NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Advanced analytical technologies for rapid screening of process and product quality attributes supporting upstream bioprocessing

TAP HERE TO RETURN TO KIOSK MENU

Yun Alelyunas, Richard Jeske, Julie Wushensky, Chandler Przybylski, Jurgen Sanes

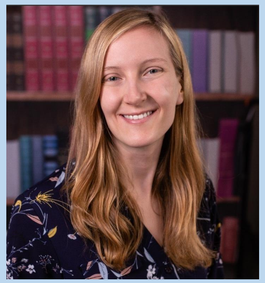
Waters Immerse Delaware, Newark, DE 19713

Please use the headings above to navigate through the different sections of the poster

Introduction

- In upstream bioprocess development, timely feedback on process and product related information are crucial to affect design-make-test cycle.
- Waters Immerse Delaware is built to mimic an upstream cell culture and analytical setting.
- The center is currently equipped with Ambr® 250 bioreactor (Sartorius AG) for DOE experiment, Andrew+™ Robots for automated sample preparation, an ACQUITY™ UPLC™ system, BioAccord™ LC-MS system, and other bioanalyzers for cell counting and other attributes.
- In this poster, automated sample preparation and rapid LC, LC-MS screening methods are detailed for titer, aggregation, media and other CQA assays. These methods can be readily adopted by process engineers allowing timely data collection and analysis.
- These analytical techniques have been applied to process optimization experiments using Ambr250 Multi-Parallel Bioreactor system. Example data are displayed.

Yun Alelyunas



Julie Wushensky

Waters™

Advanced analytical technologies for rapid screening of process and product quality attributes supporting upstream bioprocessing

TAP HERE TO RETURN TO KIOSK MENU

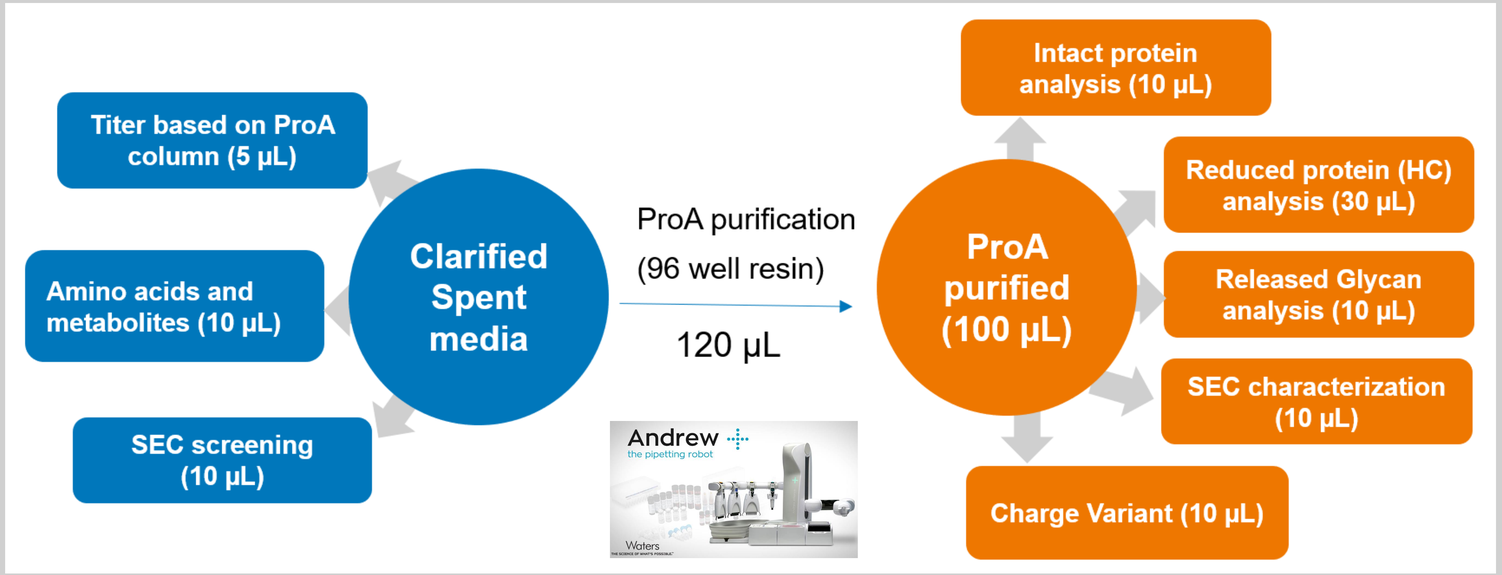
Yun Alelyunas, Richard Jeske, Julie Wushensky, Chandler Przybylski, Jurgen Sanes

Waters Immerse Delaware, Newark, DE 19713

Please use the headings above to navigate through the different sections of the poster

Automated Sample Preparations

- Samples used in various analysis for process and products attributes are either raw media or purified media based on ProA affinity capture.
- Andrew+ Pipette Robots is extensively used in sample preparation.
- Figure below shows sample generation/consumption for various analysis. Blue colored are those using raw media. Orange colored are those using ProA purified samples.



Total spent media volume used = 150 µL

Total ProA purified volume used = 70 µL

Advanced analytical technologies for rapid screening of process and product quality attributes supporting upstream bioprocessing

TAP HERE TO RETURN TO KIOSK MENU

Yun Alelyunas, Richard Jeske, Julie Wushensky, Chandler Przybylski, Jurgen Sanes

Waters Immerse Delaware, Newark, DE 19713

Please use the headings above to navigate through the different sections of the poster

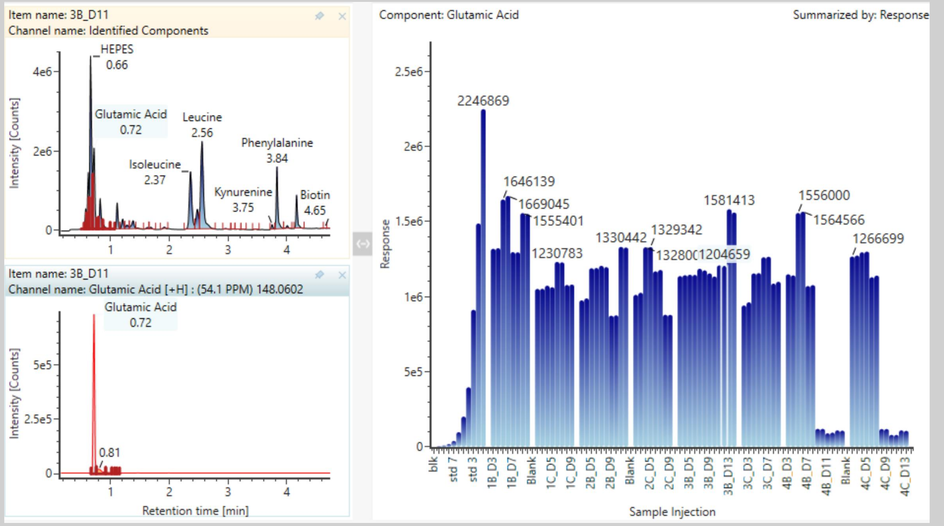
Cell culture media and metabolites analysis

- Spent culture media and metabolites analyses is based on reversed-phase chromatography using Waters ACQUITY Premier HSS T3 1.8 µm 2.1 x 100 mm column on the BioAccord HRMS system. Run time is 9 min.
- The method comes with an analysis package including 220+ compounds library and guided workflow using waters_connect™ instrument controlling software.

Column temperature = 40 °C
Mobile phase A = 0.1%FA
Mobile phase B = 90%ACN/10%IPA/0.1%FA
Run time = 9 min x 2 (pos/neg)
Injection volume = 2 µL
Desolvation temperature = 550 °C
Capillary voltage = 1 kV (pos)
Cone voltage = 20 V (pos)

Time (min)	Flow Rate (mL/min)	Composition A (%)	Composition B (%)	Curve
0.00	0.350	100.0	0.0	Initial
1.00	0.350	100.0	0.0	6
2.50	0.350	95.0	5.0	6
4.50	0.350	60.0	40.0	6
7.00	0.350	5.0	95.0	6
8.00	0.350	5.0	95.0	6
8.10	0.350	100.0	0.0	6
10.00	0.350	100.0	0.0	6

LC-MS conditions and gradient table



Example data showing TIC of all detected compound, XIC for glutamic acid and its trending plot of bioreactor sampled over the course of incubation.

Advanced analytical technologies for rapid screening of process and product quality attributes supporting upstream bioprocessing

TAP HERE TO RETURN TO KIOSK MENU

Yun Alelyunas, Richard Jeske, Julie Wushensky, Chandler Przybylski, Jurgen Sanes

Waters Immerse Delaware, Newark, DE 19713

Please use the headings above to navigate through the different sections of the poster

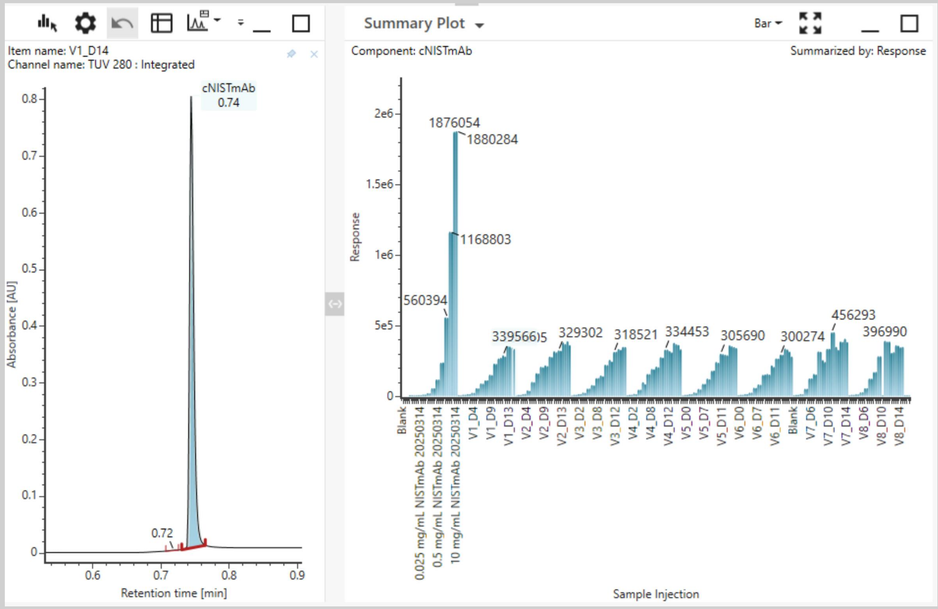
Titer determination using ProA affinity chromatography

- Titer (g/L) measurement is based on Waters ProA affinity column 2.1 x 20 mm using ACQUITY Premier LC-UV system. Run time is 3 min.
- Samples are clarified spent media from bioreactor.

Column temperature = 25 °C
Mobile phase A = 1x DPBS
Mobile phase B = 0.1%FA
UV = 280 nm
Injection volume = 1-2 µL

Time (min)	Flow Rate (mL/min)	Composition A (%)	Composition B (%)	Curve
0.00	0.750	100.0	0.0	Initial
0.50	0.750	100.0	0.0	6
0.51	0.750	0.0	100.0	6
1.00	0.750	0.0	100.0	6
1.01	0.750	100.0	0.0	6
1.20	0.750	100.0	0.0	6
1.21	0.750	0.0	100.0	6
1.40	0.750	0.0	100.0	6
1.41	0.750	100.0	0.0	6
1.60	0.750	100.0	0.0	6
1.61	0.750	0.0	100.0	6
1.80	0.750	0.0	100.0	6
1.81	0.750	100.0	0.0	6
3.00	0.750	100.0	0.0	6

LC-UV conditions, gradient cycling post elution was included for a reduced carryover.



Example data showing TUV chromatogram of mAb in question and its trending plot of bioreactor samples obtained throughout the course of an incubation.

Advanced analytical technologies for rapid screening of process and product quality attributes supporting upstream bioprocessing

TAP HERE TO RETURN TO KIOSK MENU

Yun Alelyunas, Richard Jeske, Julie Wushensky, Chandler Przybylski, Jurgen Sanes

Waters Immerse Delaware, Newark, DE 19713

Please use the headings above to navigate through the different sections of the poster

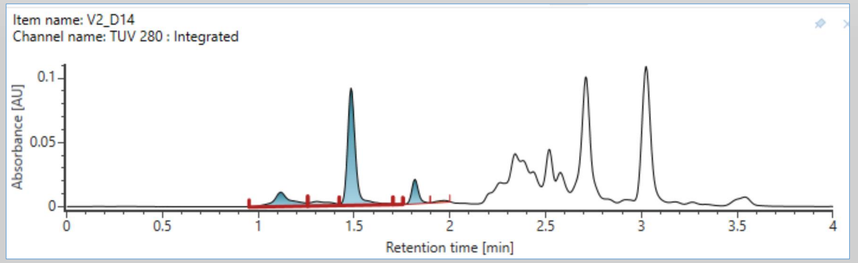
Aggregation measurement based on SEC column

- Two SEC method are used for screening or characterization.
- Screening method uses ACQUITY Premier SEC 250 Å 1.7 µm 4.6 x 100 mm column.
- Characterization method uses Acquity Premier SEC 250 Å 1.7 µm 4.6 x 300 column.

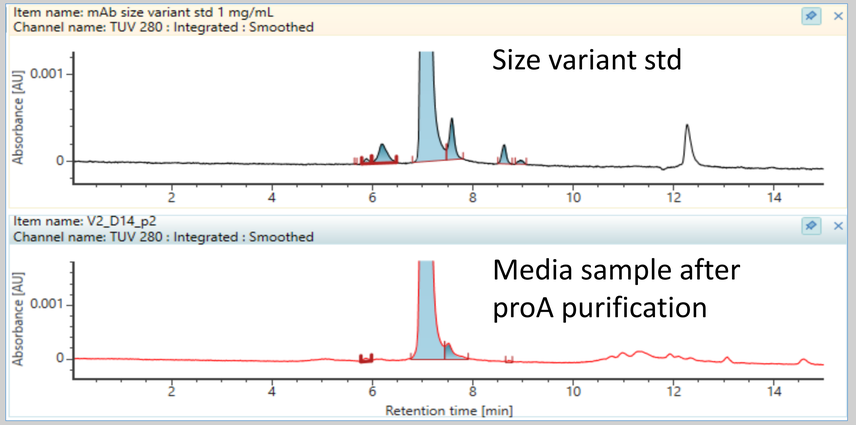
Screening method
Column = Premier SEC 4.6 x 100 mm
Column temperature = 25 °C
Isocratic mobile phase = 1 x DPBS
Flow rate = 0.5 mL/min
Run time = 4 min
Injection volume = 1 µL
UV = 280 nm
Sample = clarified Spent media or ProA purified, no dilution

Characterization method
Column = Premier SEC 4.6 x 300 mm
Column temperature = 25 °C
Isocratic mobile phase = 1xDPBS
Flow rate = 0.3 mL/min
Run time = 15 min
Injection volume = 1 µL
UV = 280 nm
Sample = ProA purified sample

LC-UV conditions



SEC analysis of raw spent media using screening method



SEC analysis of ProA purified mAb using characterization method

Advanced analytical technologies for rapid screening of process and product quality attributes supporting upstream bioprocessing

TAP HERE TO RETURN TO KIOSK MENU

Yun Alelyunas, Richard Jeske, Julie Wushensky, Chandler Przybylski, Jurgen Sanes

Waters Immerse Delaware, Newark, DE 19713

Please use the headings above to navigate through the different sections of the poster

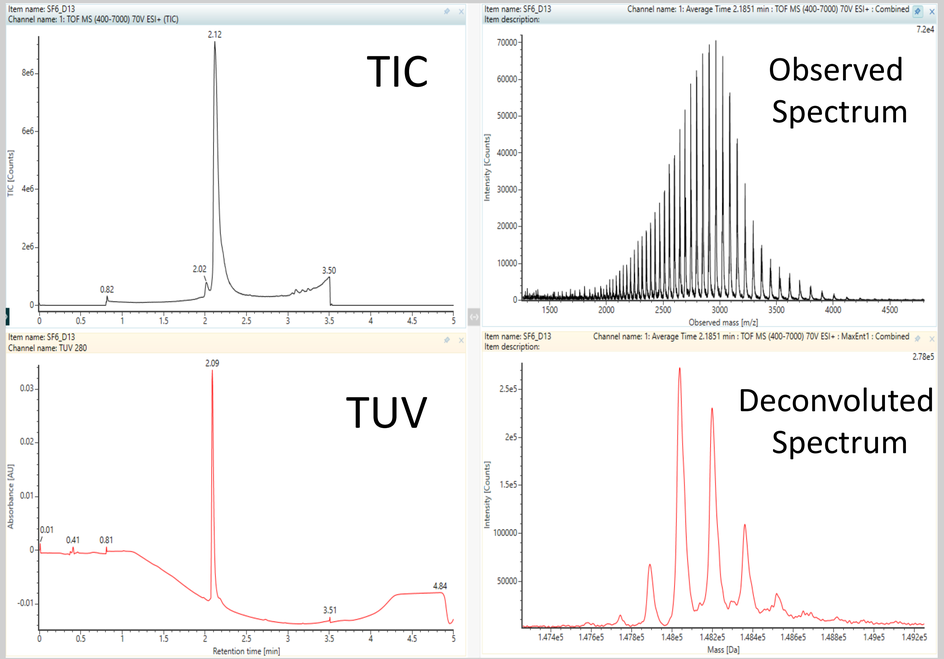
Intact Protein analysis

- Intact protein analysis uses ACQUITY Premier Protein BEH C4 300 Å 1.7 µm 2.1 x 50 mm column on the BioAccord system. Run time is 5 min.
- Media sample were purified using resin based ProA affinity capture on Andrew+ Pipette Robot and diluted 1:10 with formic acid.

Column temperature = 80 °C
Mobile phase = 0.1% FA
Mobile phase B = 90%ACN/10%IPA/0.1%FA
Injection volume = 5 µL
UV = 280 nm
Desolvation temperature = 550 °C
Capillary voltage = 1.5 kV
Cone voltage = 70 V

Time (min)	Flow Rate (mL/min)	Composition A (%)	Composition B (%)	Curve
0.00	0.400	95.0	5.0	Initial
0.50	0.400	95.0	5.0	6
1.00	0.400	85.0	15.0	6
3.50	0.400	15.0	85.0	6
3.70	0.400	5.0	95.0	6
4.30	0.400	5.0	95.0	6
4.50	0.400	95.0	5.0	6
5.00	0.400	95.0	5.0	6

LC-MS conditions and gradient table



Example data showing UV and Mass chromatogram of mAb, the observed and deconvoluted mass spectra.

Advanced analytical technologies for rapid screening of process and product quality attributes supporting upstream bioprocessing

TAP HERE TO RETURN TO KIOSK MENU

Yun Alelyunas, Richard Jeske, Julie Wushensky, Chandler Przybylski, Jurgen Sanes

Waters Immerse Delaware, Newark, DE 19713

Please use the headings above to navigate through the different sections of the poster

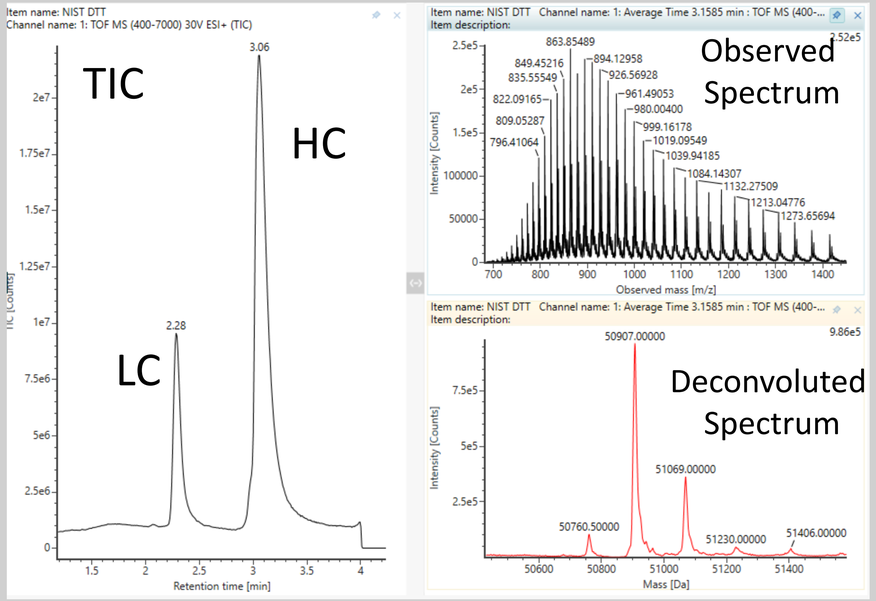
Reduced Protein analysis via LC and HC (DTT reduction)

- Reduced protein analysis is based on guanidine denaturing and DTT reduction to produce light chain (LC) and heavy chain (HC).
- The analysis uses ACQUITY Premier Protein BEH C4 300 Å 1.7 µm 2.1 x 50 mm column using the BioAccord system. Run time is 5 min.

Column temperature = 60 °C
Mobile phase = 0.1% FA
Mobile phase B = 90%ACN/ 10%IPA/ 0.1%FA
Injection volume = 2 µL
UV = 260 nm
Desolvation temperature = 450 °C
Capillary voltage = 1.2 kV
Cone voltage = 30 V

Time (min)	Flow Rate (mL/min)	Composition A (%)	Composition B (%)	Curve
0.00	0.400	95.0	5.0	Initial
0.50	0.400	95.0	5.0	6
1.00	0.400	75.0	25.0	6
3.50	0.400	65.0	35.0	6
3.70	0.400	10.0	90.0	6
4.30	0.400	10.0	90.0	6
4.50	0.400	95.0	5.0	6
5.00	0.400	95.0	5.0	6

LC-MS conditions and gradient table



Example data showing chromatogram of reduced mAb to generate HC and LC, the observed and deconvoluted mass spectra of HC.

Advanced analytical technologies for rapid screening of process and product quality attributes supporting upstream bioprocessing

TAP HERE TO RETURN TO KIOSK MENU

Yun Alelyunas, Richard Jeske, Julie Wushensky, Chandler Przybylski, Jurgen Sanes

Waters Immerse Delaware, Newark, DE 19713

Please use the headings above to navigate through the different sections of the poster

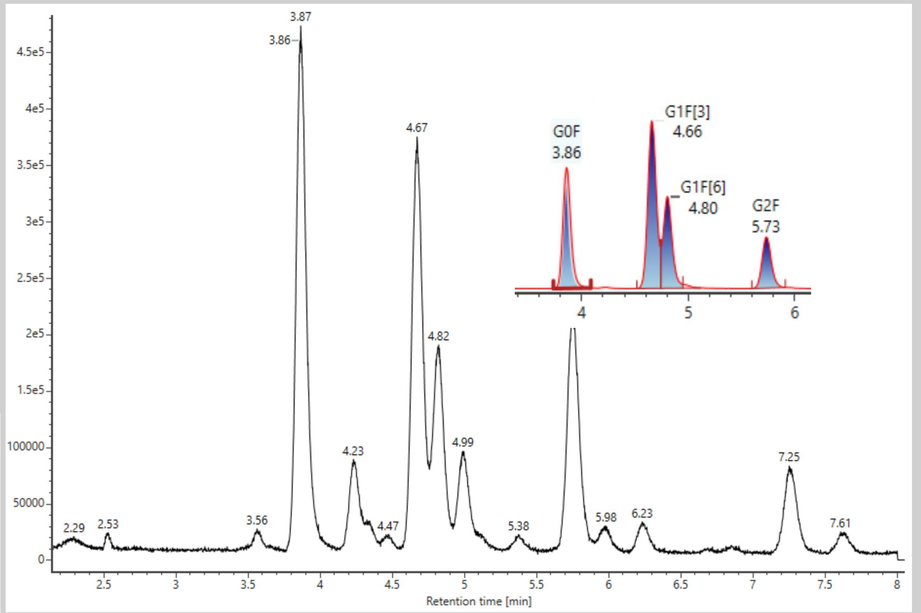
Released Glycan analysis

- Released glycan analysis uses ACQUITY Premier Glycan BEH Amide 130 Å 1.7 µm 2.1 x 150 mm on the BioAccord system. Run time is 10 min.
- Samples were prepared using Waters released Glycan kits using Andrew+ Pipetting Robots.

Column temperature = 60 °C
Mobile phase A = Ammonium format glycan solution
Mobile phase B = ACN
Injection volume = 2 µL
UV = 260 nm
Desolvation temperature = 550 °C
Capillary voltage = 1.3 kV
Cone voltage = 40 V

Time (min)	Flow Rate (mL/min)	Composition A (%)	Composition B (%)	Curve
0.00	0.400	35.0	65.0	Initial
7.00	0.400	40.0	60.0	6
7.10	0.400	60.0	40.0	6
7.50	0.400	60.0	40.0	6
7.60	0.400	35.0	65.0	6
10.00	0.400	35.0	65.0	6

LC-MS conditions and gradient table



Example data showing total ion chromatogram of released glycan of a mAb sample. Insert showed major glycans detected.

NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Advanced analytical technologies for rapid screening of process and product quality attributes supporting upstream bioprocessing

TAP HERE TO RETURN TO KIOSK MENU

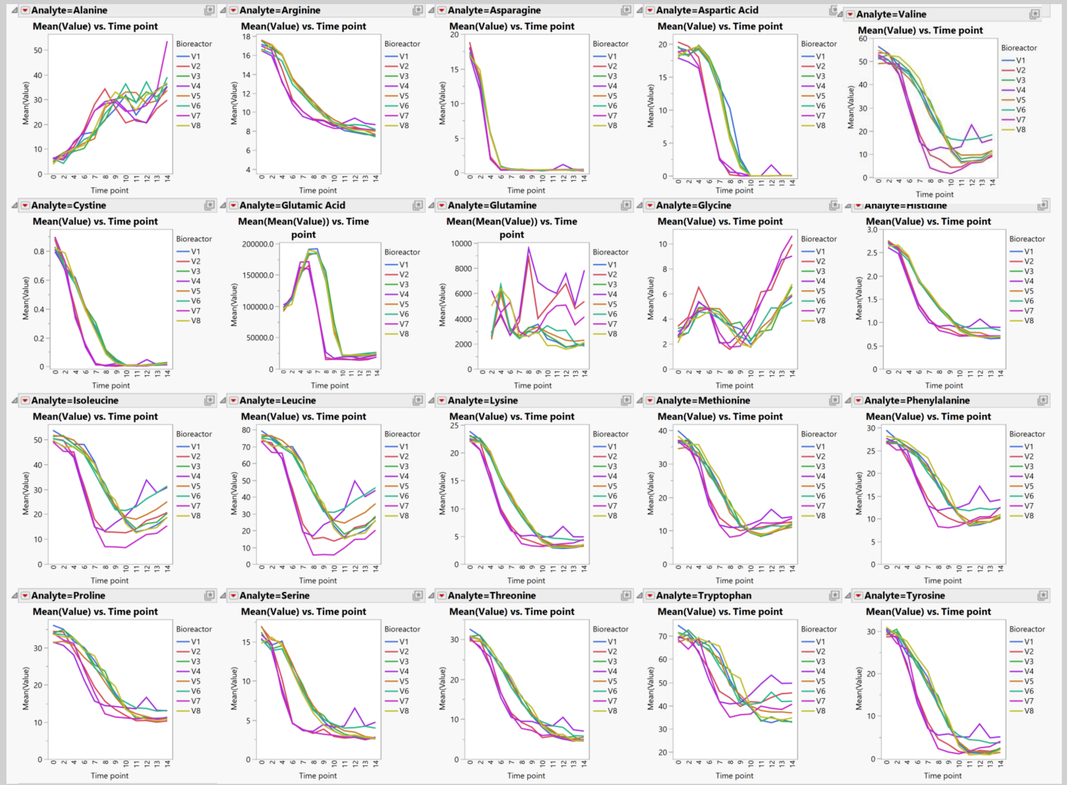
Yun Alelyunas, Richard Jeske, Julie Wushensky, Chandler Przybylski, Jurgen Sanes

Waters Immerse Delaware, Newark, DE 19713

Please use the headings above to navigate through the different sections of the poster

JMP add-in for data visualization and analysis

- Waters add-in is available in JMP® (SAS Institute Inc.) marketplace free-of-charge for download. The add-in enables data import, organize and display data based on class or biotransformation pathways. One example is shown below displaying overlaid plot of amino acid in 8 bioreactors over 14 days of incubation.



Advanced analytical technologies for rapid screening of process and product quality attributes supporting upstream bioprocessing

TAP HERE TO RETURN TO KIOSK MENU

Yun Alelyunas, Richard Jeske, Julie Wushensky, Chandler Przybylski, Jurgen Sanes

Waters Immerse Delaware, Newark, DE 19713

Please use the headings above to navigate through the different sections of the poster

Summary

- Rapid analytical methods are developed for titer, aggregation, intact protein, reduced protein, and released glycan analysis.
- When possible, same mobile phases are used across multiple assays:
  - Intact protein, reduced protein, and media and metabolite analysis share the same mobile phase A: (H_2O/0.1%FA), and Mobile phase B (90% ACN, 10% IPA, 0.1% FA).
  - Titer and SEC analysis use the same mobile phase A (DPBS buffer solution)
  - Aqueous buffer mobile phases are all from commercially available for ease of preparation.
- All sample preparations are carried out using Andrew+ Pipetting Robots.
- These methods1 have been applied in Ambr250 incubation, timely feedback of process and product related attributes have provided detailed understanding of the process in the design-make-test cycle.

720009578EN