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

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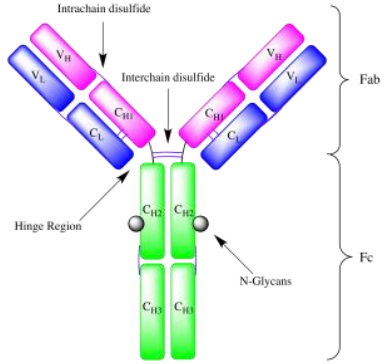
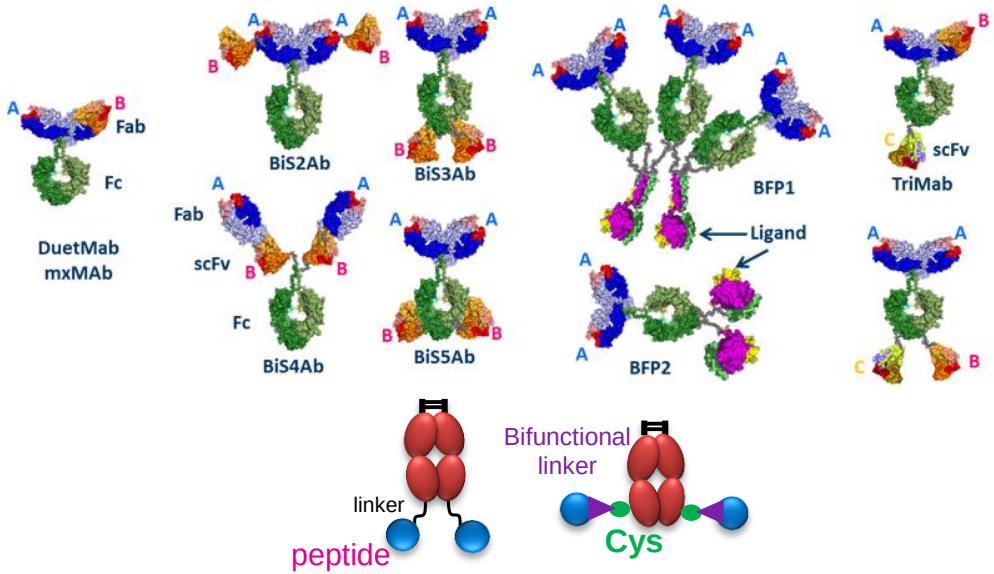
October 2022

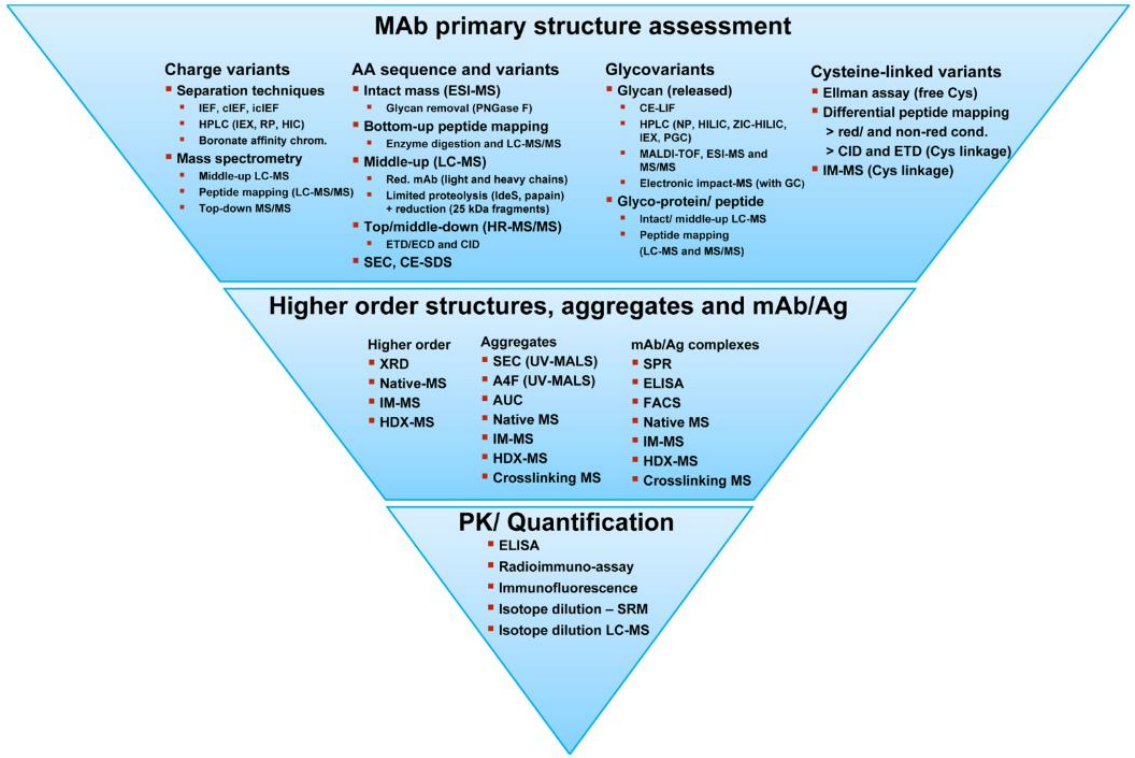
1. Therapeutic protein analysis
2. Introduction to Design-of-Experiment (DoE)
3. Application of DoE to analytical method development
4. Chemometrics tools for data analysis
5. Case studies and Key considerations

Analytical characterisation of therapeutic proteins



Monoclonal Antibody (mAb)





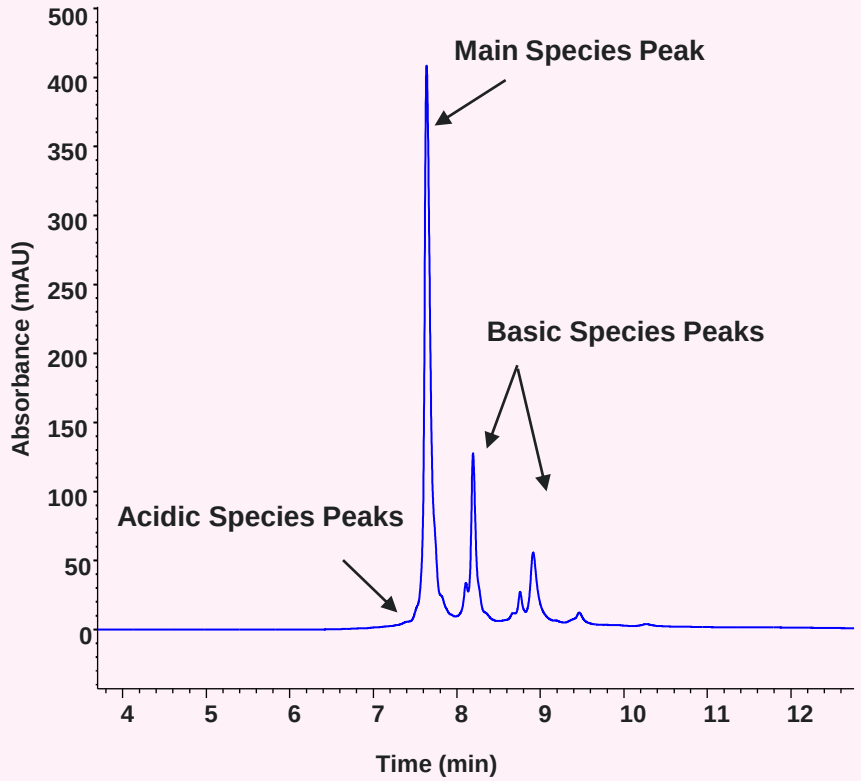
The inherent structural complexity of proteins constitutes an analytical challenge. Analytical methods are necessary to support product development, manufacturing and commercialisation.

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;

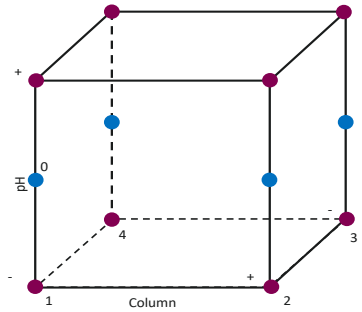
    - ***Faster, Cheaper and Smarter experiments → Stronger and Better analytical methods***

A Split-DoE Approach for Cation Exchange Chromatography Method Development



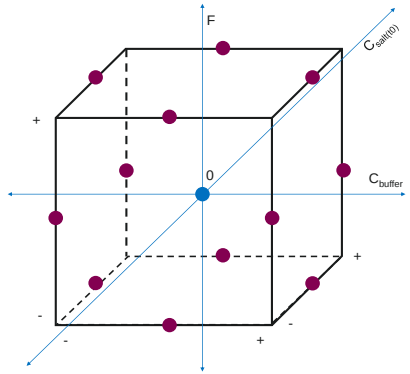
Charge variants separation of a mAb by cation exchange chromatography (CEX)

Main Effects Screening Design



- Column
- Mobile phase pH

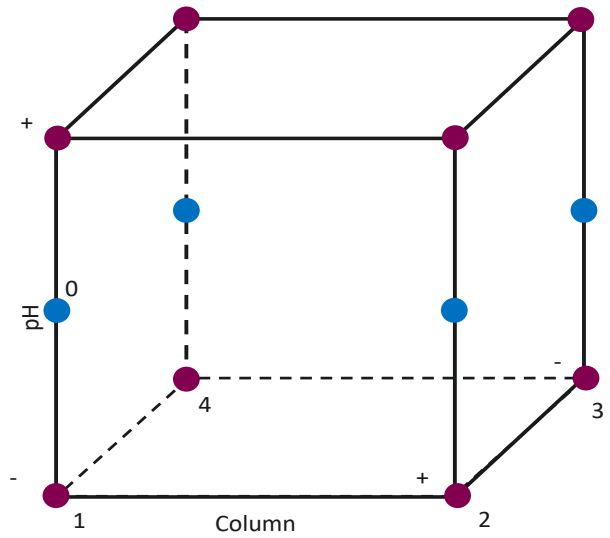
Central Composite Design



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

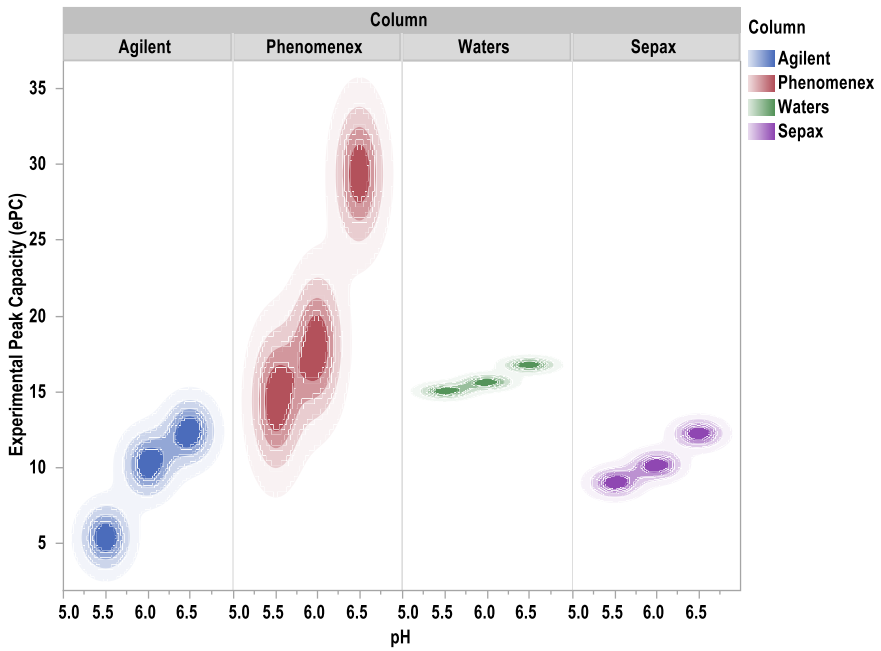
A Split-DoE Approach for Cation Exchange Chromatography Method Development

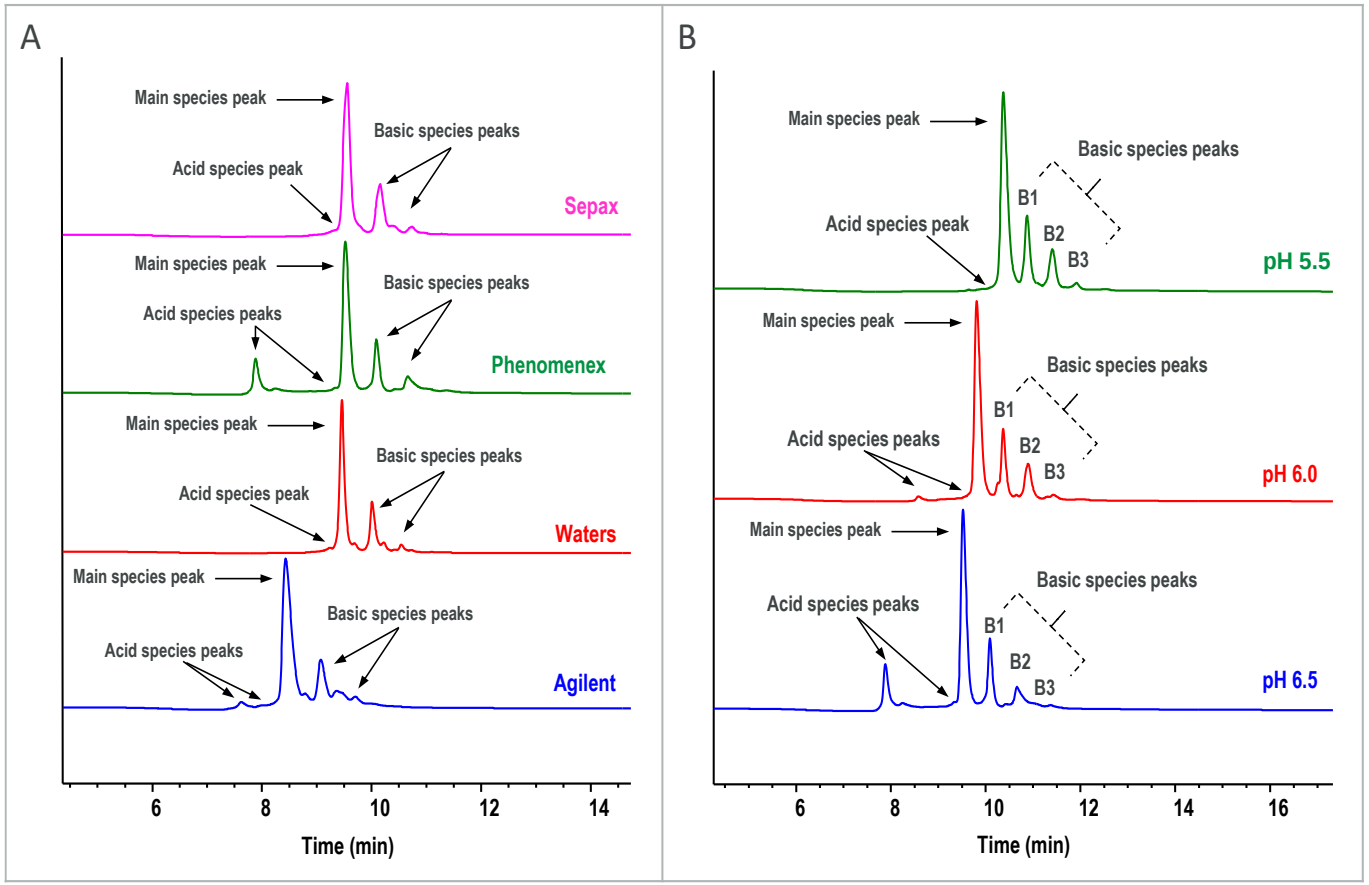
Main Effects Screening Design



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
$C_{salt(t0)}$: 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 µL
Sample concentration: 1 mg/mL
UV: 210 nm, 280 nm

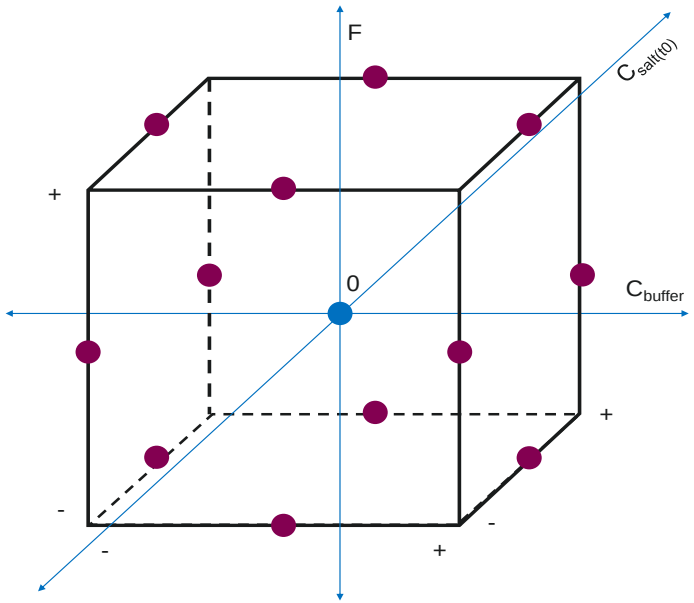
A Split-DoE Approach for Cation Exchange Chromatography Method Development



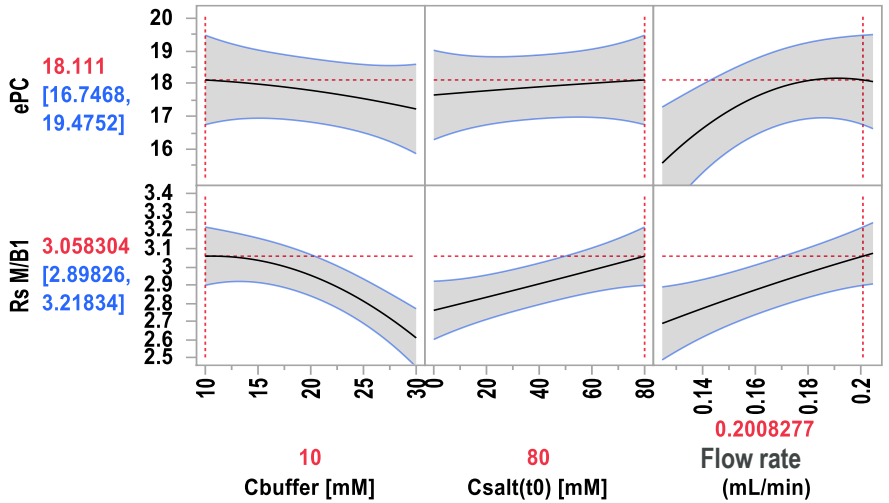


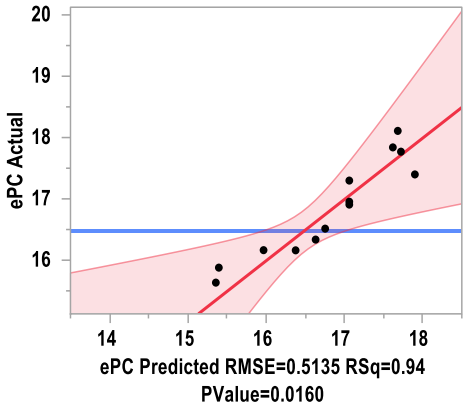
A Split-DoE Approach for Cation Exchange Chromatography Method Development

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 sodum chloridel
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





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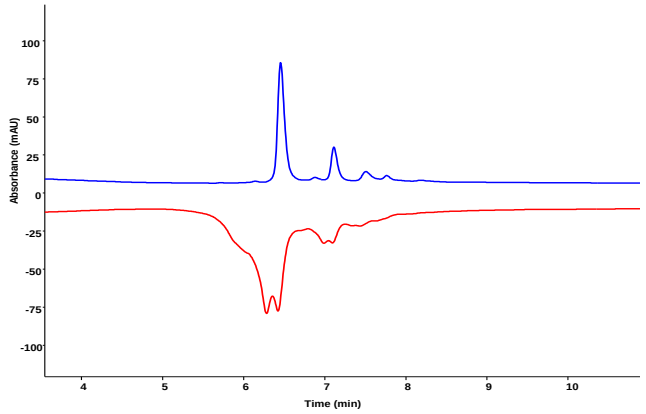


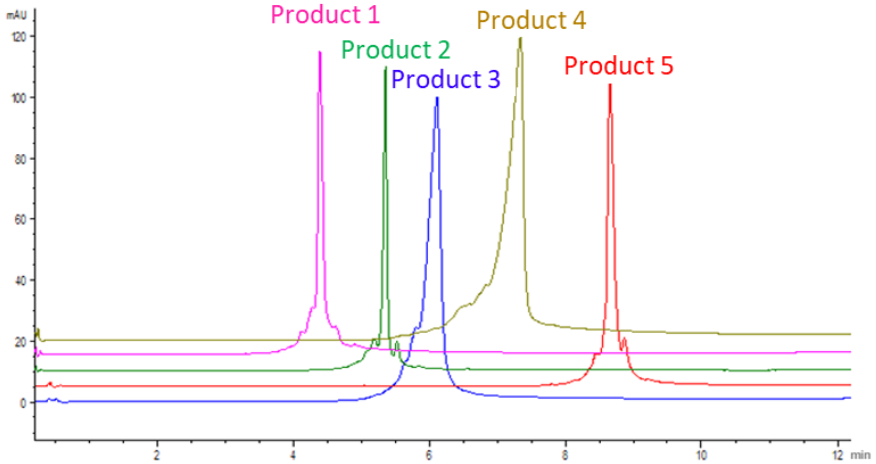
4 columns

mobile phase

pH range

Flow rate

27 experimental runs



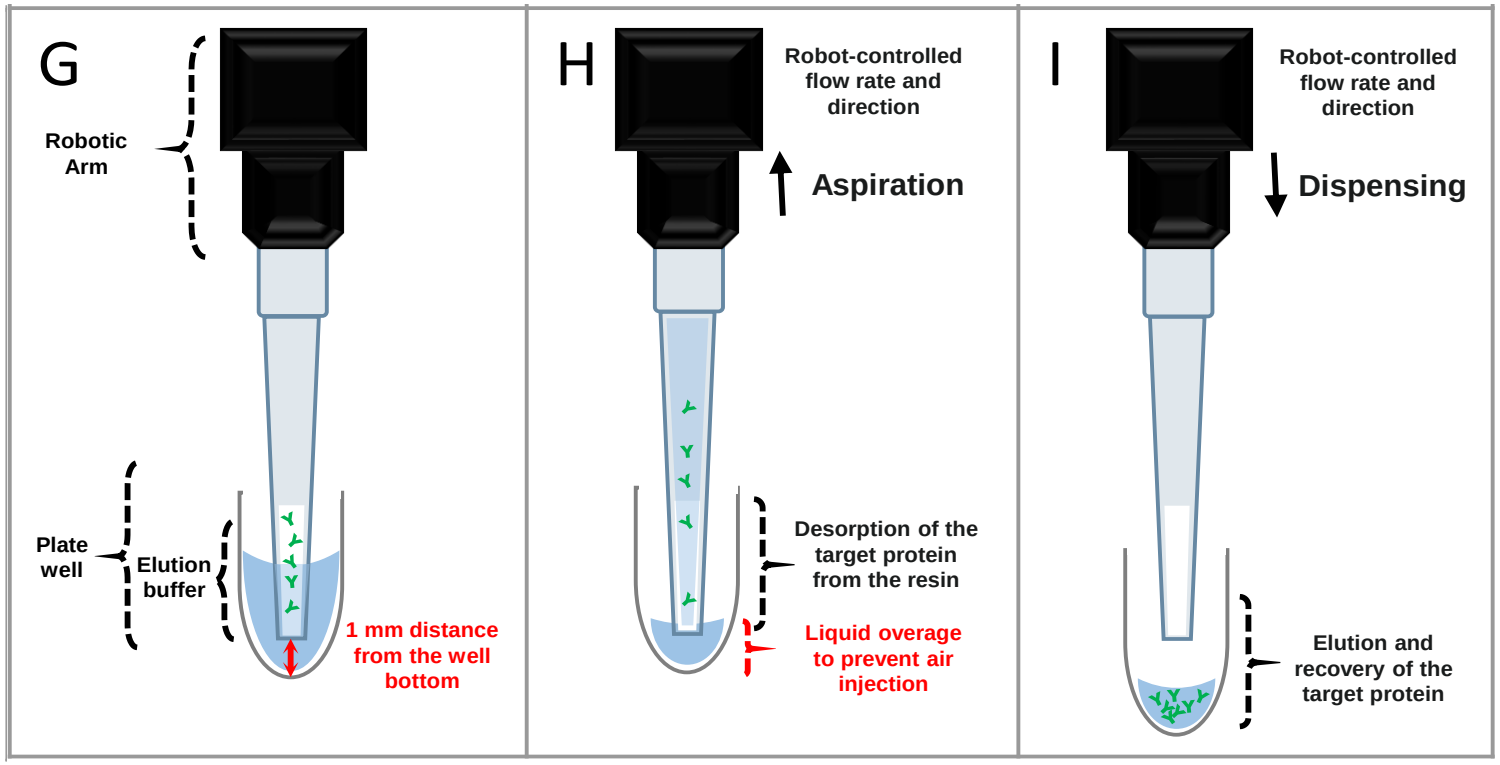
Data generated by Davide Di Girolamo and Ryte Poskute

Platform workflow to analytical method development

Microscale Chromatographic Purification of mAbs and BsAbs

DoE-assisted method development

mAbs
BsAbs

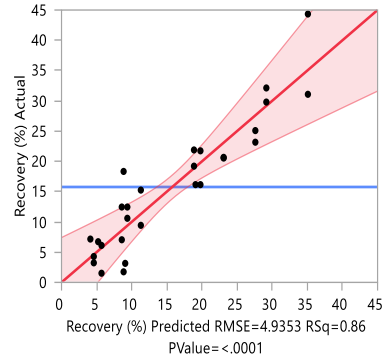


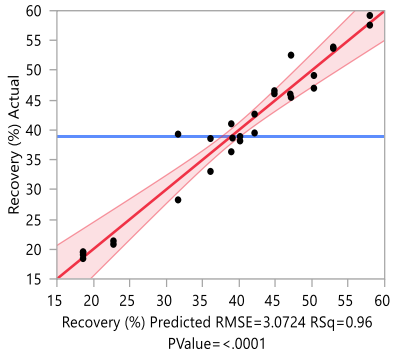
MESD Factors
Capture cycles (4 – 15)
Capture Flow rate (4 – 16 µL/sec)
Wash 1 cycles (1 – 3)
Wash 1 Flow rate (4 – 16 µL/sec)
Wash 2 cycles (1 – 3)
Wash 2 Flow rate (4 – 16 µL/sec)
Elution cycles (4 – 15)
Elution Flow rate (4 – 16 µL/sec)
Final Elution Volume (80 – 120 µL)
MESD Response
%Recovery

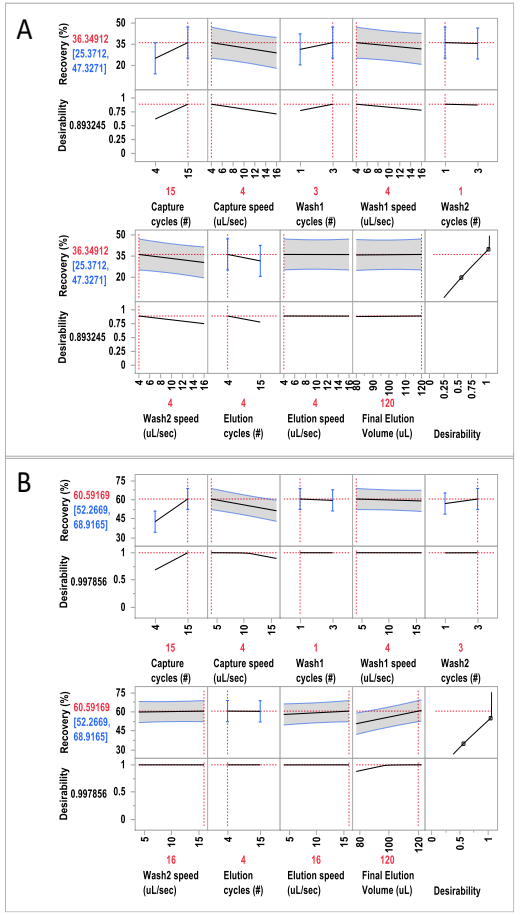
Microscale Chromatographic Purification of mAbs and BsAbs

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*



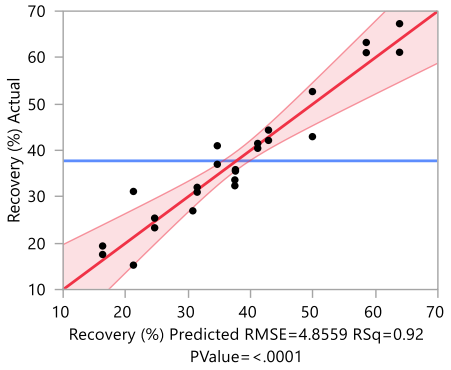


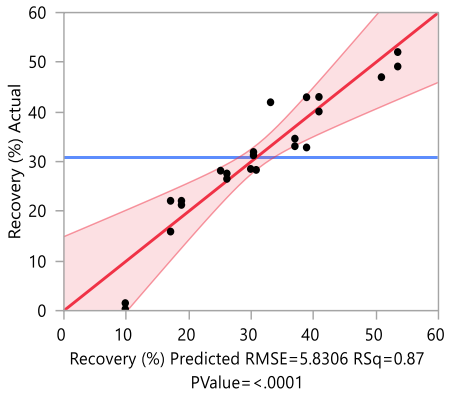


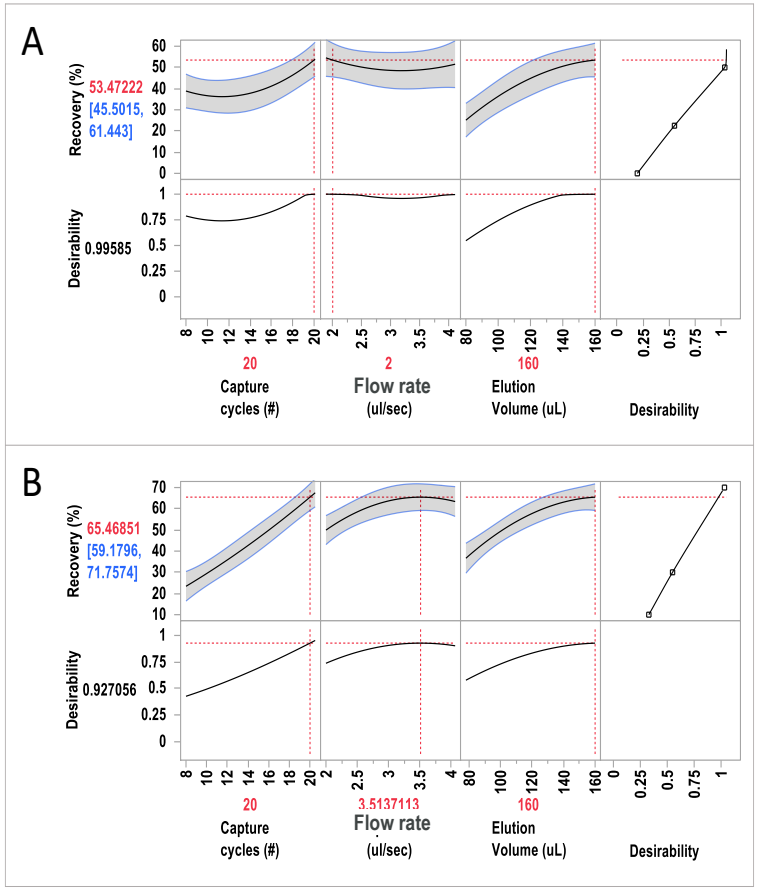
Microscale Chromatographic Purification of mAbs and BsAbs

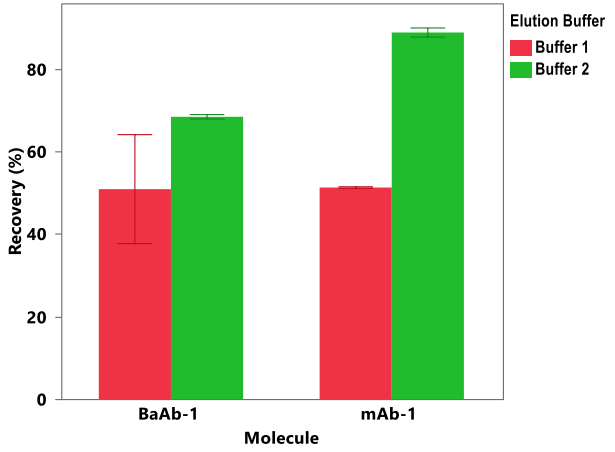
DoE-assisted method development: Central Composite Design

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









Microscale Chromatographic Purification
Method Assessment

DoE approach

Main Effect Screening Design

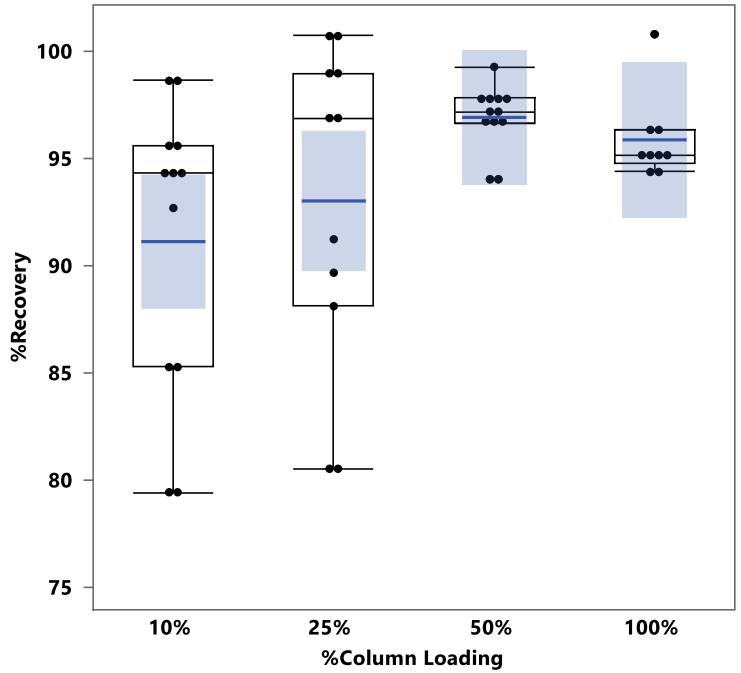
12 Treatments

Central Composite Design

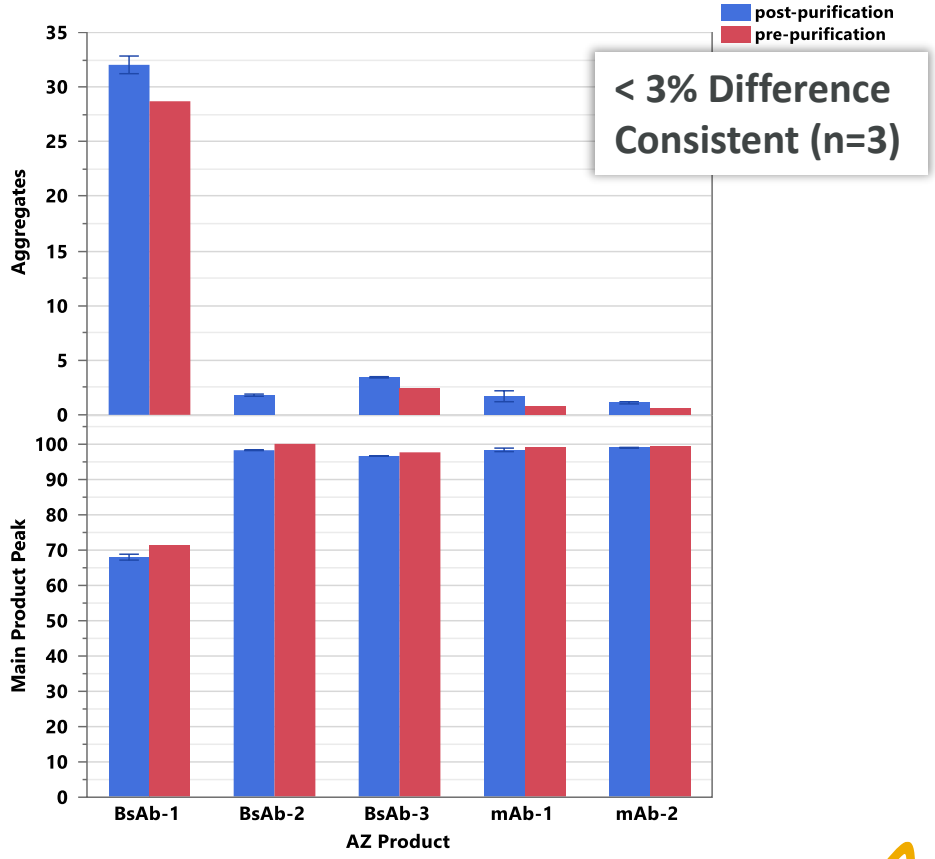
12 Treatments

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:

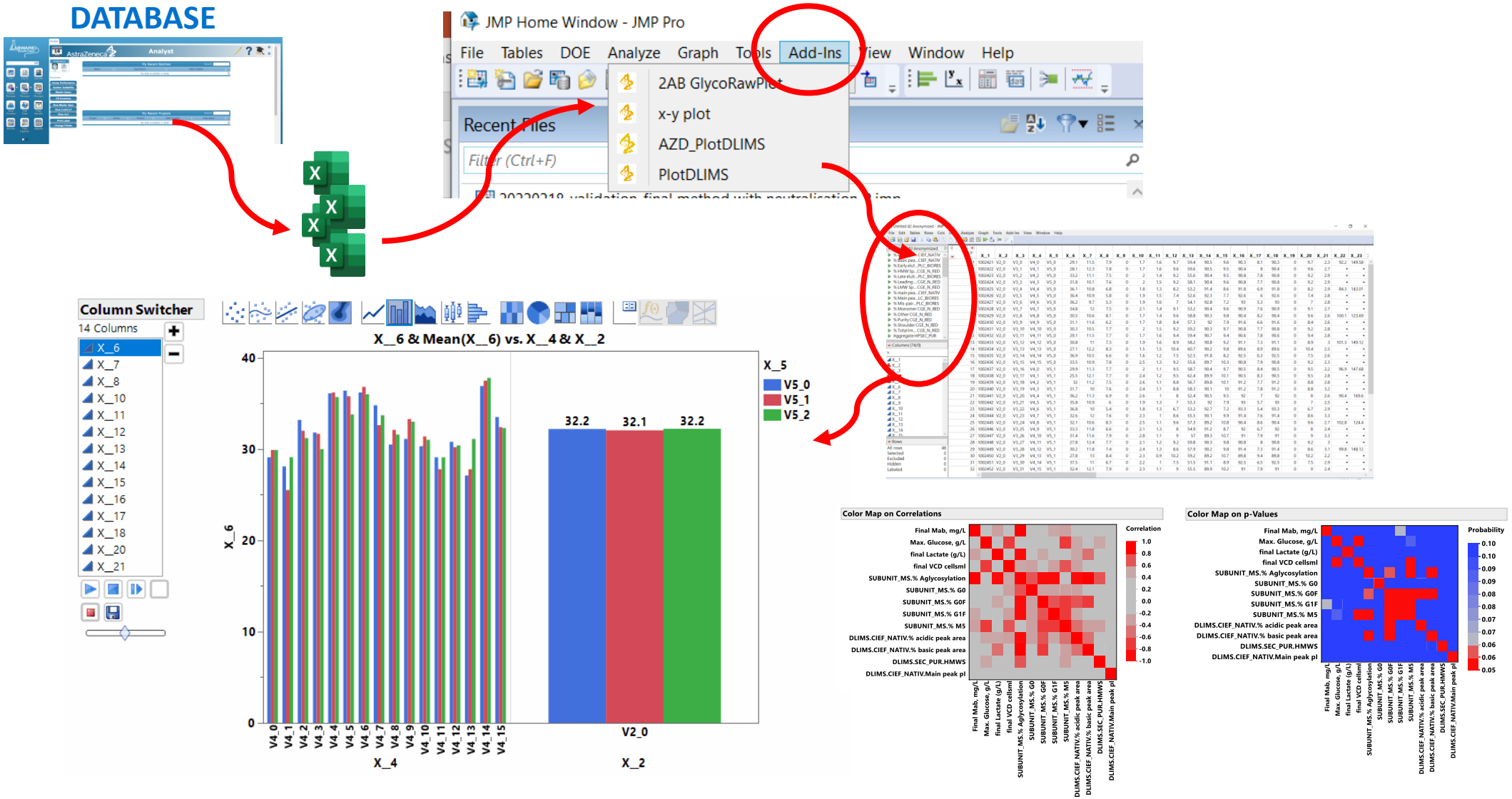
- Experimental **planning based on the** time, costs and other analytical **resources available**.
- Scheduling and execution of experiments with adequate sample size and type of data to **extrapolate maximum information** from chemical data and **efficiently address the challenges and goals of the intended 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



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Thank you

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