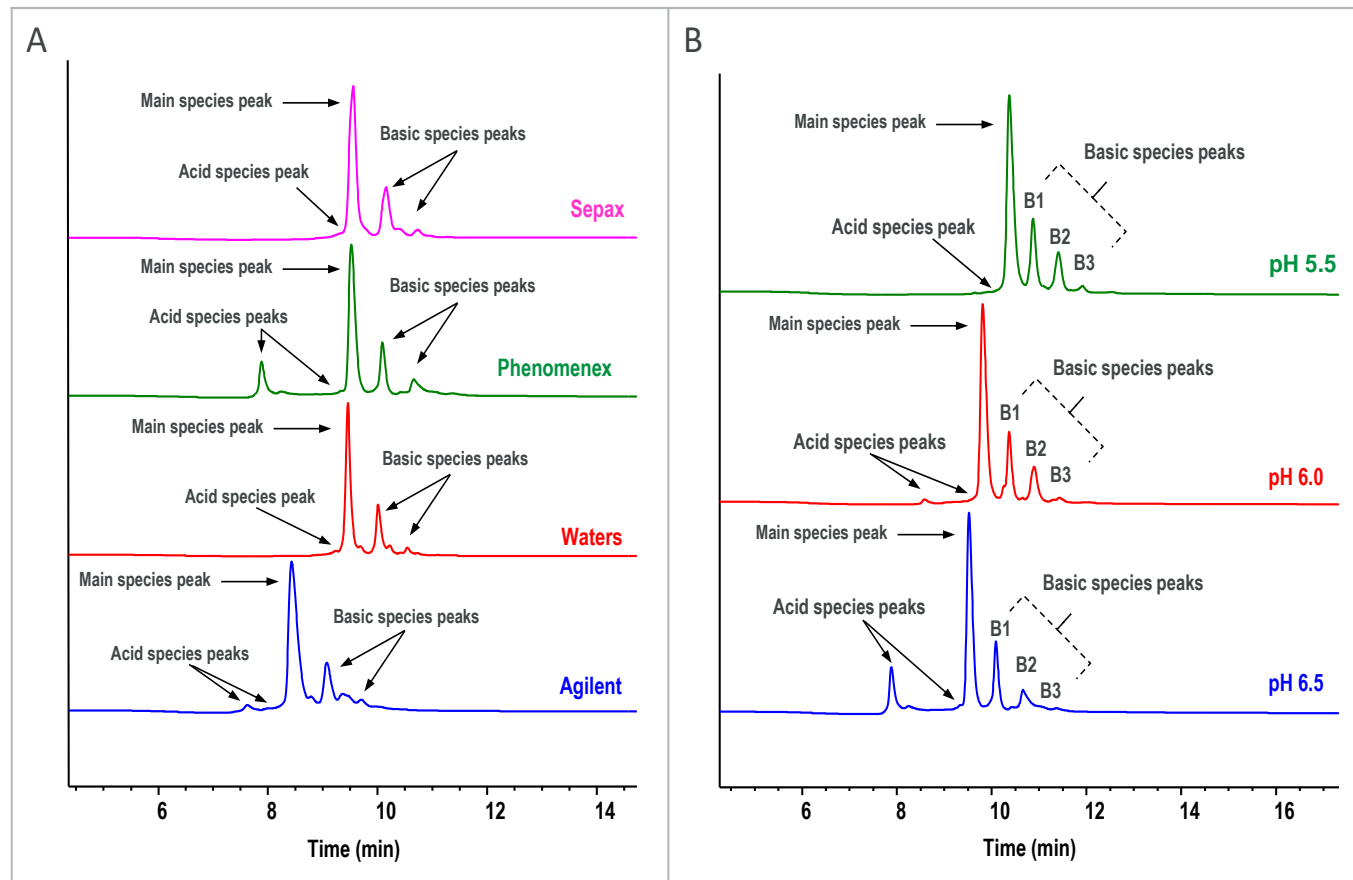
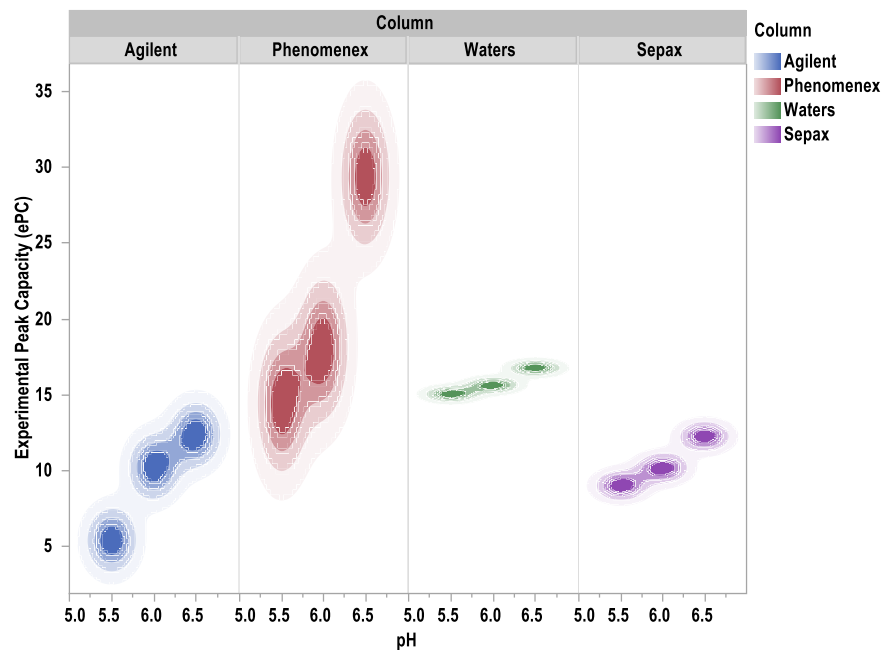
Main Effects Screening Design



Factors
Columns: Agilent, Sepax, Phenomenex, Waters
pH (5.5, 6.0, 6.5)
Response
Experimental Peak Capacity (ePC)
Constant
C_{buffer} : 20 mM sodium phosphate buffer
$C_{\text{salt}(t_0)}$: 40 mM sodium chloride
Flow rate: 0.17 mL/min
gt: 15 min
g_{shape} : linear, 40-500mM sodium chloride
Temperature: 25 °C
Injection Volume: 5 μ L
Sample concentration: 1 mg/mL
UV: 210 nm, 280 nm

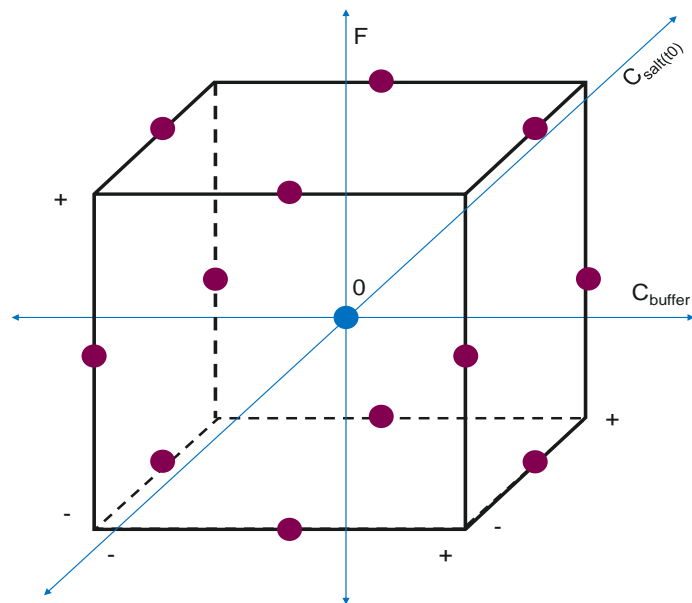


A Split-DoE Approach for Cation Exchange Chromatography Method Development

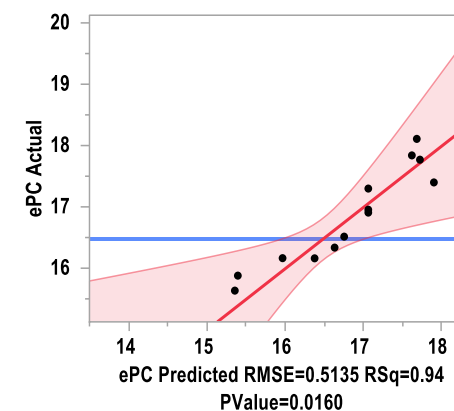
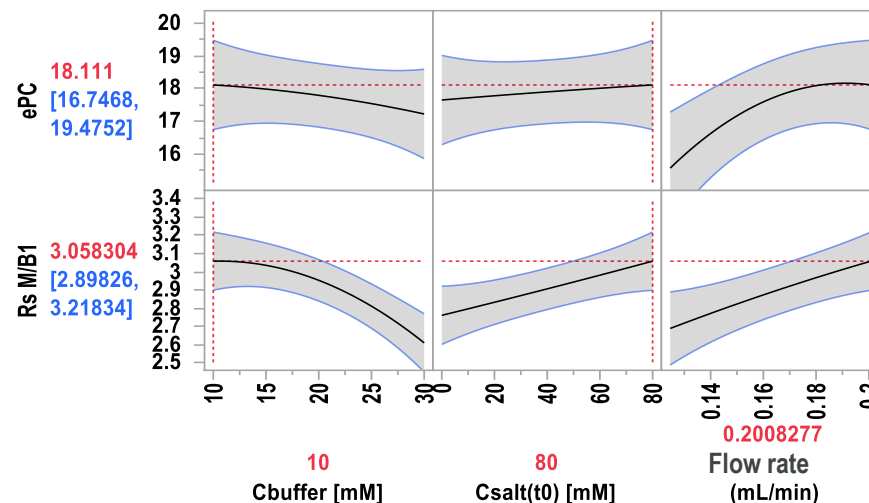


A Split-DoE Approach for Cation Exchange Chromatography Method Development

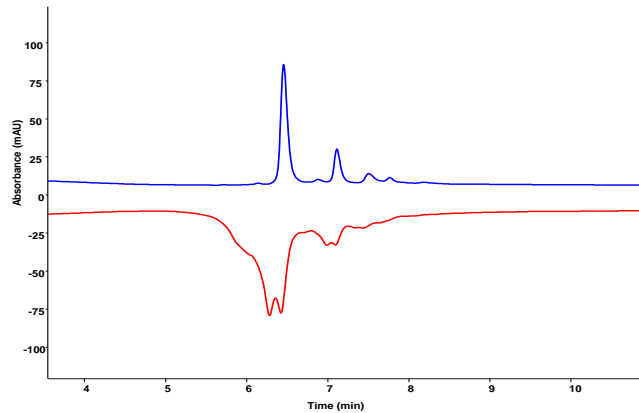
Central Composite Design



HT CEX Method
Column: Phenomenex: bioZen WCX 2.1 x 50 mm, 6 um, np
Mobile phase: A) 10 mM Na3PO4, 80 mM sodium chloride; B) 10 mM Na3PO4, 80 mM -1 M sodium chloride
Gradient: 8-40%B in 0-10 min linear ramp
gt: 10 min
F: 0.2 mL/min
Temperature: 25 °C
Injection Volume: 5 µL
Sample concentration: 1 mg/mL
UV: 210 nm, 280 nm



A Split-DoE Approach for Cation Exchange Chromatography Method Development



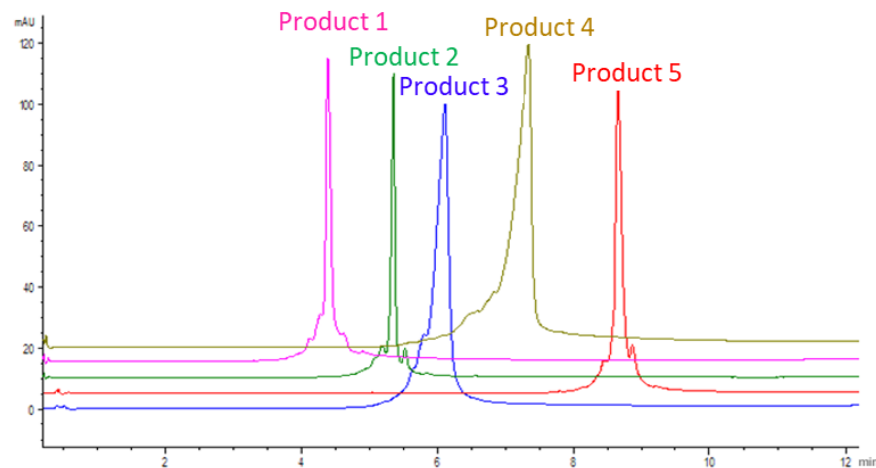
4 columns

mobile phase

pH range

Flow rate

27 experimental runs



Platform workflow to
analytical method
development

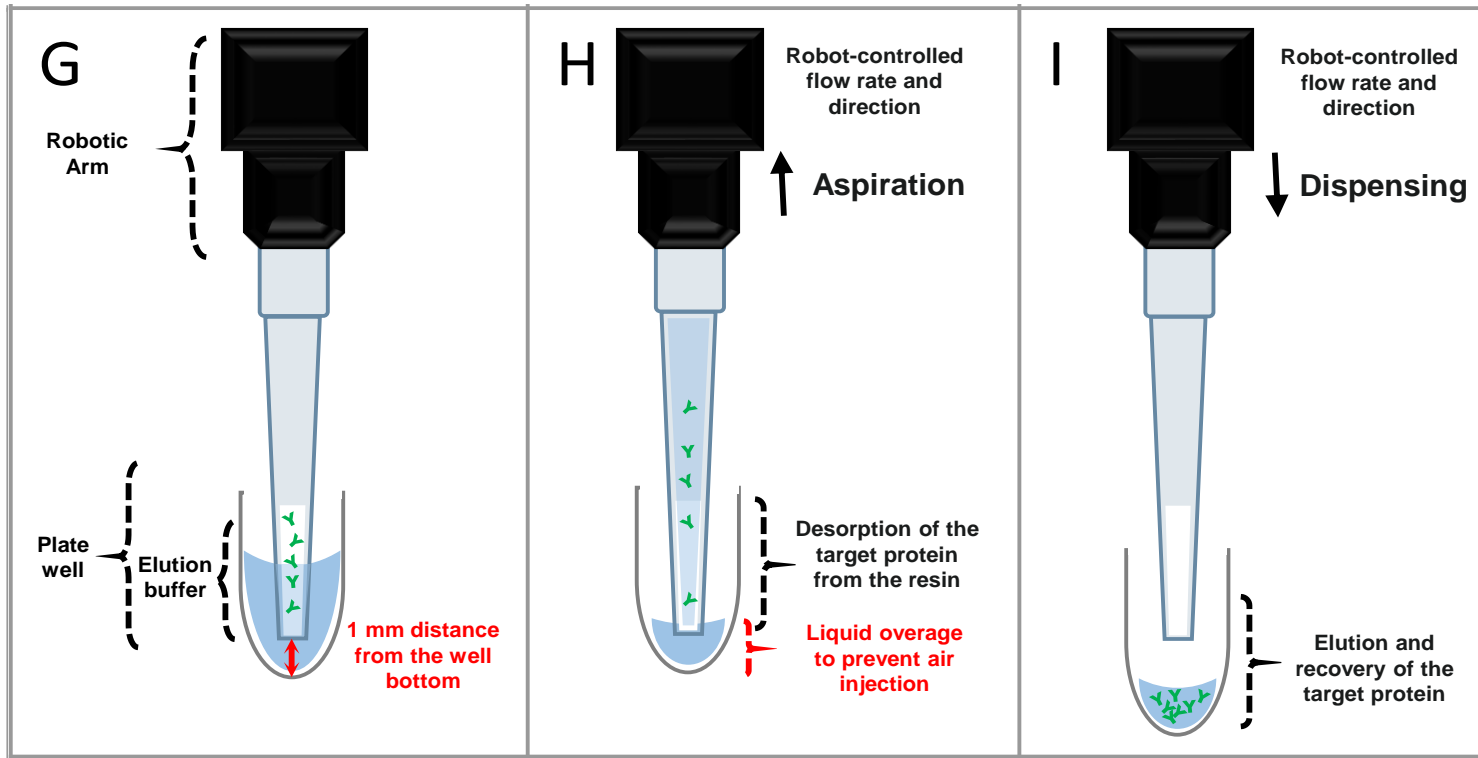
Data generated by Davide Di Girolamo and Ryte Poskute



Microscale Chromatographic Purification of mAbs and BsAbs

DoE-assisted method development

mAbs
BsAbs



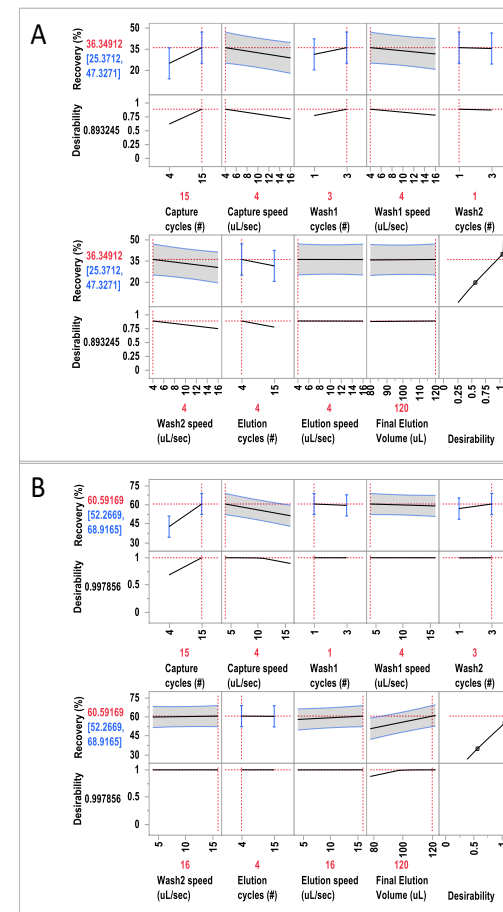
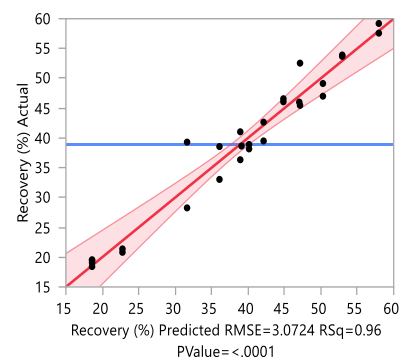
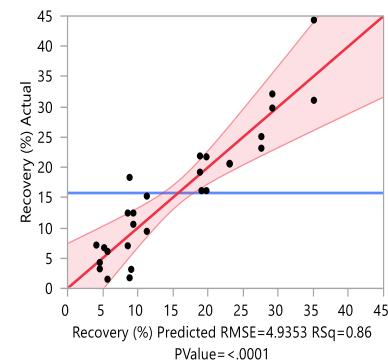
MESD Factors
Capture cycles (4 – 15)
Capture Flow rate (4 – 16 $\mu\text{L}/\text{sec}$)
Wash 1 cycles (1 – 3)
Wash 1 Flow rate (4 – 16 $\mu\text{L}/\text{sec}$)
Wash 2 cycles (1 – 3)
Wash 2 Flow rate (4 – 16 $\mu\text{L}/\text{sec}$)
Elution cycles (4 – 15)
Elution Flow rate (4 – 16 $\mu\text{L}/\text{sec}$)
Final Elution Volume (80 – 120 μL)
MESD Response
%Recovery



Microscale Chromatographic Purification of mAbs and BsAbs

DoE-assisted method development: Main Effect Screening Design

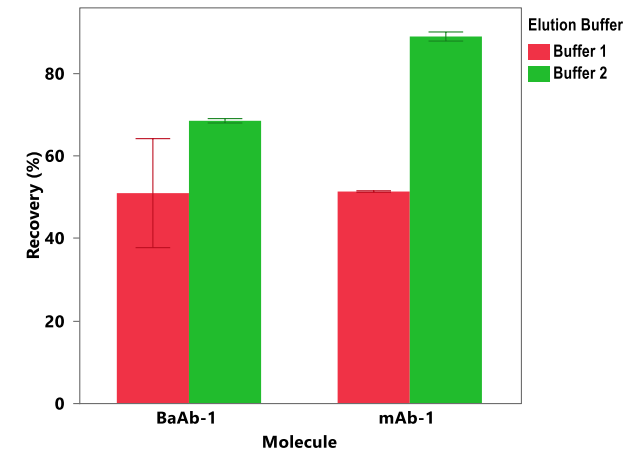
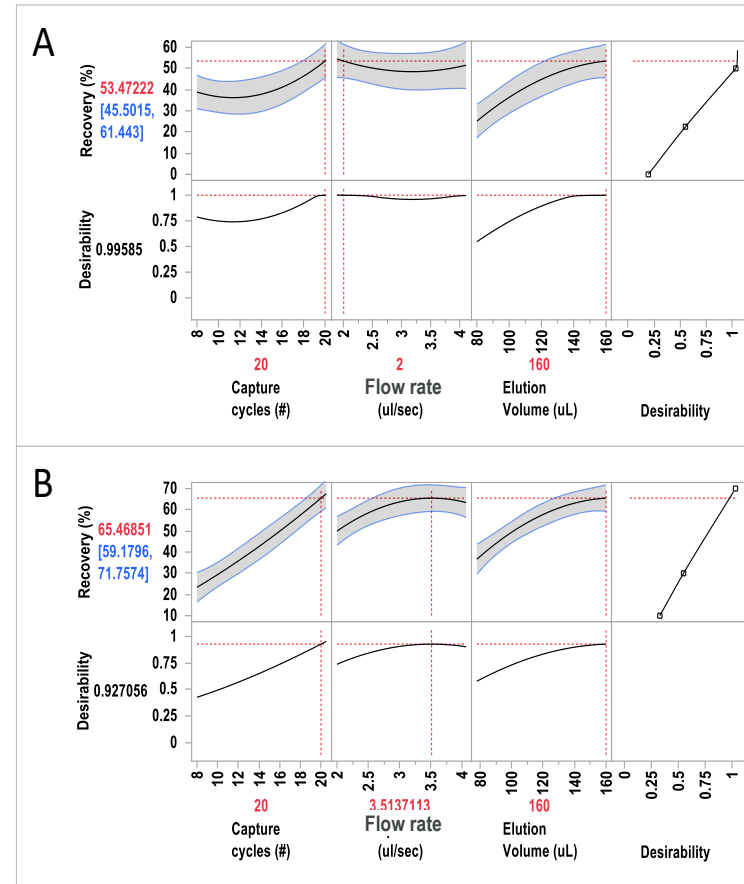
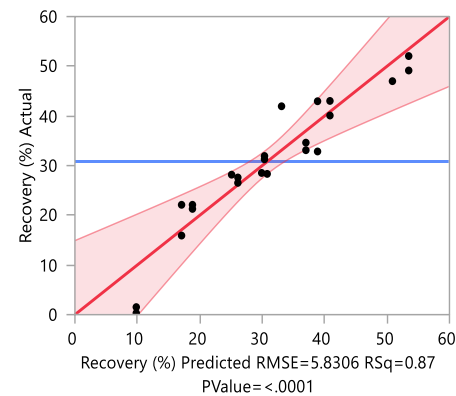
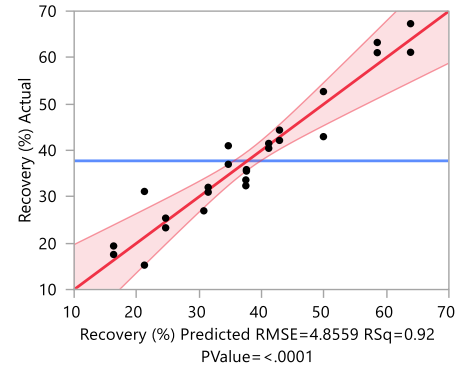
mAb-1 Parameter Estimates				
Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	16.89	1.67	10.09	<.0001*
Capture cycles (4,15)	5.56	1.67	3.32	0.0031*
Capture Flow rate (μL/sec)(4,16)	-3.63	1.67	-2.17	0.0410*
Wash1 cycles (1,3)	2.37	1.67	1.42	0.1701
Wash1 Flow rate (μL/sec)(4,16)	-2.24	1.67	-1.34	0.1949
Wash2 cycles (1,3)	-0.31	1.67	-0.18	0.8556
Wash2 (μL/sec)(4,16)	-2.84	1.67	-1.70	0.1037
Elution cycles (4,15)	-2.28	1.67	-1.36	0.1877
Elution Flow rate (μL/sec)(4,16)	-0.04	1.67	-0.02	0.9816
Final Elution Volume (μL)(80,120)	0.19	1.67	0.11	0.9115
BsAb-1 Parameter Estimates				
Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	37.54	1.27	29.57	<.0001*
Capture cycles (4,15)	8.90	1.27	7.01	<.0001*
Capture Flow rate (μL/sec)(4,16)	-4.62	1.27	-3.64	0.0014*
Wash1 cycles (1,3)	-0.54	1.27	-0.42	0.6750
Wash1 Flow rate (μL/sec)(4,16)	-0.76	1.27	-0.60	0.5574
Wash2 cycles (1,3)	1.79	1.27	1.41	0.1720
Wash2 Flow rate (μL/sec)(4,16)	0.36	1.27	0.29	0.7773
Elution cycles (4,15)	-0.09	1.27	-0.07	0.9465
Elution Flow rate (μL/sec)(4,16)	1.31	1.27	1.03	0.3124
Final Elution Volume (μL)(80,120)	4.68	1.27	3.69	0.0013*



Microscale Chromatographic Purification of mAbs and BsAbs

DoE-assisted method development: Central Composite Design

CCD Factors
Capture cycles (8 – 20)
Capture F (2 – 4 $\mu\text{L}/\text{sec}$)
Final Elution Volume (80 – 160 μL)
CCD Response
%Recovery
Constant parameters
Wash1 cycles (1) F (4 $\mu\text{L}/\text{sec}$)
Wash1 F (4 $\mu\text{L}/\text{sec}$)
Wash2 cycles (1), F (4 $\mu\text{L}/\text{sec}$)
Wash2 F (4 $\mu\text{L}/\text{sec}$)
Wash2 cycles (1)
Wash2 F (4 $\mu\text{L}/\text{sec}$)
Elution cycles (4)
Elution F 4 $\mu\text{L}/\text{sec}$



Microscale Chromatographic Purification *Method Assessment*

DoE approach

Main Effect Screening Design

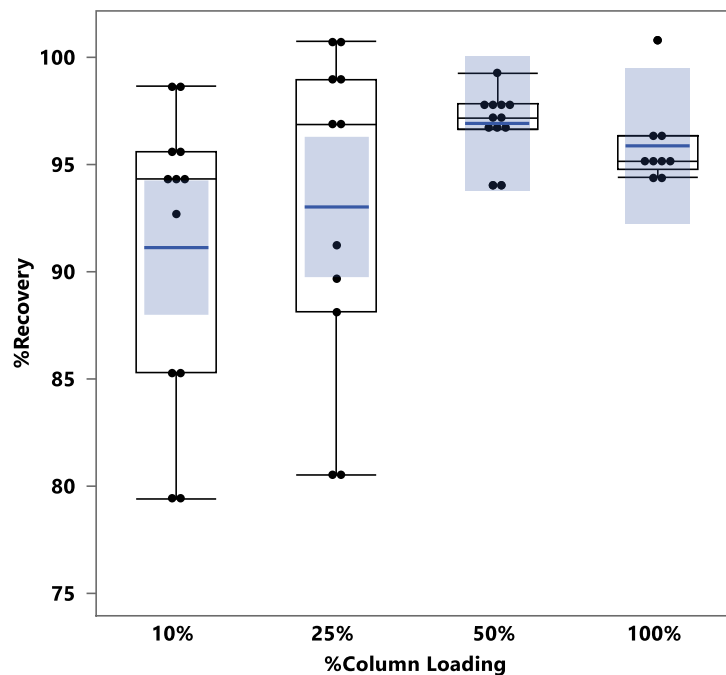
12 Treatments

Central Composite Design

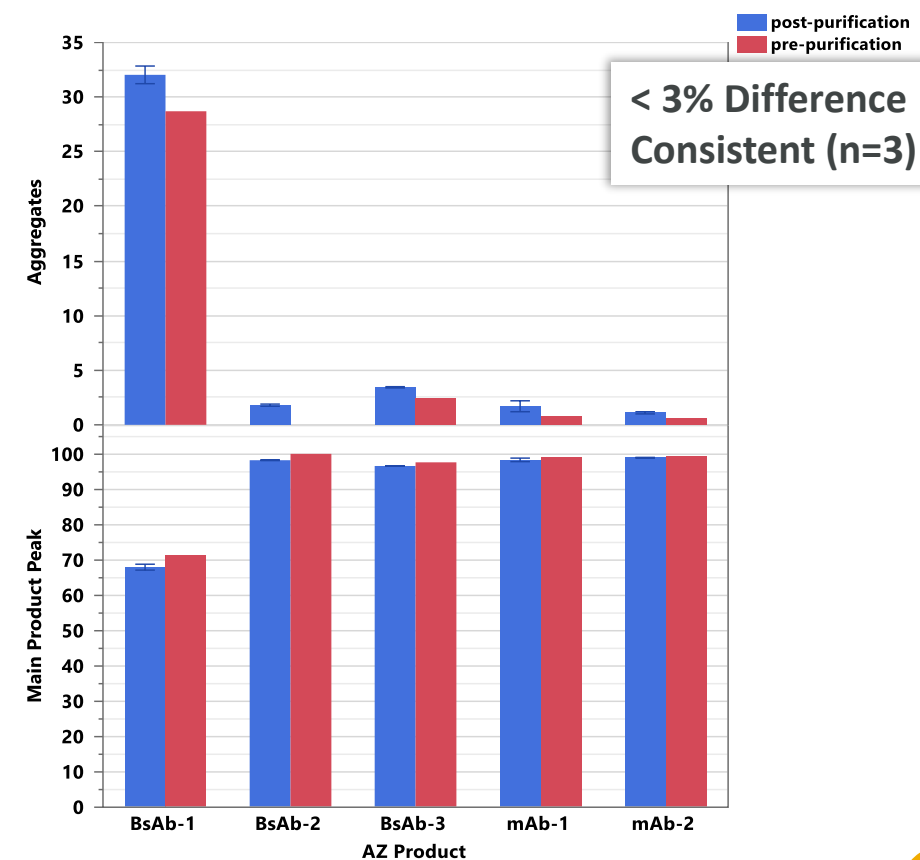
12 Treatments

24 Treatments
n=2
(2-days in the lab!)

Recovery evaluation



Product Quality Assessment



Key Considerations:

DoE-assisted method development followed by appropriate statistical analysis enabled:

- Experimental **planning based on the** time, costs and other analytical **resources available**.
- Scheduling and execution of experiments with adequate sample size and type of data to **extrapolate maximum information** from chemical data and **efficiently address the challenges and goals of the intended research**.
- Save time and costs for the experiments execution required by the standard OFAT (one-factor-at-a-time) approach.
- **Deconvolutes the complexity of analytical method development** by interrogating several factors at a time and studying the effect of both individual method parameters and their interactions on the dependent variable(s).



4


Future Work: Export files from Database and Analyse in JMP

- Expand data analytics capabilities and data automation
 - *Data Visualisation*
 - *Statistical Analysis*
 - *Multivariate Methods*
 - *Modeling*



Automated data curation and modeling

DATABASE



JMP Home Window - JMP Pro

File Tables DOE Analyze Graph Tools **Add-Ins** View Window Help

- 2AB GlycoRawPlot
- x-y plot
- AZD_PlotDLIMS
- PlotDLIMS

Recent Files

Filter (Ctrl+F)

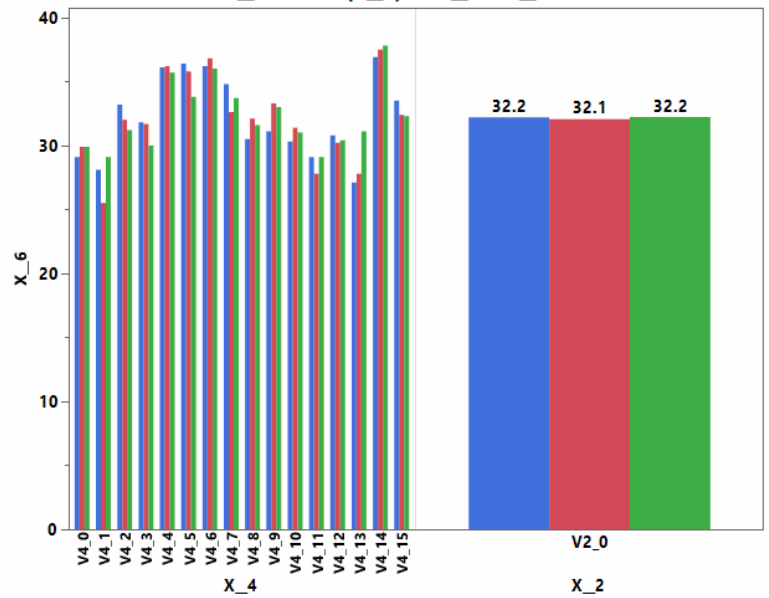
20220218_validation_final_method_with_neutralization

Column Switcher

14 Columns

- X_6
- X_7
- X_8
- X_10
- X_11
- X_12
- X_13
- X_14
- X_15
- X_16
- X_17
- X_18
- X_20
- X_21

X_6 & Mean(X_6) vs. X_4 & X_2

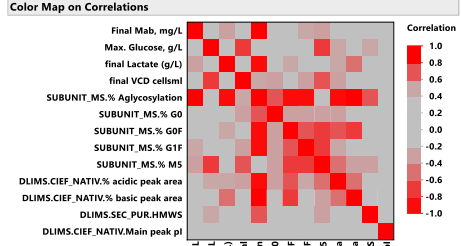


X_5

- V5.0
- V5.1
- V5.2

Color Map on Correlations

Correlation



Color Map on p-Values

Probability

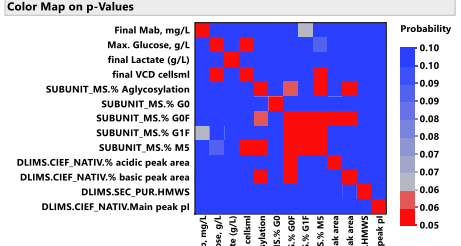


Table of Data

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_10	X_11	X_12	X_13	X_14	X_15	X_16	X_17	X_18	X_20	X_21	X_23	
% CIEF NATIV	1000401	V2.0	V5.0	V4.0	V5.0	28.1	11.2	1.0	0	1.7	1.6	1.7	1.6	1.7	1.6	1.7	1.6	1.7	1.6	1.7	1.6	1.7
% EarlyEtd_PJC_BIOWS	1000402	V2.0	V5.1	V4.1	V5.0	28.1	12.3	7.8	0	1.7	1.6	1.7	1.6	1.7	1.6	1.7	1.6	1.7	1.6	1.7	1.6	1.7
% LateEtd_PJC_BIOWS	1000403	V2.0	V5.2	V4.2	V5.0	32.2	11.1	7.5	0	2	1.4	1.2	1.4	1.2	1.4	1.2	1.4	1.2	1.4	1.2	1.4	1.2
% NonEtd_PJC_N_BIOWS	1000404	V2.0	V5.0	V4.3	V5.0	31.8	10.1	7.8	0	2	1.5	1.2	1.5	1.2	1.5	1.2	1.5	1.2	1.5	1.2	1.5	1.2
% LAMR_PJC_N_BIOWS	1000405	V2.0	V5.4	V4.4	V5.0	36.1	10.8	6.8	0	1.8	1.3	1.2	1.3	1.2	1.4	1.1	1.3	1.2	1.4	1.1	1.3	1.2
% NonEtd_PJC_Analytic	1000406	V2.0	V5.3	V4.5	V5.0	34.4	10.9	5.8	0	1.9	1.5	1.4	1.5	1.4	1.6	1.3	1.5	1.4	1.6	1.3	1.5	1.2
% Main_PJC_BIOWS	1000407	V2.0	V5.6	V4.6	V5.0	36.2	9.7	5.3	0	1.9	1.6	1.7	1.6	1.7	1.6	1.7	1.6	1.7	1.6	1.7	1.6	1.7
% Main_PJC_Analytic	1000408	V2.0	V5.7	V4.7	V5.0	36.8	12	7.5	0	2	1.4	1.1	1.2	1.1	1.3	1.0	1.2	1.1	1.3	1.0	1.2	1.1
% NonEtd_PJC_BIOWS	1000409	V2.0	V5.8	V4.8	V5.0	36.2	10.6	8.1	0	1.7	1.4	1.4	1.4	1.3	1.5	1.2	1.4	1.3	1.5	1.2	1.4	1.1
% Main_PJC_BIOWS	1000410	V2.0	V5.9	V4.9	V5.0	31.1	11.6	6.2	0	1.7	1.8	1.4	1.7	1.8	1.4	1.7	1.8	1.4	1.7	1.8	1.4	1.1
% Main_PJC_Analytic	1000411	V2.0	V5.10	V4.10	V5.0	36.3	10.5	7.7	0	2	1.5	1.2	1.5	1.2	1.5	1.2	1.5	1.2	1.5	1.2	1.5	1.2
% Other_CIEF_N_BIOWS	1000412	V2.0	V5.11	V4.11	V5.0	28.1	11.5	7.7	0	1.7	1.6	1.4	1.6	1.4	1.6	1.4	1.6	1.4	1.6	1.4	1.6	1.3
% Purified_CIEF_N_BIOWS	1000413	V2.0	V5.12	V4.12	V5.0	36.2	10.6	6.8	0	1.9	1.4	1.3	1.4	1.3	1.5	1.2	1.4	1.3	1.5	1.2	1.4	1.1
% Other_CIEF_N_BIOWS	1000414	V2.0	V5.13	V4.13	V5.0	27.1	12.2	8.3	0	1.5	1.5	1.4	1.5	1.4	1.6	1.3	1.5	1.4	1.6	1.3	1.5	1.2
% Purified_CIEF_N_BIOWS	15	1000415	V2.0	V5.14	V4.14	36.9	10.5	6.8	0	1.6	1.2	1.2	1.2	1.1	1.3	1.0	1.2	1.1	1.3	1.0	1.2	1.1
% Other_CIEF_N_BIOWS	16	1000416	V2.0	V5.15	V4.15	33.5	10.9	7.8	0	2.5	1.3	1.2	1.4	1.1	1.4	1.1	1.3	1.2	1.4	1.1	1.3	1.2
% Purified_CIEF_N_BIOWS	17	1000417	V2.0	V5.16	V4.16	28.9	11.3	7.7	0	2	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
% Other_CIEF_N_BIOWS	18	1000418	V2.0	V5.17	V4.17	25.3	12.1	1.7	0	2.4	1.2	1.1	1.2	1.1	1.3	1.0	1.2	1.1	1.3	1.0	1.2	1.1
% Purified_CIEF_N_BIOWS	19	1000419	V2.0	V5.18	V4.18	32	11.2	7.5	0	2.6	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
% Other_CIEF_N_BIOWS	20	1000420	V2.0	V5.19	V4.19	31.7	10	7.6	0	2.4	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
% Purified_CIEF_N_BIOWS	21	1000421	V2.0	V5.20	V4.20	36.2	11.3	6.9	0	2.4	1	1	1	1	1	1	1	1	1	1	1	1
% Other_CIEF_N_BIOWS	22	1000422	V2.0	V5.21	V4.21	35.8	10.9	6	0	1.9	1.3	1.2	1.3	1.2	1.4	1.1	1.3	1.2	1.4	1.1	1.3	1.2
% Purified_CIEF_N_BIOWS	23	1000423	V2.0	V5.22	V4.22	36.8	10	5.4	0	1.8	1.3	1.2	1.3	1.2	1.4	1.1	1.3	1.2	1.4	1.1	1.3	1.2
% Other_CIEF_N_BIOWS	24	1000424	V2.0	V5.23	V4.23	32.6	12	7.6	0	2.3	1	1	1	1	1	1	1	1	1	1	1	1
% Purified_CIEF_N_BIOWS	25	1000425	V2.0	V5.24	V4.24	32.1	10.6	6.5	0	2.5	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
% Other_CIEF_N_BIOWS	26	1000426	V2.0	V5.25	V4.25	33.3	11.8	6.6	0	2.1	1.3	1.2	1.3	1.2	1.4	1.1	1.3	1.2	1.4	1.1	1.3	1.2
% Purified_CIEF_N_BIOWS	27	1000427	V2.0	V5.26	V4.26	31.4	11.6	7.9	0	2.8	1.1	1	1	1	1	1	1	1	1	1	1	1
% Other_CIEF_N_BIOWS	28	1000428	V2.0	V5.27	V4.27	27.8	12.4	7.7	0	2.1	1.2	1.2	1.2	1.1	1.3	1.0	1.2	1.1	1.3	1.0	1.2	1.1
% Purified_CIEF_N_BIOWS	29	1000429	V2.0	V5.28	V4.28	30.2	11.8	7.4	0	2.4	1.3	1.2	1.3	1.2	1.4	1.1	1.3	1.2	1.4	1.1	1.3	1.2
% Other_CIEF_N_BIOWS	30	1000430	V2.0	V5.29	V4.29	27.8	13	8.4	0	2.3	0.9	1.2	1.2	1.1	1.3	1.0	1.2	1.1	1.3	1.0	1.2	1.1
% Purified_CIEF_N_BIOWS	31	1000431	V2.0	V5.30	V4.30	37.5	11	6.7	0	2.2	1	1	1	1	1	1	1	1	1	1	1	1
% Other_CIEF_N_BIOWS	32	1000432	V2.0	V5.31	V4.31	32.4	12.1	7.9	0	2.3	1.1	1	1	1	1	1	1	1	1	1	1	1



Thank you



Giulia Lambiase

LinkedIn [linkedin.com/in/giulia-lambiase-004683134](https://www.linkedin.com/in/giulia-lambiase-004683134)