Accelerating Attribute-Focused Process and Product Development Through the Development and Deployment of Autonomous Process Analytical Technology Platform System

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Abstract

Enabling real-time monitoring and control of the biomanufacturing processes through product quality insights continues to be an area of focus in the biopharmaceutical industry. The goal is to manufacture products with the desired quality attributes. To realize this rigorous attribute-focused Quality by Design (QbD) approach, it's critical to support the development of processes that consistently deliver high-quality products and facilitate product commercialization. Time delays associated with off-line analytical testing can limit the speed of process development. Thus, developing and deploying analytical technology is necessary to accelerate process development. In this study, we have developed the Micro Sequential Injection (μSI) process analyzer and the Automatic Assay Preparation Platform (A2P2) system. These innovations address the unmet need for an automatic, online, real-time sample acquisition and preparation platform system for in-process monitoring, control, and release of biopharmaceuticals. These systems can also be deployed in laboratory areas as an off-line analytical system and on the manufacturing floor to enable rapid testing and release of products manufactured in a GMP environment.

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Short Running Title:

Autonomous PAT Systems in Accelerated Product Development

ABSTRACT

Enabling real-time monitoring and control of the biomanufacturing processes through product quality insights continues to be an area of focus in the biopharmaceutical industry. The goal is to manufacture products with the desired quality attributes. To realize this rigorous attribute-focused Quality by Design (QbD) approach, it's critical to support the development of processes that consistently deliver high-quality products and facilitate product commercialization. Time delays associated with off-line analytical testing can limit the speed of process development. Thus, developing and deploying analytical technology is necessary to accelerate process development. In this study, we have developed the Micro Sequential Injection (μSI) process analyzer and the Automatic Assay Preparation Platform (A2P2) system. These innovations address the unmet need for an automatic, online, real-time sample acquisition and preparation platform system for in-process monitoring, control, and release of biopharmaceuticals. These systems can also be deployed in laboratory areas as an off-line analytical system and on the manufacturing floor to enable rapid testing and release of products manufactured in a GMP environment.

KEYWORDS

Process Analytical Technology (PAT), Quality by Design (QbD), Micro Sequential Injection (μSI), Automatic Assay Preparation Platform (A2P2)

INTRODUCTION

The biomanufacturing landscape has transformed recently, impacting labor demand, causing supply chain disruptions, and leading to persistent inflation. Workforce adaptations have led to productivity disruption and increased health concerns for those unable to work remotely. To stay competitive, innovation is crucial to adapt to the new norm and to introduce new products. Expediting the introduction of new products requires an understanding of critical product quality attributes, safety, efficacy, manufacturing, and logistics. Reliable data is essential for rapid development and speedy delivery to patients. As remote work is now part of business continuity, proactive data collection and analysis are needed for faster insights and value creation. An innovative analytical strategy is vital for building resilience and managing risks in manufacturing quality, addressing labor demand, and securing a competitive edge.

Analytical testing is frequently the critical path in the entire product manufacturing life cycle, often causing delays in product release due to long testing result turnaround times. In next-generation process development and manufacturing, real-time or near real-time analytics are crucial to streamline QbD development through assay automation, data visualization, and predictive modeling. This approach enables attribute-focused development, robust manufacturing processes, and rapid, yet reliable, process control and product release.

In developing an analytical strategy, automated systems aim to enable routine and on-demand acquisition of key product quality and process performance attributes in laboratories and manufacturing facilities. The proposed autonomous PAT platform offers 24/7 monitoring, reliable performance, and resource agility, as opposed to traditional analytical approaches which require significant analyst intervention. Carefully designed fluidic conduits and strategically placed material handling mechanisms provide resource conservation, optimized assay efficiency, controlled conditions, reproducible results, and long-term system robustness. The system contains multiple functional unit operations, working together through programmable algorithms to manage sample and reagent logistics, direct processes, and reactions, and enable self-directed assay protocol selection with rule-based parameter adjustment for optimal output.

Drawing inspiration from next-generation manufacturing and the Industry 4.0 concept, analytical testing is being moved from laboratories to the manufacturing floor. This paradigm shift enables in-situ testing for faster results, improved process understanding, and greater cost-effectiveness in process development and manufacturing [Ref. 1]. Sample acquisition and preparation workflows are typically prone to errors due to manual operations. Meanwhile, liquid handlers require substantial initial investment, extensive development, and can be costly to adapt to new requirements. Chip-based fluidic miniaturization serves as another option, offering advantages such as low material consumption, design freedom, and rapid analysis [Ref. 2].

Portability is another advantage of chip-based analytical systems, allowing the co-location of the manufac-

turing process and analytical instrument. This eliminates delays related to sample logistics and laboratory testing. However, minimizing sample and reagent consumption to the nanoliter or lower range on a microfabricated scale can introduce sampling errors, analysis errors through evaporation, and assay errors due to the precipitation of solids.

Instead, designing a compact and robust instrument that reduces sample and reagent consumption to a few microliters is more advantageous. These factors have led to a practical level of miniaturization through the integrated μSI fluidic design. This design maintains short fluidic conduit geometry instead of narrow ones, decreasing sample and reagent consumption without the inherent issues of microfabricated fluidic channels [Ref. 3].

Τηε μΣΙ Μετηοδολογψ

The μSI methodology, the successor to the flow injection (FI) methodology, was introduced by Ruzicka and Marshall et al. [Ref. 4]. It introduces computer control capability to the system, allowing for better integration with modern process automation. A barebones μSI system comprises a bi-directional syringe pump, a holding coil, a multi-position valve, a reactor coil, and a detector. Conventional FI protocols require physical reconfiguration of the flow manifold to perform different assays; however, a μSI system allows all experimental protocols to be scripted and selected by users.

In a reagent-based μSI assay protocol, the sample and the reagent are injected sequentially through the solvent selector valve into the holding coil, forming a layered solution profile in the tubing. The solution profile is then transported through the mixing coil and into the detector by the flow reversal action of the syringe pump after the valve switches to the flow cell. A flow-through detector records the changes in a desired physical parameter when the reaction product reaches the flow cell.

Flow programming, along with random access to sample and reagent solution ports by the valve, provides versatility since all operating protocols such as sample injection, solute dispersion, and timing are controlled by software protocols. Assays with similar characteristics can be implemented on the same μSI system without physical reconfiguration of the μSI system.

μΣΙ Προςεσς Αναλψζερ Σψστεμ

The biomanufacturing industry has long aspired to achieve real-time monitoring and control of biological manufacturing processes to efficiently produce final products with desired product quality attribute (PQA) profiles. With the aim of developing a system capable of automatic end-to-end sample preparation, and to interface online/at-line sample sources with analytical instruments to achieve multi-fold acceleration in acquiring process performance and product quality insights, the μSI process analyzer system was designed and engineered. The μSI process analyzer system comprises two compartments: the upper sample processing compartment and the lower power supply compartment. The sample processing compartment consists of four quadrants equipped with syringe pumps with glass syringes, solvent selection valves, heated coils, a degasser, solenoid valves, and a reagent cooler, all integrated to minimize the fluidic path. This compact design reduces the footprint and enables on-the-floor technology implementation.

Μυλτι-Αττριβυτε Ασσαψ Σαμπλε Πρεπαρατιον ιν τηε μΣΙ Προςεσς Αναλψζερ Σψστεμ

The multi-attribute method (MAM) assay utilizes peptide mapping methodology to identify and quantify critical quality attributes (CQAs). It is typically performed off-line through manual sample handling, followed by Liquid Chromatography-Mass Spectroscopy (LC-MS) operation, and generating results can take several days. Transitioning the off-line, manual MAM assay into a real-time online LC-MS integrated μSI process analyzer system, where results can be obtained within 2 to 3 hours, can guide process development activities, and provide valuable insights into the performance of a biomanufacturing process.

Γλψςοσψλατιον Προφιλε Σαμπλε Πρεπαρατιον ιν τηε μΣΙ Προςεσς Αναλψζερ Σψστεμ

Monitoring the glycosylation profile is critical for biosimilar products, where matching the glycosylation profile with the reference product is essential to establish similarity [Ref. 5]. Off-line glycosylation analysis

involves manual bioreactor sampling followed by the execution of the following three assays in tandem: (1) product titer, (2) affinity purification, and (3) sample preparation and analysis using Hydrophilic Interaction Chromatography (HILIC), resulting in assay execution times of several days. In contrast, analysis times for the on-line glycosylation assay using the HILIC-LC integrated µSI process analyzer system are approximately 2 hours, providing rapid feedback on product quality and process performance.

Τηε Σεχονδ Γενερατιον µΣΙ Προχεσς Αναλψζερ Σψστεµ: Τηε Α2Π2 Σψστεµ

To minimize the instrument's footprint, the µSI process analyzer system was integrated into a commercially available Agilent 1290 Infinity II Ultra-High-Performance Liquid Chromatography (UHPLC) system and an optional auxiliary mass spectrometer. This system enables both peptide mapping and glycosylation assays and can function as a standalone sample purification platform, complement the Tecan platform, or be used for subsequent product quality assays like aggregation and charge variants. This new system is referred to as the A2P2 system (**Figure 3**).

Innovative designs enable user-selectable sample preparation protocols to be executed on a platformed fluidic design, without physically reconfiguring flow paths inside the A2P2 system. The design transforms a typical UHPLC system into an A2P2-enabled LC system while maintaining its original functionalities as a standalone LC system.

The A2P2 methodology enables the integration of modality agnostic analytics with automatic sample acquisition, preparation, and analysis in a compact form factor, resulting in a more than 50% reduction in footprint while providing enhanced functionalities. Additionally, the A2P2 methodology can be used for online, at-line, or off-line applications, enabling both on-the-floor and laboratory implementations.

MATERIALS AND METHODS

µΣΙ Προχεσς Αναλψζερ Σψστεµ φορ ΜΑΜ Σαµπλε Πρεπ

The detailed µSI process analyzer fluidic design for MAM sample preparation is described in **Figure 1** . It consists of a series of columns, reaction chambers, tubing, liquid redirect valves, a multi-position solvent selector valve, and a syringe pump. Software scripting capabilities enable the µSI process analyzer system to robustly execute the process steps described in **Table 1** .

Test samples can be acquired directly from the bioreactor supernatant or purified material. The sample is aspirated into the system through the solvent selector valve and passed through an affinity purification column where the protein of interest is bound, and the sample matrix is removed as column flow-through. The purified protein is then eluted and delivered to a thermostatic incubator where denaturation, reduction, and alkylation reactions occur. This chemically processed sample undergoes buffer exchange and is enzymatically digested in a second thermostatic incubator before chromatographic separation and mass detection.

µΣΙ Προχεσς Αναλψζερ Σψστεµ φορ Γλψχαν Μαπ Σαµπλε Πρεπ

In this implementation, N-linked oligosaccharides are enzymatically released from samples using Peptide-N-Glycosidase F (PNGase F). The free oligosaccharides are then derivatized with the fluorophore 2-aminobenzoic acid (2-AA) on the reducing terminal N-acetylglucosamine (GlcNAc). The fluorescently labeled oligosaccharides are separated by the HILIC analytical column and detected by fluorescence. In**Figure 2** , the µSI process analyzer system replaces manual processes with automated sampling, sample purification and matrix removal, enzymatic release of glycans, fluorescent labeling of glycans, enrichment of labeled glycans, and synchronized injection, along with chromatographic separation and quantitation of labeled glycans.

Table 2 describes the glycan map sample preparation with protein capture, sample matrix removal, and glycan release by on-column enzymatic digestion in the µSI process analyzer system. In**Figure 2a** , cell-free cell culture samples are acquired by the aspiration action of syringe pump 1 and sent to the affinity purification column, where the protein of interest is retained in the column, and the sample matrix is removed as column flow-through. The enzyme is subsequently introduced into the affinity column, and glycans are released in the interstitial solution of the affinity column. This design eliminates the need for protein

precipitation. The released glycans are carried out by syringe pump 1 and mixed with the glycan reagent from syringe pump 2 before the degasser. The degasser removes microbubbles formed when the aqueous solution from the affinity column meets the organic solution of the 2-AA reagent prepared in methanol. The degassed glycans and 2-AA reagent mixture are delivered to the 80°C reaction coil to accelerate the fluorescent labeling reaction in flow-through mode. The 2-AA labeled glycans are air-cooled and enriched by a porous graphitic carbon (PGC) column while the excess 2-AA reagent and non-labeled glycans are cleared as the PGC column flow-through. The enriched labeled glycans are released from the PGC column and transferred to an LC injection valve, where glycan quantitation is accomplished through HILIC chromatography.

The Flow-Thru Vial (FTV) in the A2P2 system

The FTV enables all sample processing functions in the A2P2 system and directs processed intermediate and/or fully processed samples to return from the sample preparation unit to the autosampler for further processing and/or chromatographic injection. The FTV can also act as a sample receptacle connected to the bioreactor or process stream, enabling online sample processing. The design and fabrication of the FTV were carried out in-house to ensure proper testing and quick turnaround time for prototyping. As shown in **Figure 4** , a threaded hole was used to connect the vial to the system, and an angled gutter at the top of the vial allows liquid to flow directly to waste. As liquid flows into the bottom of the vial, it forces excess liquid into the gutter, where it can gravity drain to waste. Because the vial has unique features, a vial holder was also fabricated in-house to ensure proper vial alignment. The FTV was made from polyether ether ketone (PEEK) for its chemical inertness, and the vial holder was made from aluminum.

Process Execution in the A2P2 System

The aim of creating the autonomous workflow in **Figure 5** is to illustrate the A2P2 system's ability to conduct a lengthy laboratory process autonomously while eliminating the need for an analyst's input: titer measurement for the starting material, affinity purification for the starting material, titer measurement for the purified sample, and subsequent PQ analysis, in this case, the protein aggregation assay. This workflow serves as a foundation for independent operation of all applications documented in this paper, as well as for future A2P2 uses. The System Suitability Testing (SST) and Intelligent Run Control (IRC) functions are applied in concert, automating the process of sample coordination, operating parameter generation, and transfer among the A2P2 system actions. This substantially reduces human involvement, mitigates transcription errors, enhances assay reliability, and accelerates the decision-making process.

RESULTS AND DISCUSSIONS

Real-time MAM Readout

The LC-MS integrated μSI process analyzer system was introduced into a manufacturing facility and connected to a process stream for continuous product quality monitoring. The robustness of the system was first verified by processing reference standard daily for 6 days, and the results in **Figure 6a** were comparable to off-line analysis. The system was then programmed for daily bioreactor sampling. As depicted in**Figure 6b** , each bioreactor sample was bracketed by two reference standards as an assay acceptance check, and product deamidation and clips were measured. The staggering of sample processing and LC-MS analyses resulted in a throughput of approximately 7 samples per day.

Real-time Glycosylation Data

Figure 7a displays a typical glycan map generated using the HILIC UPLC integrated μSI process analyzer system from preliminary proof-of-concept experiments; the results were comparable to those obtained off-line using the manual approach. Subsequently,**Figure 7b** verifies the integrated system's performance over a 32-day period, using daily processing of the reference standard, indicating good system repeatability and robustness. **Figure 7c**showcases results from online measurements over a 32-day period from a production-scale bioreactor. Each data point represents one bioreactor sample acquired daily online, each sample is bracketed by two reference standards to ensure assay acceptance and accuracy. This data provides insights that can guide process development and aid in manufacturing consistent, high-quality products.

Bioreactor Product Titer Measurement by the A2P2 System

In **Figure 8a** , biological samples, whether manually acquired or automatically delivered, can be introduced into the autosampler via regular sample vials or the FTV. The autosampler initiates sample aspiration from these sources and carries out the injection into an affinity chromatography column within the column compartment. A spectrophotometric detector, such as a UV detector, then establishes the product titer/concentration by comparing it with known concentration standards.

Bioreactor Product Aggregation Assessment by the A2P2 System

Before protein aggregation assessment of any cell culture sample, an analyst must perform affinity purification prior to injection into a size exclusion (SEC) chromatography column. Matrix removal and protein enrichment are essential before sample preparation and PQ assays. Compared to the Tecan-Atoll process, the A2P2 system significantly improves efficiency, reducing sample turnaround time by 30%-64%, saving staff hands-on time by up to 92%, and increasing sample processing capacity by 90%-154%. **Figure 8a** depicts how the autosampler needle takes either manually collected or online samples from the vial or FTV and directs them through the affinity capture column. The column captures the protein of interest, while the sample matrix is flushed to waste through the FTV. The eluted protein of interest accumulates in the FTV as the affinity-purified sample, which can subsequently be injected into the analytical sizing column for aggregation level quantification by a UV detector. Samples that are free from cell culture matrix or have undergone affinity purification in downstream processes require no further affinity capture/cleanup. Analysts can directly inject these samples into the analytical sizing column, and a UV detector quantifies their aggregation level.

Bioreactor Product Charge Variant Profile Assessment by the A2P2 System

To evaluate charge variant profile in a cell culture sample, the sample must undergo affinity purification and buffer exchange to reduce the salt content of the sample matrix before injection into an ion exchange column. As shown in **Figure 8b** , the autosampler needle takes manually collected or online samples from the vial or FTV and directs them through an affinity capture column for cleanup. The column captures the protein of interest, flushing the sample matrix to waste via the FTV. The protein of interest is then eluted from the affinity capture column and accumulates in the FTV as an affinity-purified sample. The sample is subsequently diluted with the mobile phase of the charge variant assay inside intermediate vials to lower the salt concentration before injection into the analytical ion exchange column. If dilution doesn't sufficiently reduce the sample's salt concentration, a desalting column can perform a buffer exchange on the sample before injection for analysis. However, samples that are free from cell culture medium and meet the assay requirements for salt content require no affinity capture/cleanup, dilution, or buffer exchange. These samples can be directly injected into the ion exchange column for analysis.

Bioreactor Product Glycosylation Profile Assessment by the A2P2 System

In the case of sample preparation for a glycosylation profile assay, if the starting material is a cell culture sample, the process includes affinity protein cleanup, enzymatic release of glycans from the protein, chemical labeling of the released glycans with fluorescent tags, and subsequent HILIC chromatography column injection and analysis. As depicted in **Figure 8c** , the autosampler receives manually collected or automatically delivered bioreactor cell-free samples in vials or through the FTV. The autosampler needle takes the sample and injects it into the affinity capture column for cell culture matrix removal. The autosampler needle then delivers PNGase F to the capture column for on-column glycan release. The released glycans are transferred to the FTV, mixed with 2-AA, and directed to the 80°C reaction coil, where the fluorescent labeling reaction takes place. The fluorescently labeled glycans are then conveyed back to the FTV, ready for normal-phase chromatographic separation and quantitation.

Bioreactor Product Quality Assessment using MAM by the A2P2 System

A cell-free cell culture sample must undergo affinity purification, denaturation, reduction, alkylation, buffer exchange, and enzymatic digestion before analysis. Occasionally, a secondary enzyme is required to fully

investigate product quality attributes. As shown in**Figure 8d** , the autosampler receives manually collected or automatically delivered bioreactor cell-free samples in vials or through the FTV. The autosampler needle takes the sample and injects it into the affinity capture column for cell culture matrix removal. The autosampler then transports the purified sample into one of the intermediate vials and sequentially adds denaturation and reduction reagents. Or, as an alternative approach, the reagents can be directly added to the FTV to streamline the process. The mixture is then delivered to and incubated inside the 40°C reaction coil, in either the stopped-flow mode or the preferred continuous-flow mode. After the protein is denatured and reduced, it is transferred to the FTV, where the alkylation reagent is added either directly or using a new intermediate vial. The mixture is then directed through the 40°C reaction coil for incubation to complete protein alkylation and is subsequently buffer exchanged using the desalting column. The desalted, reduced, and alkylated protein returns to the FTV, where it awaits mixing with an enzyme and incubation in the 30°C reaction coil. The digested protein is then ready for LC injection and mass spectrometry detection.

Synthetic Process Parameters Monitoring and Product Quality Assessment by the A2P2 System

For synthetic applications, the A2P2 system can carry out reaction quenching, dilution, and chemical reactions before preparing the samples for LC or LC-MS injection. An analyst can manually deliver samples to the autosampler or use an automatic sampling system to do so. If additional sample preparations are required, two reaction coils, each with different temperature settings, are available for pre-injection sample preparation. If desalting is necessary, a desalting column can replace one of the coil positions to fulfill this task.

CONCLUSIONS

We have developed and deployed the LC-MS and LC integrated μSI process analyzer systems for online MAM and glycan measurements in addition to designing the turn-key A2P2 system to accelerate development of attribute-centric biotherapeutic manufacturing processes. Key design considerations included instrument footprint reduction, faster result turnaround, software-driven features, capability consolidation, and operational simplification. These systems are designed to be simple to use and enable rapid execution of complex assays that can inform process development. Additionally, they can be readily integrated into a strategy thereby enabling real-time, attribute-focused process development, monitoring, and control. Rapid availability of critical quality attribute data provides insights that can guide purposeful planning of subsequent experiments. Efficiency gains through the integration of fluidic miniaturization, autonomous diagnostics, and advanced analytics are substantial and lead to efficient development of processes that consistently manufacture high quality products.

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TABLES

Table 1: High level example of process steps for MAM sample preparation in the μSI process analyzer system

Process Step	Action
Sampling	Aspirate 2 mg protein sample from source
Affinity Cleanup	Protein retained, purified, and eluted from the affinity column and deposit into the holding coil dow
Denaturation	Deliver the purified protein from the holding coil into the 30C incubator. Add denaturation solution
Reduction	Add reduction solution into the 30C incubator. Incubate for 30 minutes
Alkylation	Add alkylation solution into the 30C incubator. Incubate for 20 minutes.
Quenching	Add quenching solution. Incubate for 5 minutes.
Desalting	Transfer the entire amount of mixture from the 30C incubator to the desalting column. Capture the
Enzymatic Digestion	Add enzyme solution into the 40C incubator. Incubate for 10 minutes.
LC Injection	Deliver the digested protein mixture to the LC sample loop and initiate LC-MS method.

Table 2: μSI process analyzer high-level process steps for glycan map sample preparation

Process Step	**Action**
Affinity Capture & Cleanup	Protein sample is captured, and its matrix components are removed.
Release of Glycan	Enzyme PNGase F is pushed into the affinity column where purified prote
Glycan Labeling	The release free glycans and glycan labeling reagent are mixed, degassed, a
Cooling and Enrichment of the labeled glycans	The reaction mixture is delivered to the cooling coil and labeled glycans a
Prepare for LC Injection	Labeled glycans are eluted from the PGC column. The organic content of

FIGURES

Figure 1 : The μSI process analyzer system for MAM sample preparation. (a) The fluidic diagram, (b) *Left* : The μSI process analyzer system connected to a LC-MS system on the manufacturing floor.*Right* : Close up view of the upper compartment inside the functionalized μSI process analyzer system.

Figure 2 : The μSI process analyzer system for glycosylation profile assay sample preparation. (a) The fluidic diagram, (b)*Left* : The μSI process analyzer system connected to a Waters Acquity UPLC system in development lab. *Right* : Close up to the upper compartment inside the functionalized μSI process analyzer system.

Figure 3 : The A2P2 system. (a) The fluidic diagram, (b) A configured A2P2 system utilizes a commercially available LC system. All components are integrated vertically for efficient use of laboratory footage. (c) The sample preparation unit operation beneath the autosampler in the A2P2 system. (RxN Coil: reaction coil)

Figure 4 : Engineering design of the FTP and its holder. (a)*Left* : FTV isometric view, cross-section of vial with arrows indicating liquid flow. *Middle* : FTV holder. *Right* : Full assembly of vial holder and FTV. (b) The FTV is a key part for the breadth of functionalities in the A2P2 system. The extended gutter and added lip to help prevent surface tension. Extrusion at the end of the gutter is conveniently placed directly over the waste port.

Figure 5 : The logical process developed by utilizing the LC chromatography data system software to enable autonomous operation in the A2P2 system. The uses of SST and IRC workflows enable autonomous and self-directed sample preparation and/or assay execution. (LLOQ: lower limit of quantitation, ULOQ: upper limit of quantitation, SEC: size exclusion, SST: system suitability testing, IRC: intelligent run control)

Figure 6 : (a) The TIC readout from the LC-MS connected μSI process analyzer system demonstrated good robustness over the course of 6 days continuous reference standard processing. (b) The real-time product quality output from a production bioreactor. The trending curves for deamidation and clips generated from the real-time MAM results can be used for process decisions, in-process control, and product release.

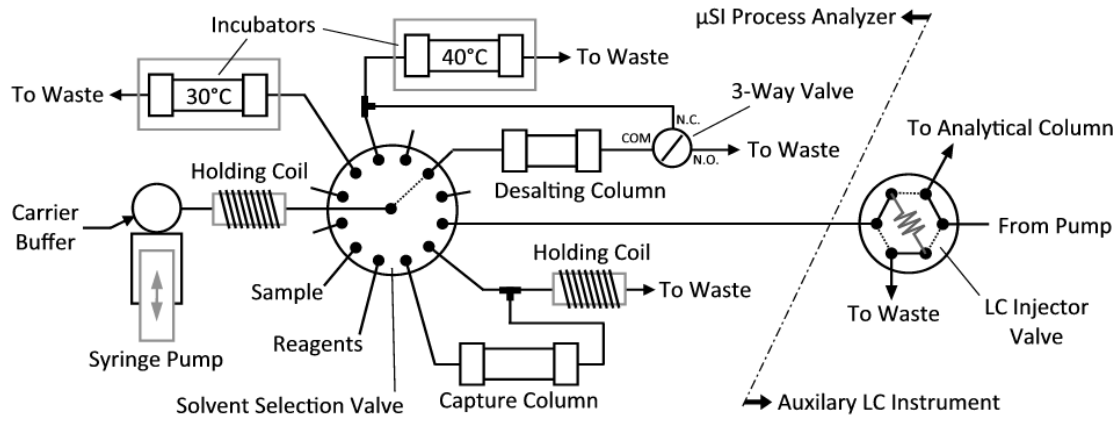
Figure 7 : (a) Glycan chromatogram generated from a reference standard processed by the integrated HILIC UPLC μSI process analyzer, full and zoomed view. (b) The performance of the system was verified by daily processing of the reference standard for 32 days. (c) Continuous glycan monitoring of a production bioreactor by the system over the duration of entire upstream bioproduction process.

Figure 8 : Capabilities of the A2P2 system. (a) Aggregation assessment for biological products, (b) Charge variant profile for biological products, (c) Glycosylation profile for biological products, (d) Multi-attribute method for biological products. Out-of-scope flow paths were masked for simplicity.

FIGURES

Figure 1:

(a)



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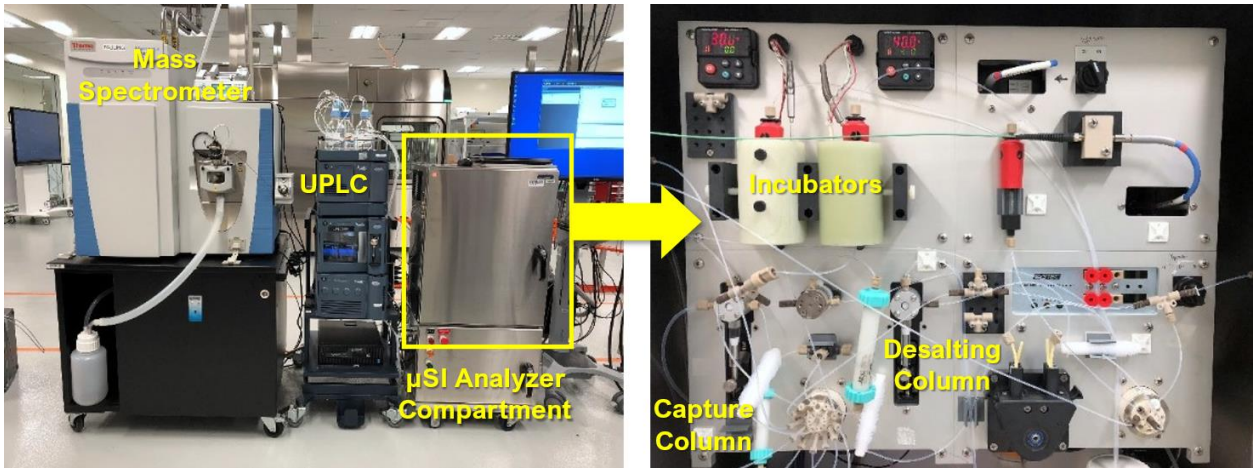
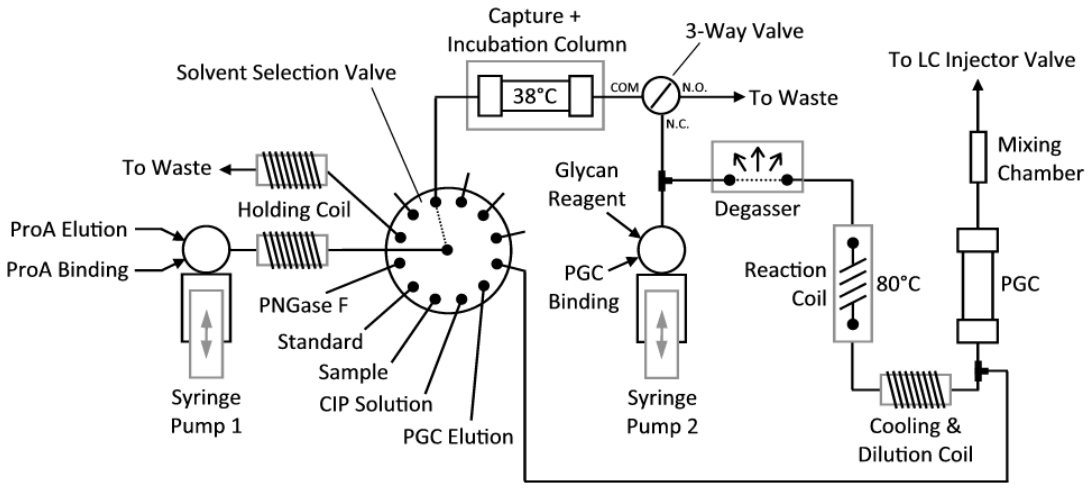


Figure 2:

(a)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ProA Binding
PGC
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80°C
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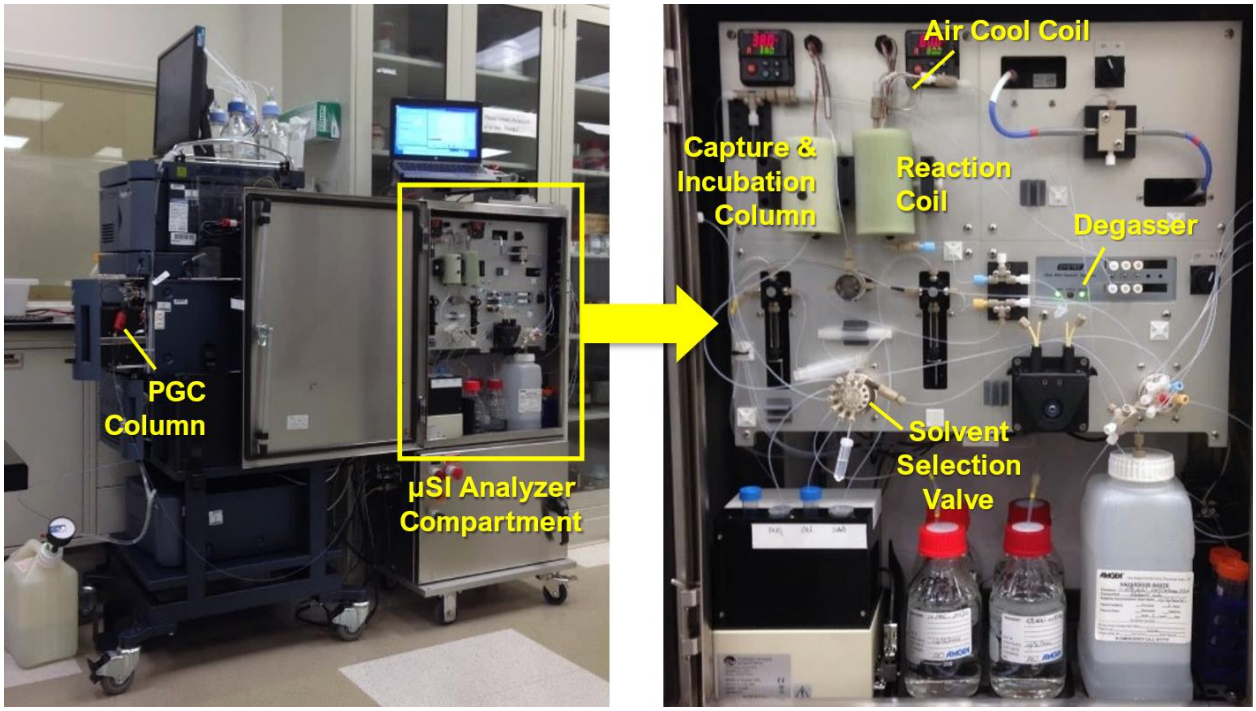
(b)


PGC
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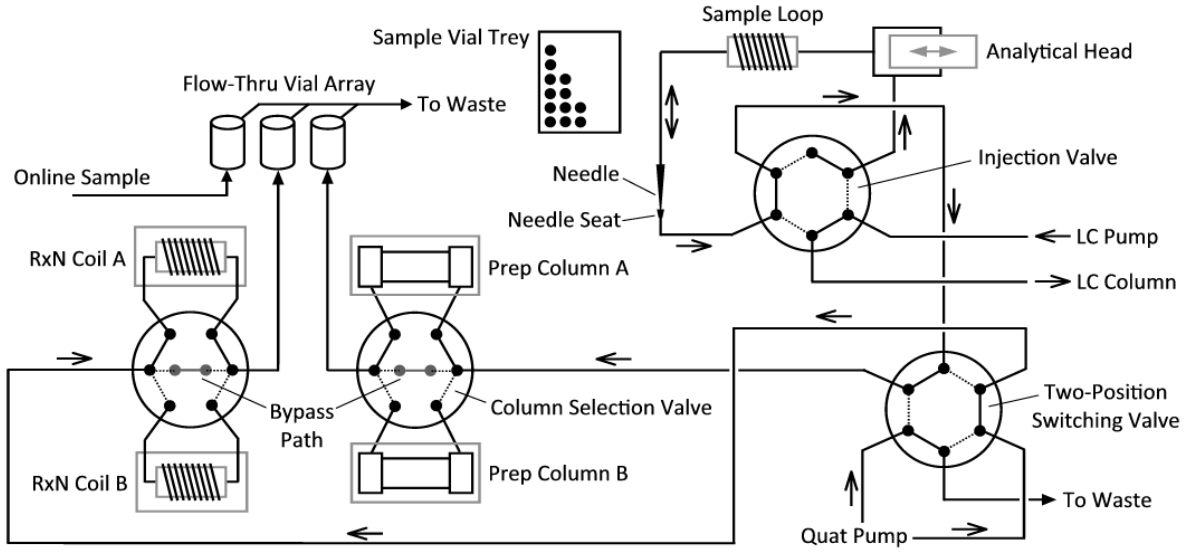
µSI Analyzer
Compartment
Air Cool Coil
Capture &
Incubation
Column
Reaction
Coil
Degasser
Solvent
Selection
Valve

Figure 3:

(a)


Sample Loop
Sample Vial Trey
Analytical Head
Flow-Thru Vial Array
To Waste
Injection Valve
Online Sample
Needle
Needle Seat
LC Pump
RxN Coil A
Prep Column A
LC Column
Two-Position
Switching Valve
Bypass
Path
Column Selection Valve
RxN Coil B
Prep Column B
To Waste
Quat Pump

(b)

(c)

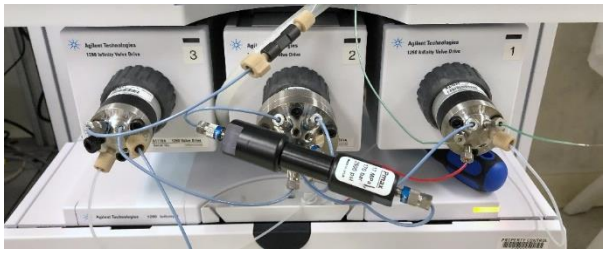
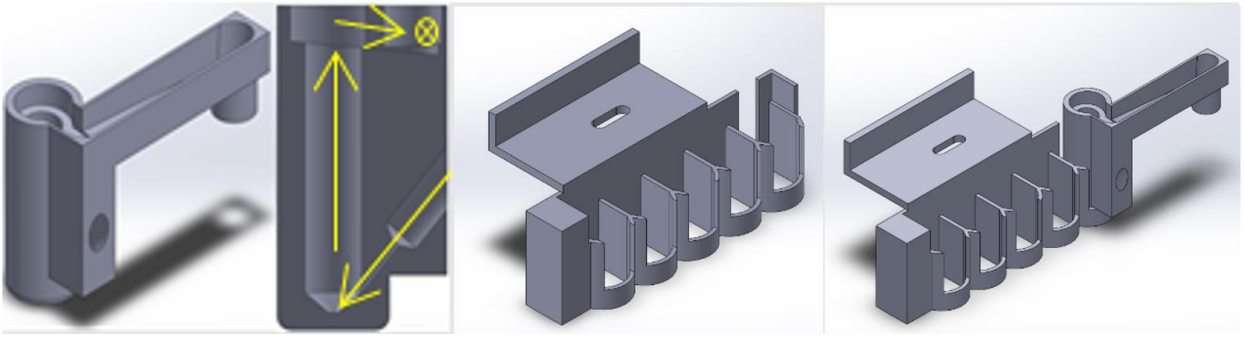

Agilent Technologies
3
2
1

Figure 4:

(a)



(b)

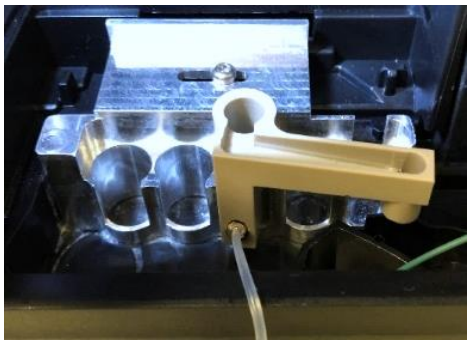


Figure 5:

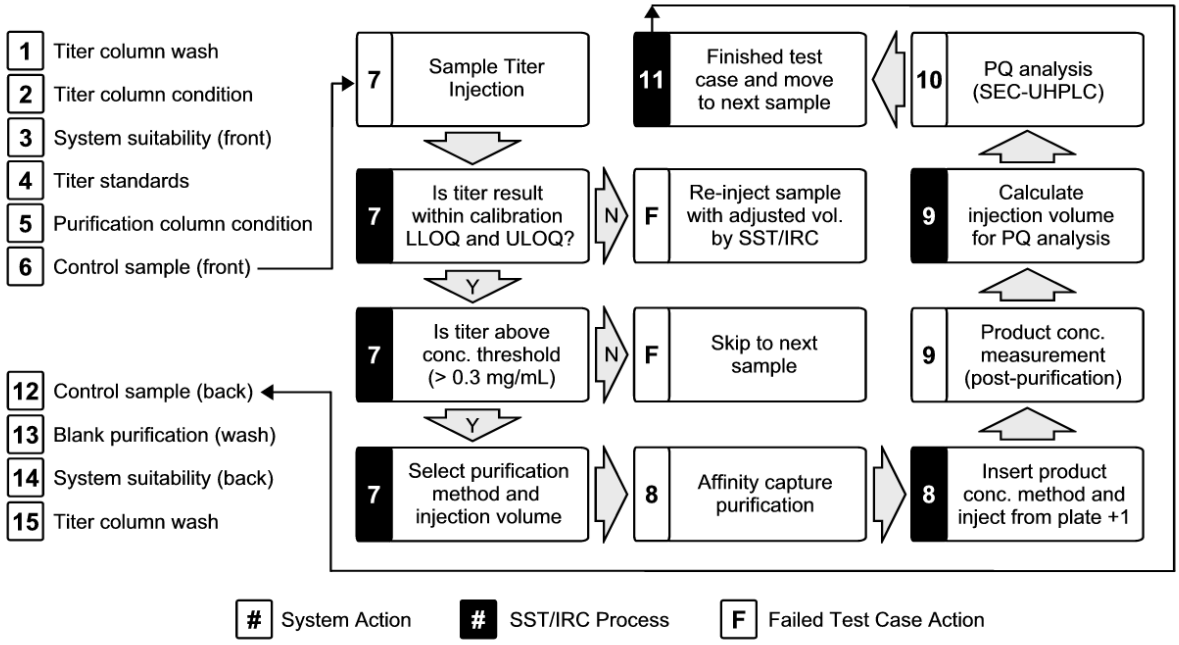
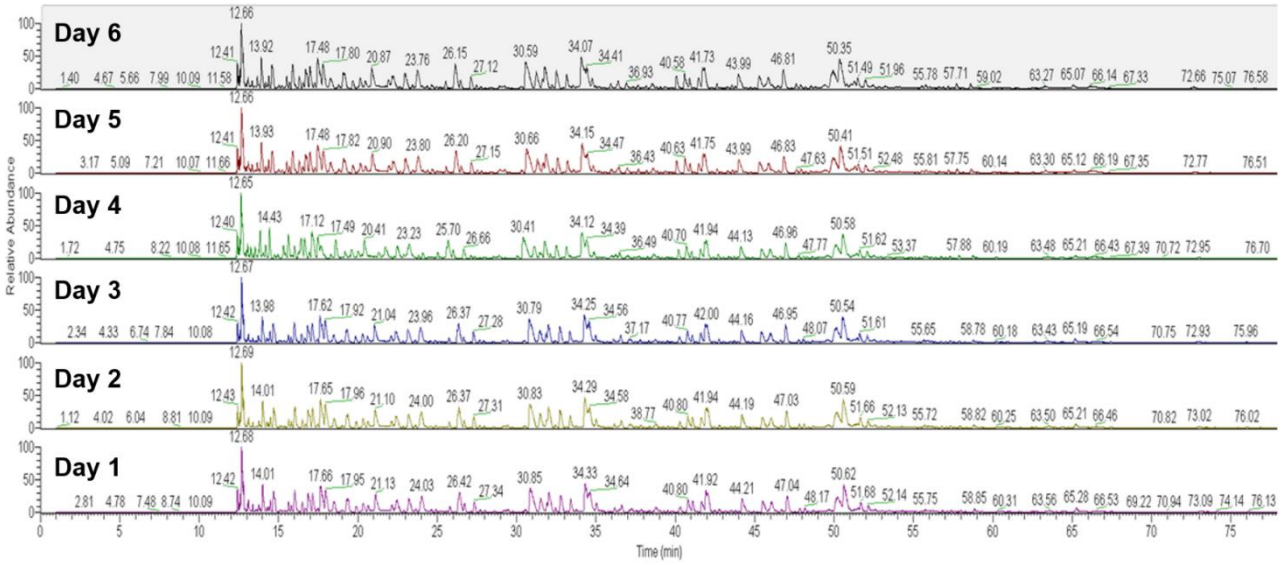

1 Titer column wash
2 Titer column condition
3 System suitability (front)
4 Titer standards
5 Purification column condition
6 Control sample (front)
12 Control sample (back)
13 Blank purification (wash)
14 System suitability (back)
15 Titer column wash
7 Sample Titer Injection
7 Is titer result within calibration LLOQ and ULOQ?
N
F Re-inject sample with adjusted vol. by SST/IRC
Y
7 Is titer above conc. threshold (> 0.3 mg/mL)
N
F Skip to next sample
Y
7 Select purification method and injection volume
8 Affinity capture purification
8 Insert product conc. method and inject from plate +1
9 Product conc. measurement (post-purification)
9 Calculate injection volume for PQ analysis
10 PQ analysis (SEC-UHPLC)
11 Finished test case and move to next sample
System Action
SST/IRC Process
F Failed Test Case Action

Figure 6:

(a)


Day 6
Day 5
Day 4
Day 3
Day 2
Day 1
Relative Abundance
Time (min)

(b)

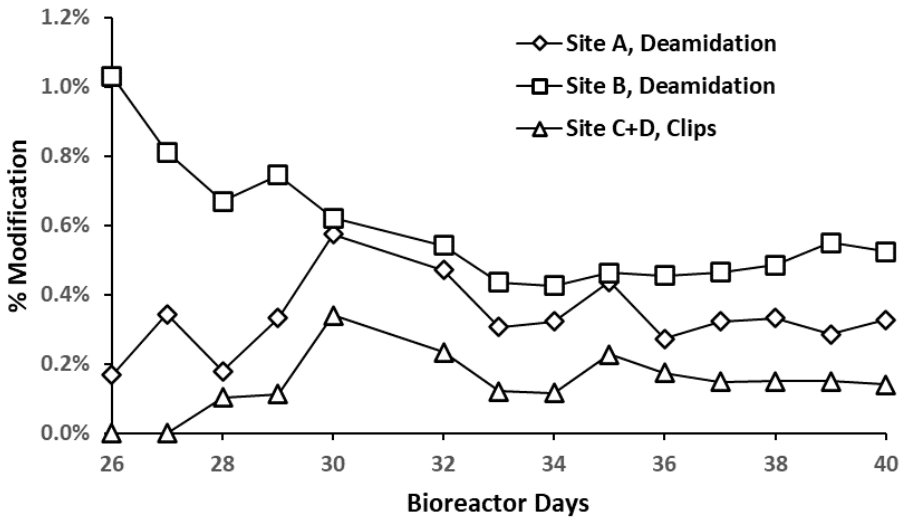
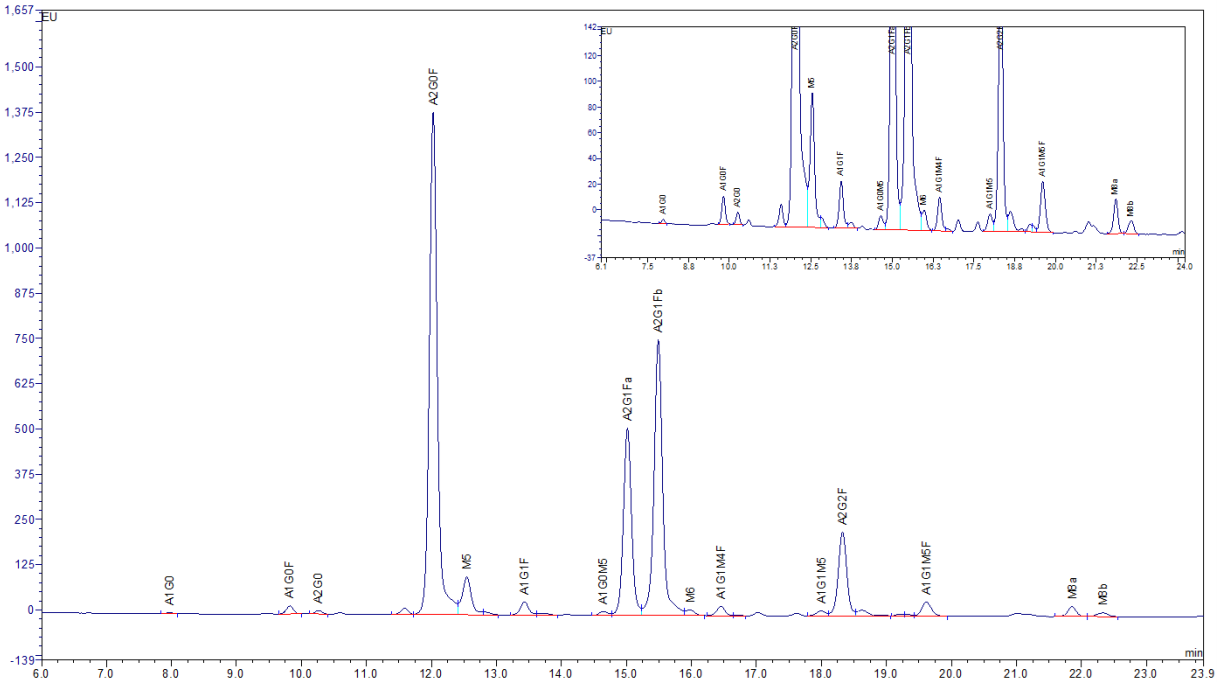
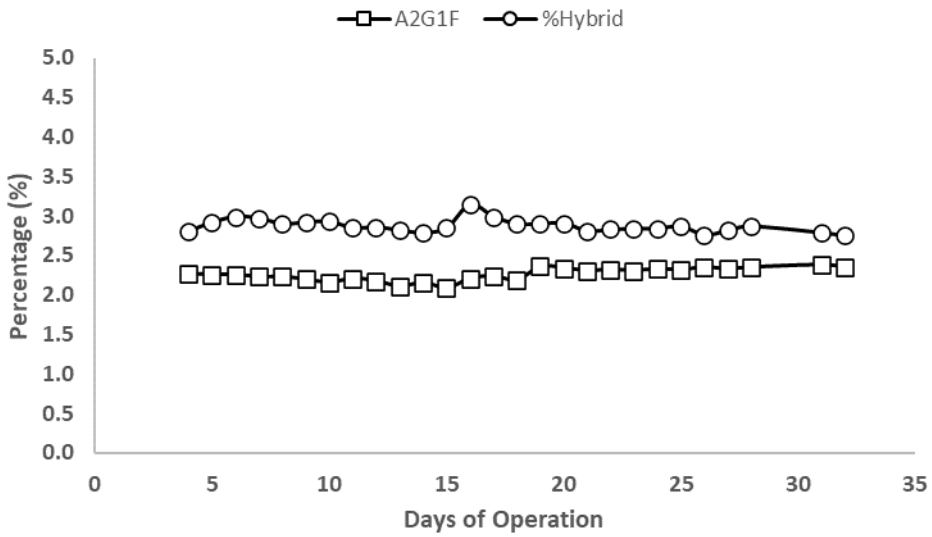

Site A, Deamidation
Site B, Deamidation
Site C+D, Clips
% Modification
Bioreactor Days

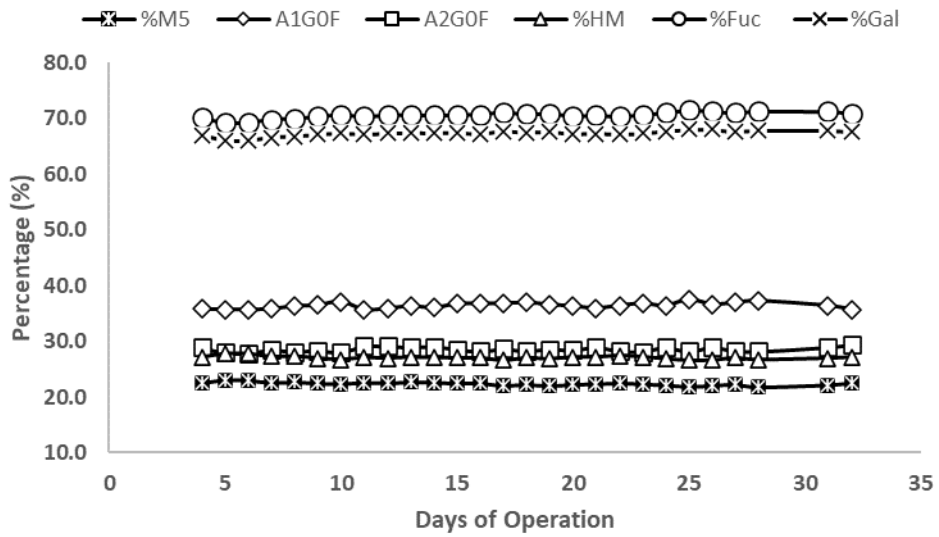
Figure 7:

(a)

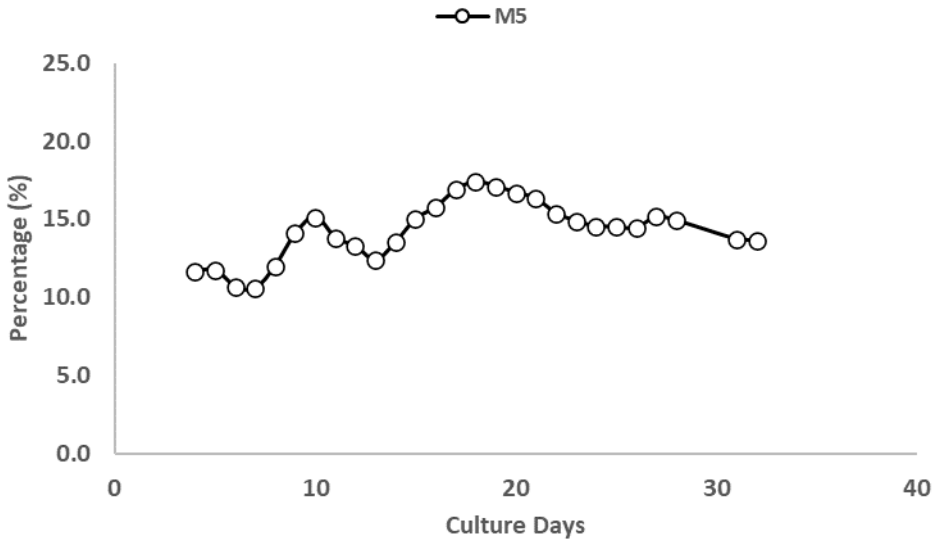

EU
A2G0F
M5
A1G1F
A1G0M5
A2G1Fb
A2G1Fa
M6
A1G1M4F
A1G1M5
A2G2F
A1G1M5F
M8a
M8b
min

(b)


A2G1F
%Hybrid
Percentage (%)
Days of Operation


%M5
A1G0F
A2G0F
%HM
%Fuc
%Gal
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
Percentage (%)
0
5
10
15
20
25
30
35
Days of Operation

(**c**)


M5
25.0
20.0
15.0
10.0
5.0
0.0
Percentage (%)
0
10
20
30
40
Culture Days

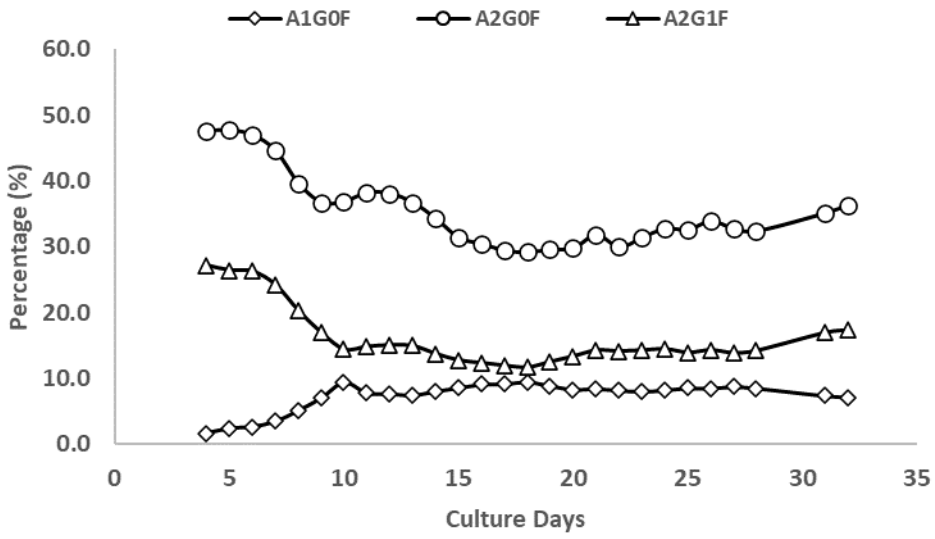
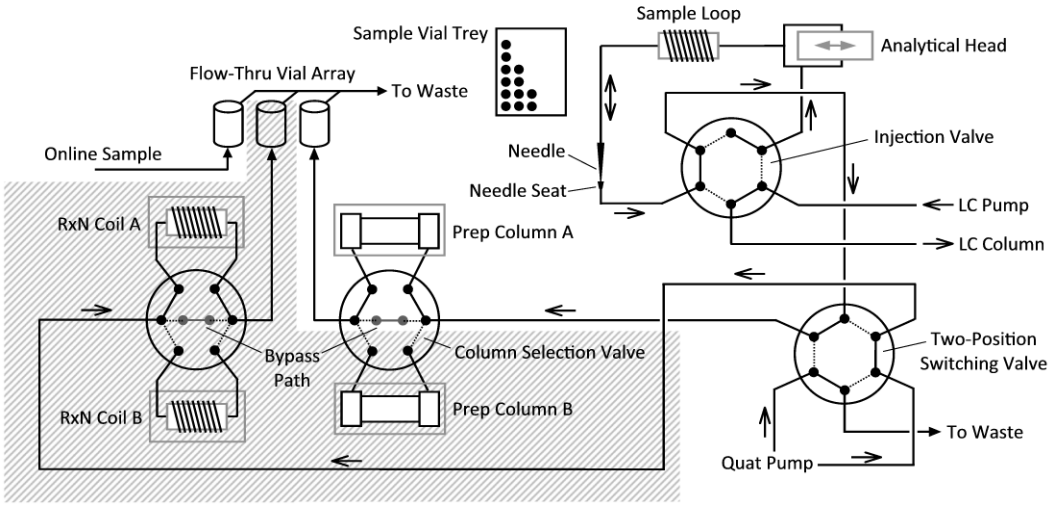
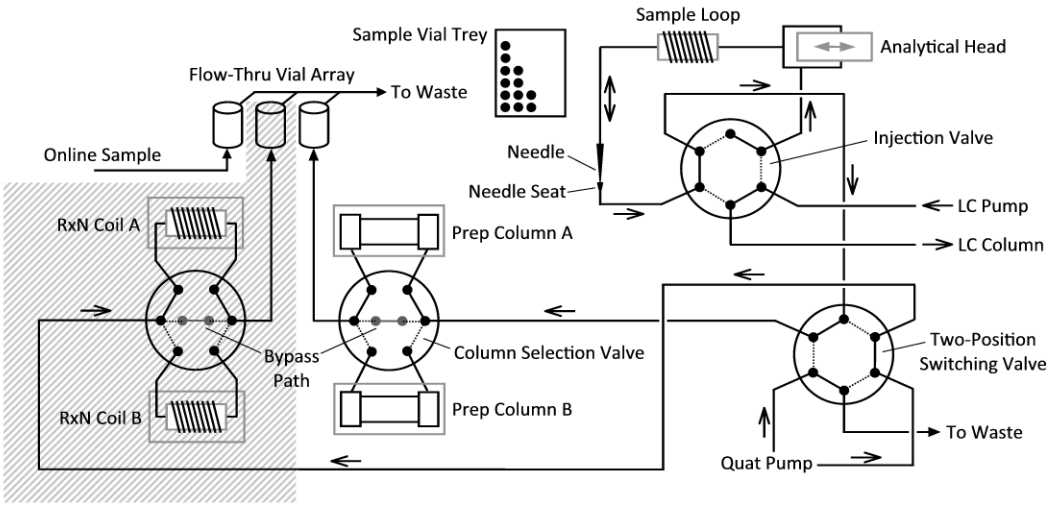

A1G0F
A2G0F
A2G1F
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Percentage (%)
0
5
10
15
20
25
30
35
Culture Days

Figure 8:

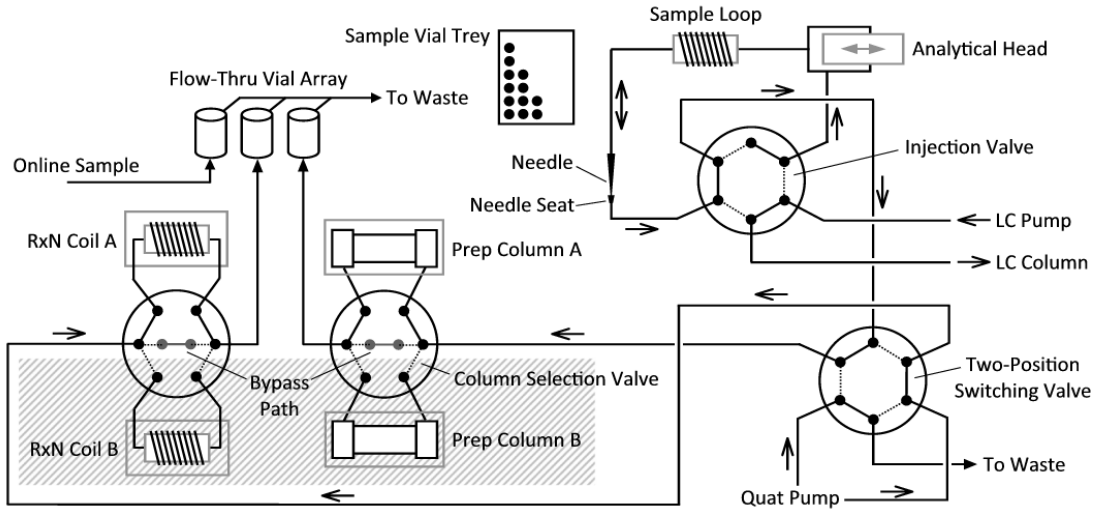
(a)


Sample Vial Trey
Sample Loop
Analytical Head
Flow-Thru Vial Array
To Waste
Online Sample
Needle
Injection Valve
Needle Seat
LC Pump
LC Column
RxN Coil A
Prep Column A
Bypass Path
Column Selection Valve
Two-Position Switching Valve
RxN Coil B
Prep Column B
To Waste
Quat Pump

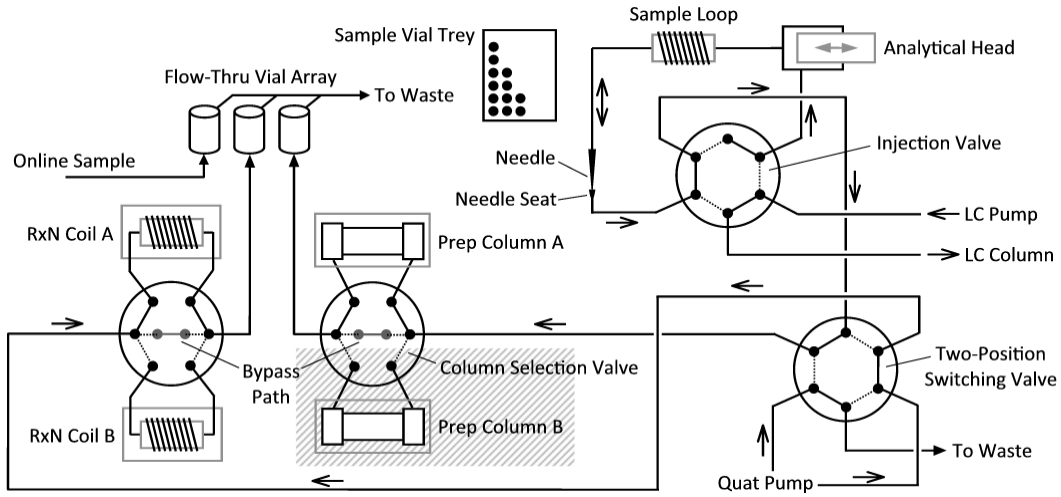
(b)


Sample Vial Trey
Sample Loop
Analytical Head
Flow-Thru Vial Array
To Waste
Online Sample
Needle
Injection Valve
Needle Seat
LC Pump
LC Column
RxN Coil A
Prep Column A
Bypass Path
Column Selection Valve
Two-Position Switching Valve
RxN Coil B
Prep Column B
To Waste
Quat Pump

(c)


Sample Vial Trey
Sample Loop
Analytical Head
Flow-Thru Vial Array
To Waste
Online Sample
Needle
Injection Valve
Needle Seat
LC Pump
LC Column
RxN Coil A
Prep Column A
Bypass Path
Column Selection Valve
Two-Position Switching Valve
RxN Coil B
Prep Column B
To Waste
Quat Pump

(d)


Sample Loop
Sample Vial Trey
Analytical Head
Flow-Thru Vial Array
To Waste
Injection Valve
Online Sample
Needle
Needle Seat
LC Pump
RxN Coil A
Prep Column A
LC Column
Two-Position
Switching Valve
Bypass
Path
Column Selection Valve
RxN Coil B
Prep Column B
To Waste
Quat Pump