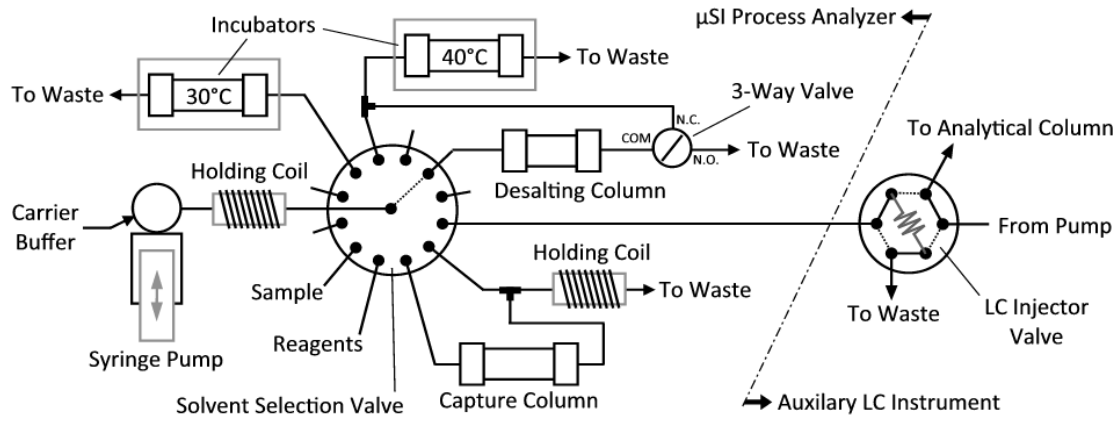
FIGURES

Figure 1:

(a)



(b)

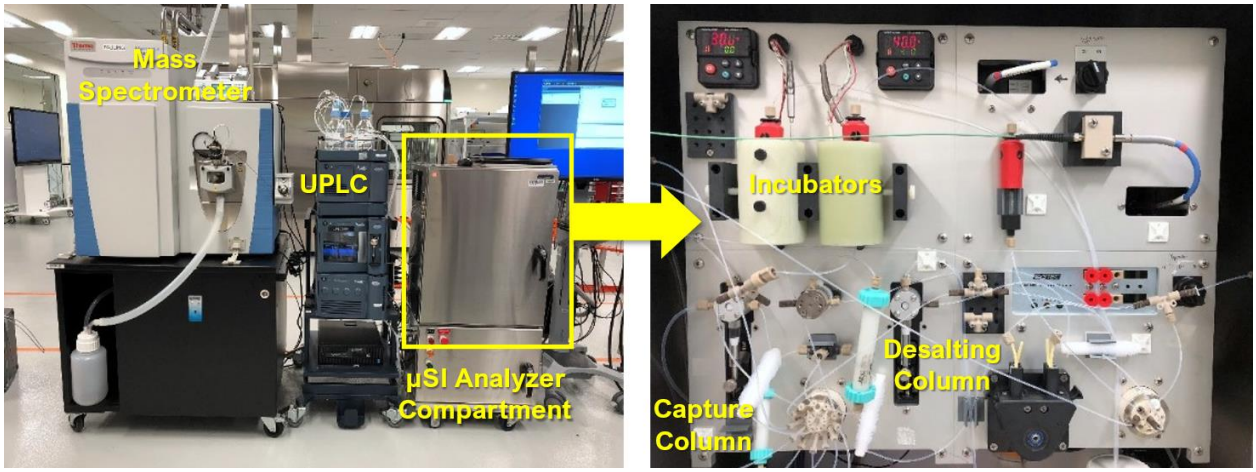
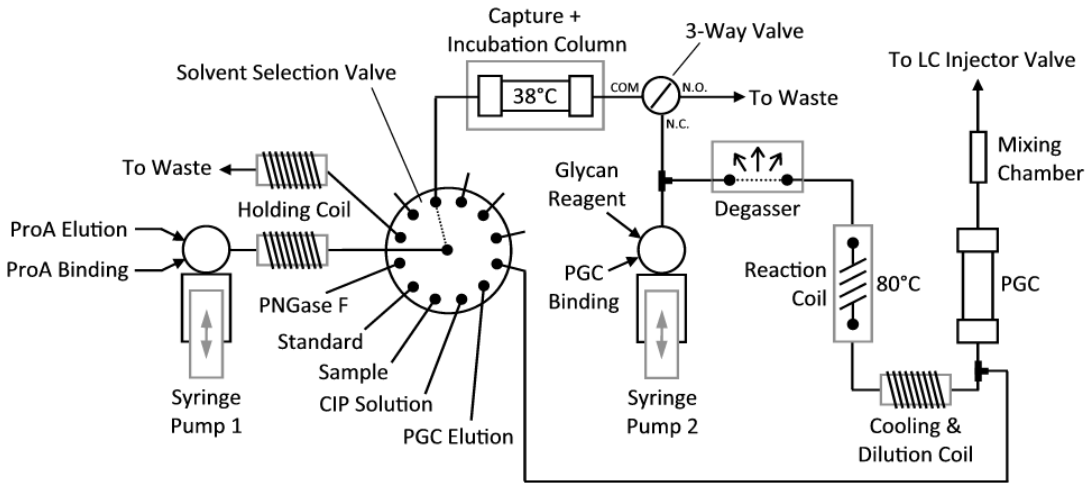


Figure 2:

(a)


Capture +
Incubation Column
3-Way Valve
Solvent Selection Valve
38°C
COM
N.O.
N.C.
To Waste
To LC Injector Valve
Mixing
Chamber
To Waste
Holding Coil
Glycan
Reagent
Degasser
ProA Elution
ProA Binding
PGC
Binding
Reaction
Coil
80°C
PGC
PNGase F
Standard
Sample
CIP Solution
PGC Elution
Syringe
Pump 1
Syringe
Pump 2
Cooling &
Dilution Coil

(b)

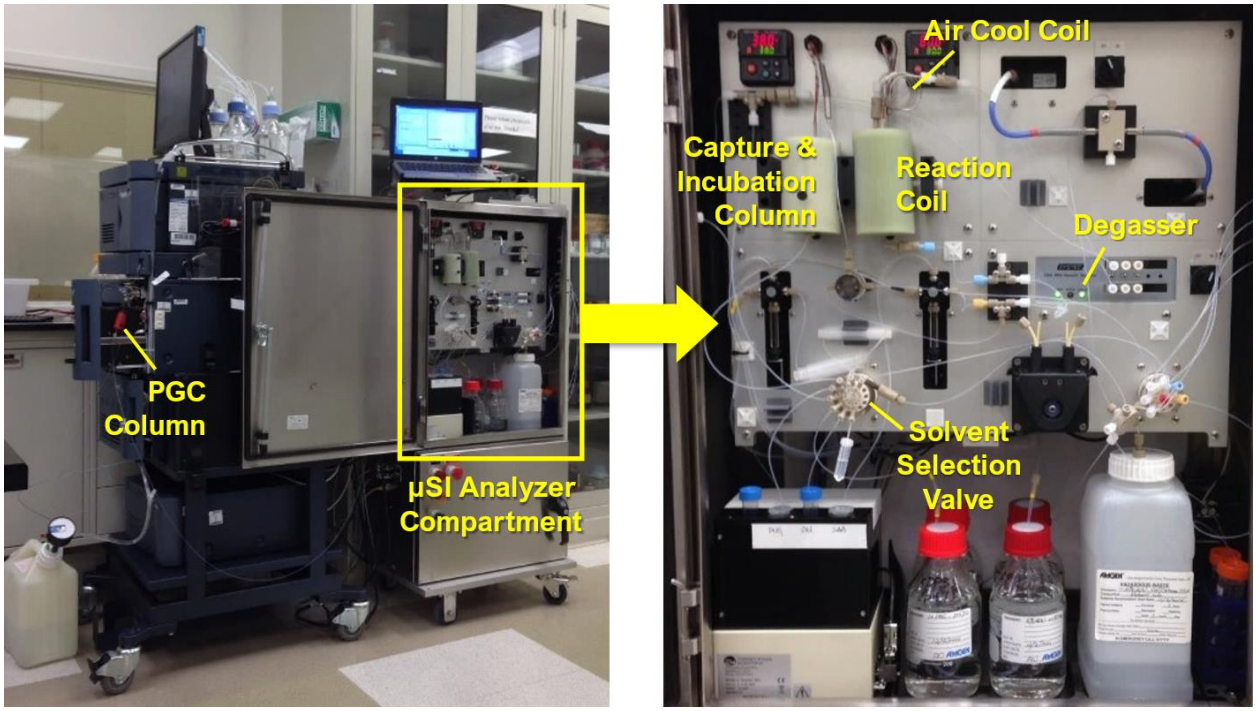
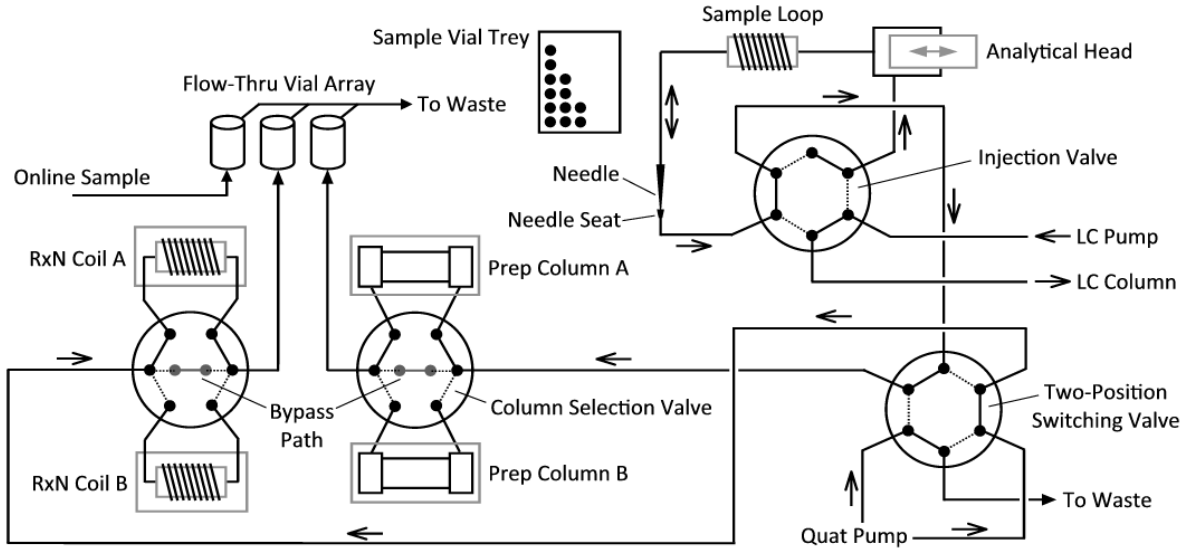

PGC
Column
µSI Analyzer
Compartment
Air Cool Coil
Capture &
Incubation
Column
Reaction
Coil
Degasser
Solvent
Selection
Valve

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)

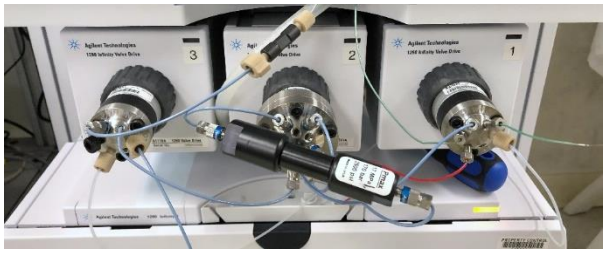
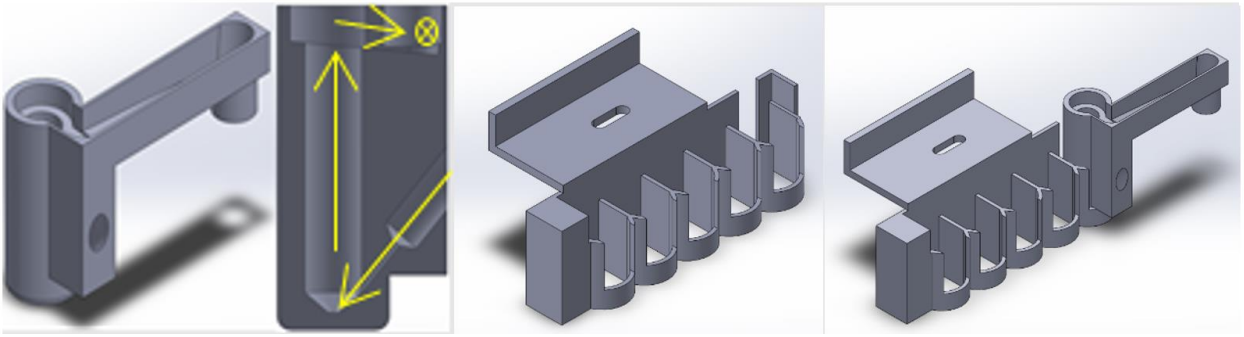

Agilent Technologies
3
2
1

Figure 4:

(a)



(b)

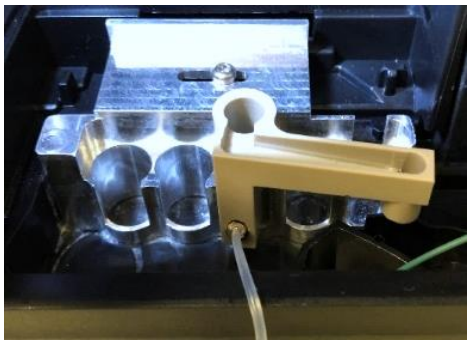


Figure 5:

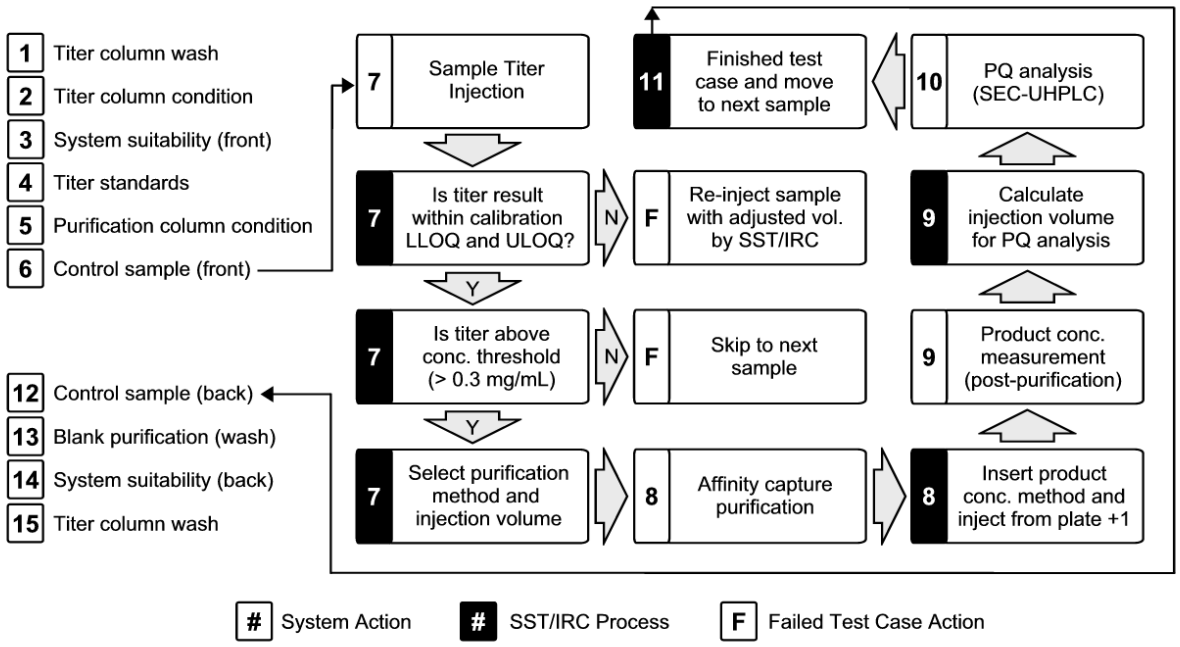
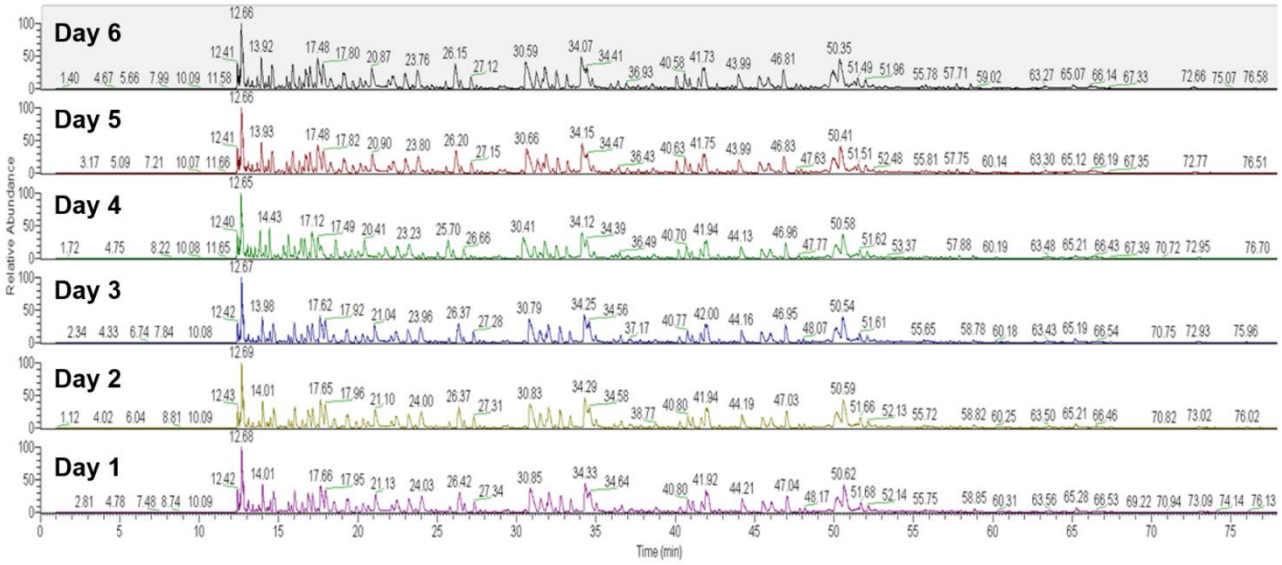

1 Titer column wash
2 Titer column condition
3 System suitability (front)
4 Titer standards
5 Purification column condition
6 Control sample (front)
12 Control sample (back)
13 Blank purification (wash)
14 System suitability (back)
15 Titer column wash
7 Sample Titer Injection
7 Is titer result within 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)

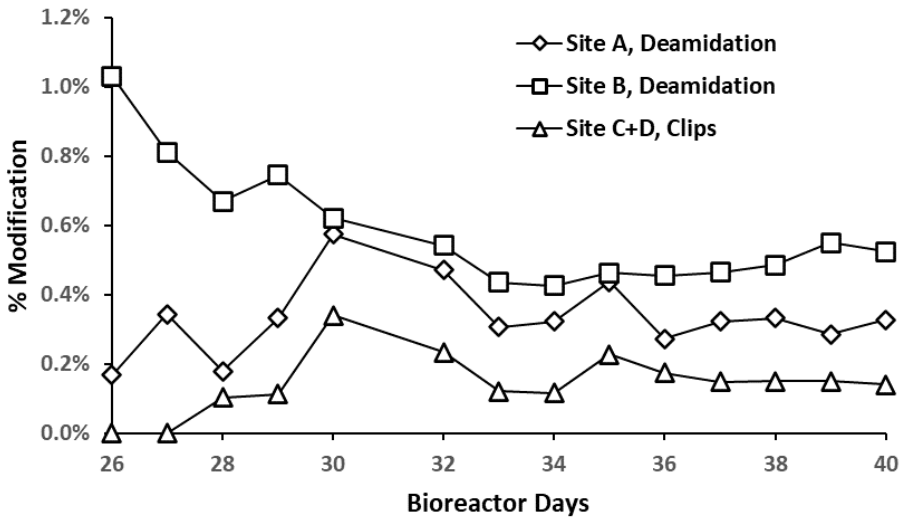
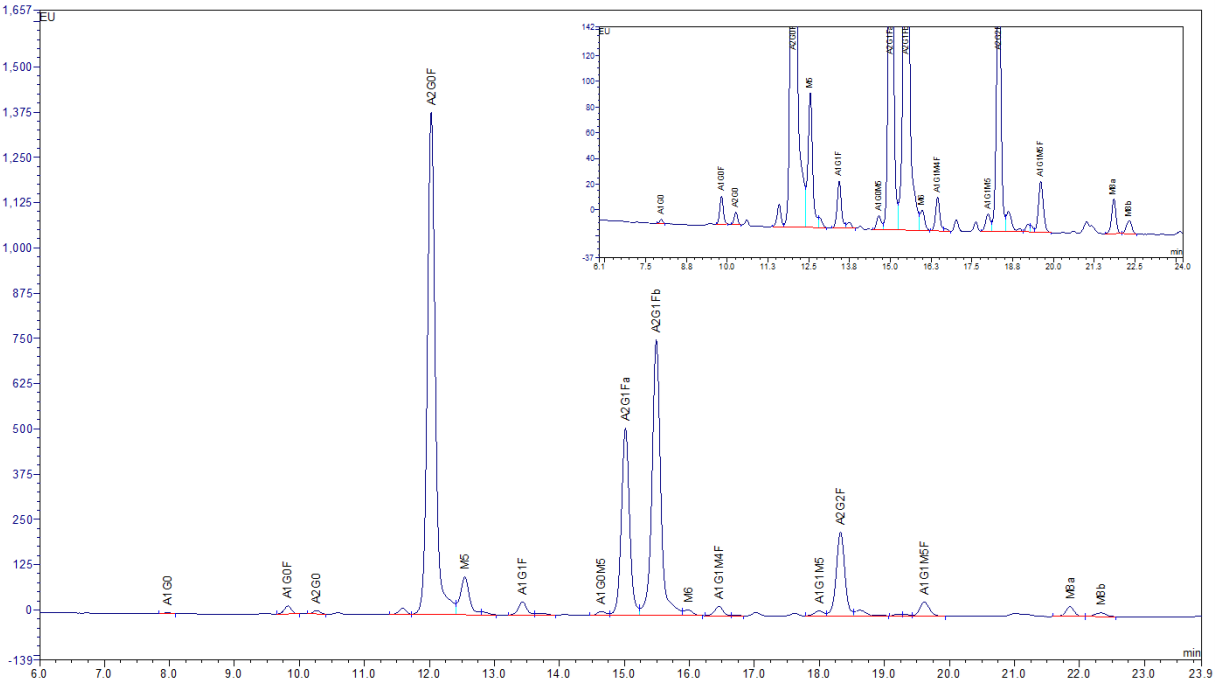
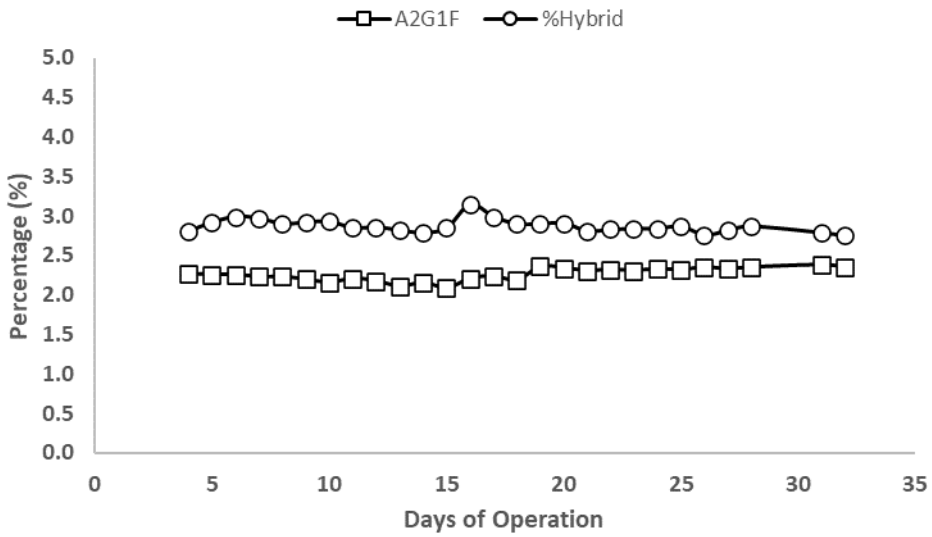

Site A, Deamidation
Site B, Deamidation
Site C+D, Clips
% Modification
Bioreactor Days

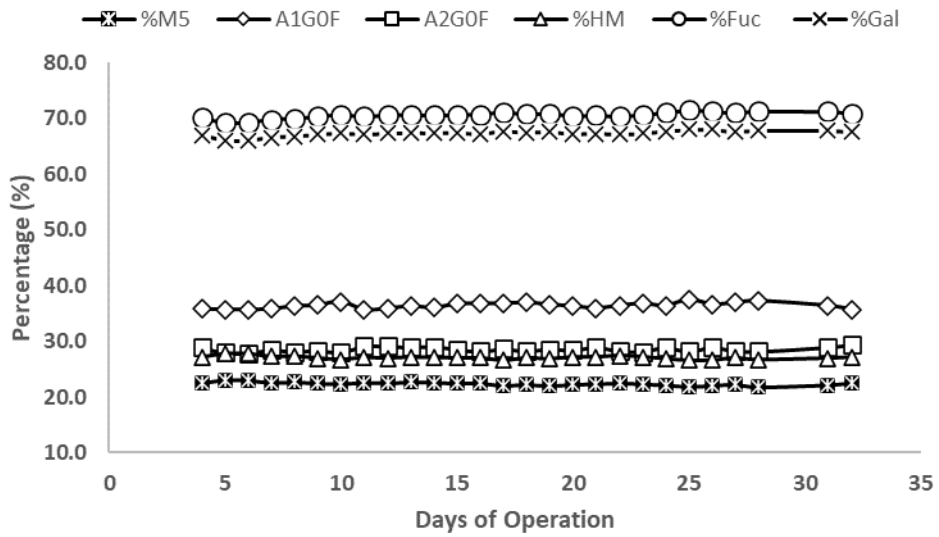
Figure 7:

(a)

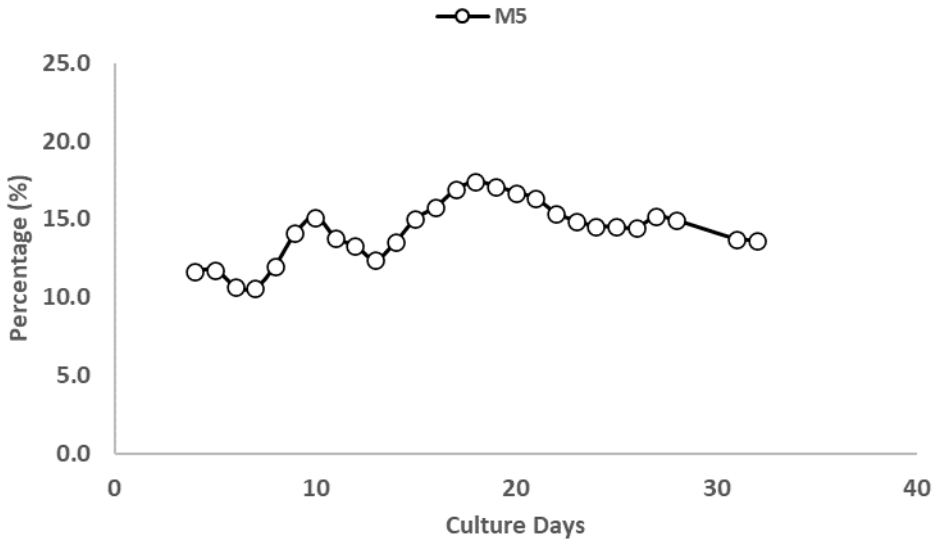

EU
A2G0F
M5
A1G1F
A2G1Fa
A2G1Fb
M6
A1G1M4F
A2G2F
A1G1M5F
M8a
M8b
min

(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

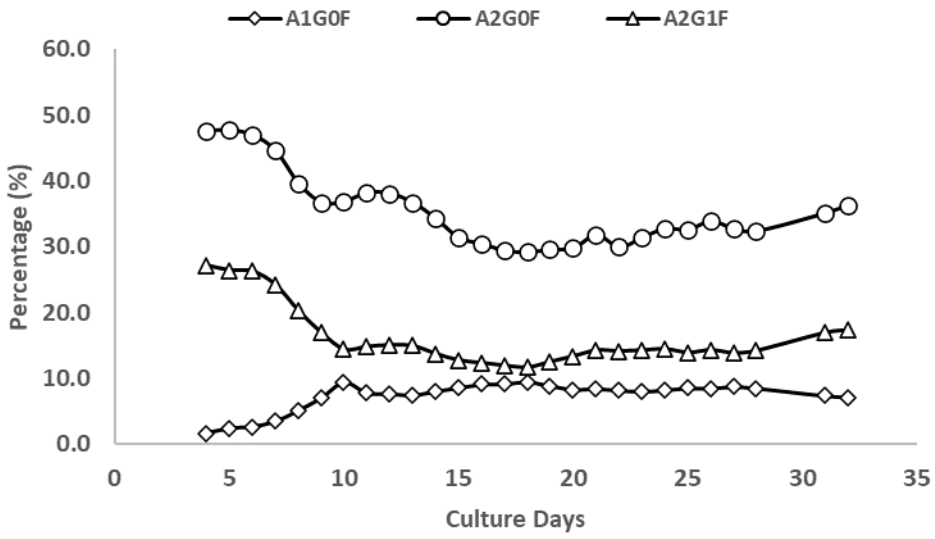
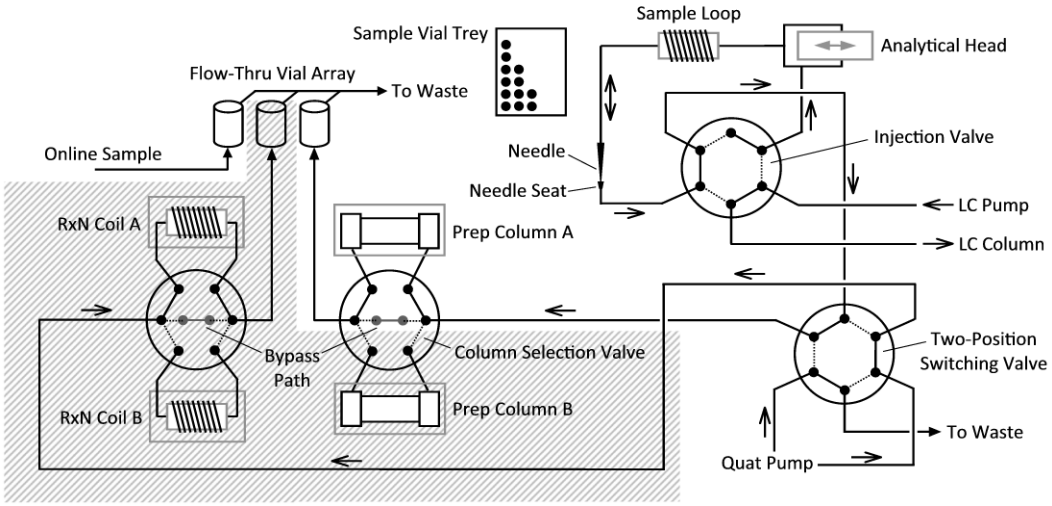
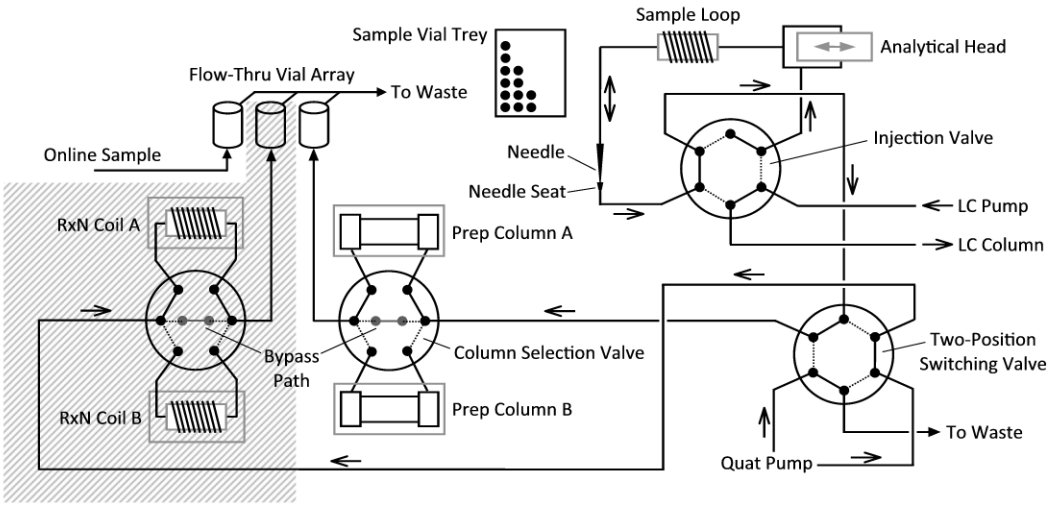

A1G0F
A2G0F
A2G1F
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Percentage (%)
0
5
10
15
20
25
30
35
Culture Days

Figure 8:

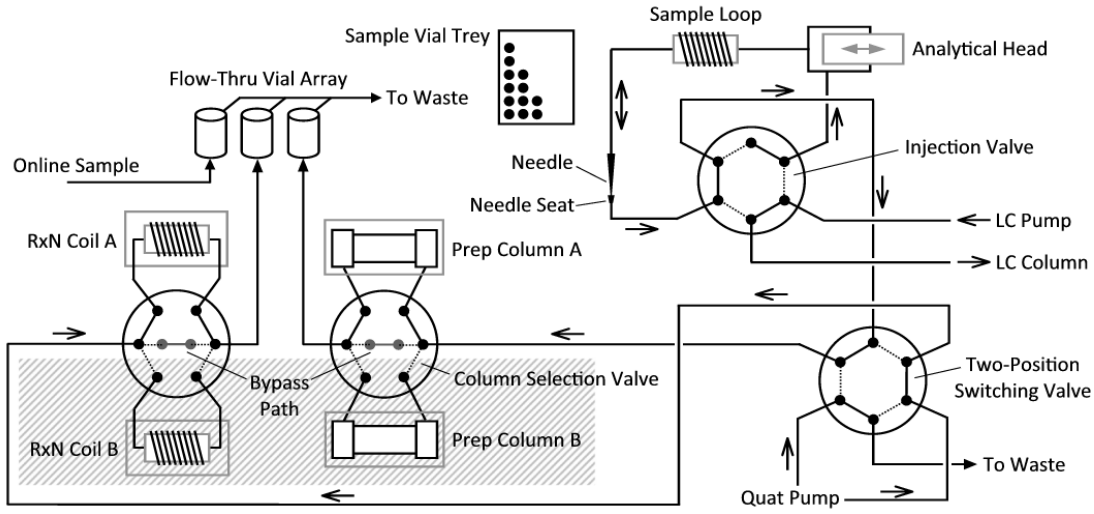
(a)


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

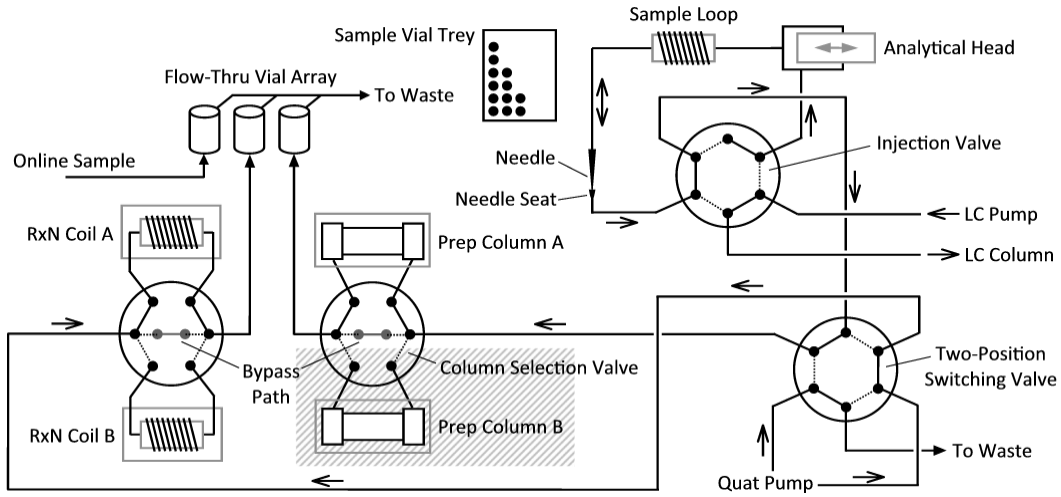
(b)


Sample Vial Trey
Sample Loop
Analytical Head
Flow-Thru Vial Array
To Waste
Online Sample
Needle
Injection Valve
Needle Seat
LC Pump
RxN Coil A
Prep Column A
LC Column
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
RxN Coil A
Prep Column A
LC Column
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
Needle
Online Sample
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