

## Acquisition Method Info

Method Name: BileAcids.m  
 Method Path: D:\MassHunter\methods\User\Cristina\BileAcids.m  
 Method Description: BA and AlloBA 2019-05-07 CGC+ CYP7A1 activity metabolites updated 2020-06-19

Device List  
 HiP Sampler  
 Binary Pump  
 Column Comp.  
 DAD  
 QQQ

### MS QQQ Mass Spectrometer

Ion Source: AJS ESI      Tune File: D:\MassHunter\Tune\QQQ\G6490A\atunes.TUNE.XML  
 Stop Mode: No Limit/As Pump      Stop Time (min): No limit  
 Time Filter: On      Time Filter Width (min): 0.07

#### Time Segments

Index	Start Time (min)	Scan Type	Ion Mode	Div Valve	Delta EMV (+)	Store	Delta EMV (-)
1	0	MS2 Scan	ESI+Agilent Jet Stream	To Waste	0	No	
2	2	MRM	ESI+Agilent Jet Stream	To MS	300	Yes	
3	4.5	MRM	ESI+Agilent Jet Stream	To MS	300	Yes	300
4	5.95	MRM	ESI+Agilent Jet Stream	To MS	300	Yes	300
5	7.15	MRM	ESI+Agilent Jet Stream	To MS	300	Yes	300
6	9	MRM	ESI+Agilent Jet Stream	To MS	300	Yes	300
7	11	MRM	ESI+Agilent Jet Stream	To MS	300	Yes	300
8	12.6	MRM	ESI+Agilent Jet Stream	To MS	300	Yes	300
9	15	MRM	ESI+Agilent Jet Stream	To MS	300	Yes	
10	18	MRM	ESI+Agilent Jet Stream	To MS	300	Yes	
11	22.5	MRM	ESI+Agilent Jet Stream	To Waste	0	No	

#### Time Segment 1

##### Scan Segments

Segment Name	Start Mass	End Mass	Scan Time	Frag (V)	Cell Acc (V)	Polarity
	100	1000	500	380	5	Positive

##### Scan Parameters

Step Size (amu): 0.1  
 Data Stg: Profile  
 Threshold: 0

##### Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	15	15
Nebulizer (psi)	20	20
SheathGasHeater	250	250
SheathGasFlow	11	11
Capillary (V)	3000	0
VCharging	2000	0

## Time Segment 2

## Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
	T_alpha_M CA	No	480.3	Wide / Unit (6490)	480.3	Wide / Unit (6490)	20	380	0	5	Positive
	T_alpha_M CA	No	480.3	Wide / Unit (6490)	462.1	Wide / Unit (6490)	80	380	8	5	Positive
	T_alpha_M CA	No	480.3	Wide / Unit (6490)	126.1	Wide / Unit (6490)	80	380	24	5	Positive
	T_beta_M CA	No	480.3	Wide / Unit (6490)	126	Wide / Unit (6490)	80	380	24	5	Positive
	T_beta_M CA	No	480.2	Wide / Unit (6490)	462.2	Wide / Unit (6490)	80	380	8	5	Positive
	T_omega_ MCA	No	480.2	Wide / Unit (6490)	462.1	Wide / Unit (6490)	80	380	8	5	Positive
	T_omega_ MCA	No	480.2	Wide / Unit (6490)	126	Wide / Unit (6490)	80	380	24	5	Positive

## Scan Parameters

Data Stg	Threshold
Centroid	0

## Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	15	15
Nebulizer (psi)	20	20
SheathGasHeater	250	250
SheathGasFlow	11	11
Capillary (V)	4000	0
VCharging	2000	0

## Ion Funnel Parameters

Pos High Pressure RF	120	Neg High Pressure RF	0
Pos Low Pressure RF	40	Neg Low Pressure RF	0

## Time Segment 3

## Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
	Tauro_7ox oLCA	No	480.3	Wide / Unit (6490)	480.3	Wide / Unit (6490)	20	380	0	5	Positive
	Tauro_7ox oLCA	No	480.3	Wide / Unit (6490)	462.1	Wide / Unit (6490)	60	380	8	5	Positive
	Tauro_7ox oLCA TCA	No	480.3	Wide / Unit (6490)	461.9	Wide / Unit (6490)	60	380	8	5	Positive
	Tauro_7ox oLCA	No	480.3	Wide / Unit (6490)	126	Wide / Unit (6490)	60	380	24	5	Positive
	TUDCA	No	464.2	Wide / Unit (6490)	464.2	Wide / Unit (6490)	15	380	0	5	Positive
	TUDCA	No	464.2	Wide / Unit (6490)	126	Wide / Unit (6490)	60	380	28	5	Positive
	TCA	No	514.2	Wide / Unit (6490)	124.2	Wide / Unit (6490)	60	380	46	5	Negative
	TUDCA	No	498.2	Wide / Unit (6490)	124.2	Wide / Unit (6490)	60	380	47	5	Negative

## Scan Parameters

Data Stg	Threshold
Centroid	0

## Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	15	15
Nebulizer (psi)	20	20
SheathGasHeater	250	250
SheathGasFlow	11	11
Capillary (V)	4500	4500
VCharging	2000	2000

## Ion Funnel Parameters

Pos High Pressure RF	140	Neg High Pressure RF	140
Pos Low Pressure RF	40	Neg Low Pressure RF	40

Time Segment 4

Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion MS1 Res	Prod Ion MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
	beta_MCA	No	391.3 Wide / Unit (6490)	355.2 Wide / Unit (6490)	35	380	16	5	Positive
	omega_MCA	No	373.3 Wide / Unit (6490)	355.2 Wide / Unit (6490)	35	380	20	5	Positive
	beta_MCA	No	373.3 Wide / Unit (6490)	355.2 Wide / Unit (6490)	35	380	8	5	Positive
	alpha_MCA	No	373.3 Wide / Unit (6490)	355.1 Wide / Unit (6490)	15	380	15	5	Positive
	omega_MCA	No	373.3 Wide / Unit (6490)	159.1 Wide / Unit (6490)	35	380	20	5	Positive
	alpha_MCA	No	373.3 Wide / Unit (6490)	105.1 Wide / Unit (6490)	35	380	58	5	Positive
	7oxoDCA	No	371.3 Wide / Unit (6490)	353.2 Wide / Unit (6490)	35	380	8	5	Positive
	7oxoDCA	No	371.3 Wide / Unit (6490)	335.2 Wide / Unit (6490)	35	380	12	5	Positive
	d4GCA	Yes	468.2 Wide / Unit (6490)	74 Wide / Unit (6490)	35	380	49	5	Negative
	GCA	No	464.2 Wide / Unit (6490)	464.2 Wide / Unit (6490)	15	380	0	5	Negative
	GCA	No	464.2 Wide / Unit (6490)	74 Wide / Unit (6490)	35	380	37	5	Negative
	d4GUDCA	Yes	452.2 Wide / Unit (6490)	74 Wide / Unit (6490)	35	380	37	5	Negative
	GUDCA	No	448.2 Wide / Unit (6490)	404.1 Wide / Unit (6490)	35	380	40	5	Negative
	GUDCA	No	448.2 Wide / Unit (6490)	386.1 Wide / Unit (6490)	35	380	35	5	Negative
	GUDCA	No	448.2 Wide / Unit (6490)	74 Wide / Unit (6490)	35	380	37	5	Negative

Scan Parameters

Data Stg	Threshold
Centroid	0

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	15	15
Nebulizer (psi)	40	40
SheathGasHeater	250	250
SheathGasFlow	11	11
Capillary (V)	5000	5000
VCharging	2000	2000

Ion Funnel Parameters

Pos High Pressure RF	100	Neg High Pressure RF	100
Pos Low Pressure RF	60	Neg Low Pressure RF	60

Time Segment 5

Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion MS1 Res	Prod Ion MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
	TDCA	No	464.2 Wide / Unit (6490)	464.2 Wide / Unit (6490)	20	380	0	5	Positive
	TCDCa	No	464.2 Wide / Unit (6490)	464.1 Wide / Unit (6490)	20	380	0	5	Positive
	TDCA	No	464.2 Wide / Unit (6490)	126.1 Wide / Unit (6490)	50	380	28	5	Positive
	TCDCa	No	464.2 Wide / Unit (6490)	126 Wide / Unit (6490)	50	380	28	5	Positive
	gamma_M CA	No	391.3 Wide / Unit (6490)	355.2 Wide / Unit (6490)	50	380	16	5	Positive
	gamma_M CA	No	373.3 Wide / Unit (6490)	355 Wide / Unit (6490)	50	380	10	5	Positive
	gamma_M CA	No	373.3 Wide / Unit (6490)	304.8 Wide / Unit (6490)	50	380	4	5	Positive
	TCDCa	No	498.2 Wide / Unit (6490)	124.2 Wide / Unit (6490)	50	380	45	5	Negative
	TDCA	No	498.2 Wide / Unit (6490)	124.1 Wide / Unit (6490)	50	380	45	5	Negative
	G7oxoLCA	No	446.2 Wide / Unit (6490)	330.1 Wide / Unit (6490)	50	380	49	5	Negative
	G7oxoLCA	No	446.2 Wide / Unit (6490)	74 Wide / Unit (6490)	50	380	37	5	Negative

Scan Parameters

Data Stg	Threshold
Centroid	0

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	15	15
Nebulizer (psi)	20	20
SheathGasHeater	250	250
SheathGasFlow	11	11
Capillary (V)	4000	4000
VCharging	2000	2000

Ion Funnel Parameters

Pos High Pressure RF	120	Neg High Pressure RF	120
Pos Low Pressure RF	60	Neg Low Pressure RF	60

Time Segment 6

Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion MS1 Res	Prod Ion MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
	d4CA	Yes	377.3 Wide / Unit (6490)	359.2 Wide / Unit (6490)	30	380	8	5	Positive
	CA	No	373.3 Wide / Unit (6490)	355.2 Wide / Unit (6490)	30	380	8	5	Positive
	AlloCA	No	373.3 Wide / Unit (6490)	355.2 Wide / Unit (6490)	30	380	12	5	Positive
	CA	No	373.3 Wide / Unit (6490)	159.1 Wide / Unit (6490)	30	380	20	5	Positive
	d4UDCA	Yes	361.2 Wide / Unit (6490)	95.1 Wide / Unit (6490)	30	380	40	5	Positive
	HDCA	No	357.3 Wide / Unit (6490)	104.8 Wide / Unit (6490)	30	380	50	5	Positive
	HDCA	No	357.2 Wide / Unit (6490)	95.1 Wide / Unit (6490)	30	380	40	5	Positive
	UDCA	No	357.2 Wide / Unit (6490)	95 Wide / Unit (6490)	30	380	35	5	Positive
	UDCA	No	357.2 Wide / Unit (6490)	80.9 Wide / Unit (6490)	30	380	50	5	Positive
	d4GCDCA	Yes	452.2 Wide / Unit (6490)	74 Wide / Unit (6490)	30	380	37	5	Negative
	GCDCA	No	448.2 Wide / Unit (6490)	404.1 Wide / Unit (6490)	30	380	32	5	Negative
	GCDCA	No	448.2 Wide / Unit (6490)	74 Wide / Unit (6490)	30	380	30	5	Negative
	AlloCA	No	407.3 Wide / Unit (6490)	361.2 Wide / Unit (6490)	30	380	36	5	Negative
	Allo3bDCA	No	391.3 Wide / Unit (6490)	345.1 Wide / Unit (6490)	30	380	40	5	Negative
	Allo3bDCA	No	391.3 Wide / Unit (6490)	342.8 Wide / Unit (6490)	30	380	40	5	Negative

Scan Parameters

Data Stg	Threshold
Centroid	0

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	15	15
Nebulizer (psi)	30	30
SheathGasHeater	250	250
SheathGasFlow	11	11
Capillary (V)	4500	4500
VCharging	2000	2000

Ion Funnel Parameters

Pos High Pressure RF	100	Neg High Pressure RF	100
Pos Low Pressure RF	80	Neg Low Pressure RF	80

Time Segment 7

Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
	6.7diketoL CA	No	405.3	Wide / Unit (6490)	351.1	Wide / Unit (6490)	35	380	12	5	Positive
	6.7diketoL CA	No	405.3	Wide / Unit (6490)	333.1	Wide / Unit (6490)	35	380	16	5	Positive
	12oxoLCA	No	391.3	Wide / Unit (6490)	309.3	Wide / Unit (6490)	35	380	20	5	Positive
	12oxoLCA	No	391.3	Wide / Unit (6490)	145.1	Wide / Unit (6490)	35	380	32	5	Positive
	7oxoLCA	No	373.3	Wide / Unit (6490)	355.1	Wide / Unit (6490)	35	380	8	5	Positive
	7oxoLCA	No	373.3	Wide / Unit (6490)	335.1	Wide / Unit (6490)	35	380	12	5	Positive
	7oxoLCA	No	373.3	Wide / Unit (6490)	105.1	Wide / Unit (6490)	35	380	58	5	Positive
	GDCA	No	448.2	Wide / Unit (6490)	404.1	Wide / Unit (6490)	35	380	32	5	Negative
	GDCA	No	448.2	Wide / Unit (6490)	386.1	Wide / Unit (6490)	35	380	35	5	Negative
	GDCA	No	448.2	Wide / Unit (6490)	74	Wide / Unit (6490)	35	380	30	5	Negative
	Allo12bDC A	No	391.3	Wide / Unit (6490)	345.1	Wide / Unit (6490)	35	380	36	5	Negative
	Allo12bDC A	No	391.3	Wide / Unit (6490)	327.3	Wide / Unit (6490)	35	380	40	5	Negative

Scan Parameters

Data Stg	Threshold
Centroid	0

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	15	15
Nebulizer (psi)	30	30
SheathGasHeater	250	250
SheathGasFlow	11	11
Capillary (V)	4500	4500
VCharging	2000	2000

Ion Funnel Parameters

Pos High Pressure RF	100	Neg High Pressure RF	100
Pos Low Pressure RF	60	Neg Low Pressure RF	60

Time Segment 8

Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
	d4CDCA	Yes	361.3	Wide / Unit (6490)	95.2	Wide / Unit (6490)	40	380	40	5	Positive
	d4DCA	Yes	361.3	Wide / Unit (6490)	95.1	Wide / Unit (6490)	40	380	40	5	Positive
	CDCA	No	357.2	Wide / Unit (6490)	104.9	Wide / Unit (6490)	40	380	50	5	Positive
	DCA	No	357.2	Wide / Unit (6490)	104.8	Wide / Unit (6490)	40	380	50	5	Positive
	DCA	No	357.2	Wide / Unit (6490)	95.1	Wide / Unit (6490)	40	380	40	5	Positive
	CDCA	No	357.2	Wide / Unit (6490)	81.1	Wide / Unit (6490)	40	380	48	5	Positive
	TLCA	No	482.2	Wide / Unit (6490)	124.2	Wide / Unit (6490)	40	380	45	5	Negative
	TLCA	No	482.2	Wide / Unit (6490)	107.2	Wide / Unit (6490)	40	380	50	5	Negative
	TLCA	No	482.2	Wide / Unit (6490)	80	Wide / Unit (6490)	40	380	56	5	Negative
	GLCA	No	432.2	Wide / Unit (6490)	388.1	Wide / Unit (6490)	40	380	37	5	Negative
	GLCA	No	432.2	Wide / Unit (6490)	74	Wide / Unit (6490)	40	380	41	5	Negative
	AlloDCA	No	391.3	Wide / Unit (6490)	345	Wide / Unit (6490)	40	380	36	5	Negative
	AlloDCA	No	391.3	Wide / Unit (6490)	327.1	Wide / Unit (6490)	40	380	40	5	Negative

Scan Parameters

Data Stg	Threshold
Centroid	0

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	15	15
Nebulizer (psi)	20	20
SheathGasHeater	250	250
SheathGasFlow	11	11
Capillary (V)	4000	4000
VCharging	2000	2000

Ion Funnel Parameters

Pos High Pressure RF	120	Neg High Pressure RF	120
Pos Low Pressure RF	60	Neg Low Pressure RF	60

Time Segment 9

Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion MS1 Res	Prod Ion MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
	AlloLCA	No	377.3 Wide / Unit (6490)	295.1 Wide / Unit (6490)	35	380	16	5	Positive
	Allo3bLCA	No	377.3 Wide / Unit (6490)	295 Wide / Unit (6490)	50	380	12	5	Positive
	d4LCA	Yes	363.3 Wide / Unit (6490)	139.1 Wide / Unit (6490)	50	380	24	5	Positive
	d4LCA	Yes	363.3 Wide / Unit (6490)	99.1 Wide / Unit (6490)	50	380	32	5	Positive
	LCA	No	359.3 Wide / Unit (6490)	135.1 Wide / Unit (6490)	50	380	24	5	Positive
	AlloLCA	No	359.3 Wide / Unit (6490)	135 Wide / Unit (6490)	35	380	25	5	Positive
	LCA	No	359.3 Wide / Unit (6490)	95.1 Wide / Unit (6490)	50	380	32	5	Positive
	Allo3bLCA	No	359.2 Wide / Unit (6490)	135.1 Wide / Unit (6490)	50	380	25	5	Positive
	3oxoLCA	No	357.3 Wide / Unit (6490)	104.8 Wide / Unit (6490)	50	380	60	5	Positive
	3oxoLCA	No	357.3 Wide / Unit (6490)	95 Wide / Unit (6490)	50	380	40	5	Positive
	3oxoLCA	No	357.3 Wide / Unit (6490)	80.9 Wide / Unit (6490)	50	380	48	5	Positive

Scan Parameters

Data Stg      Threshold  
Centroid      0

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	15	15
Nebulizer (psi)	30	30
SheathGasHeater	250	250
SheathGasFlow	11	11
Capillary (V)	5500	0
VCharging	2000	0

Ion Funnel Parameters

Pos High Pressure RF	120	Neg High Pressure RF	120
Pos Low Pressure RF	80	Neg Low Pressure RF	80



Time Segment 10

Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion MS1 Res	Prod Ion MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
	7a,12a-C4	No	417.3 Wide / Unit (6490)	381.1 Wide / Unit (6490)	75	380	17	5	Positive
	7a,12a-C4	No	417.3 Wide / Unit (6490)	253 Wide / Unit (6490)	75	380	53	5	Positive
	7a,12a-C4	No	417.3 Wide / Unit (6490)	97 Wide / Unit (6490)	75	380	45	5	Positive
	7a-C4	No	401.3 Wide / Unit (6490)	383.2 Wide / Unit (6490)	75	380	17	5	Positive
	7a-C4	No	401.3 Wide / Unit (6490)	177 Wide / Unit (6490)	75	380	30	5	Positive
	7a-C4	No	401.3 Wide / Unit (6490)	121.2 Wide / Unit (6490)	75	380	57	5	Positive
	7a-C4	No	401.3 Wide / Unit (6490)	97 Wide / Unit (6490)	75	380	57	5	Positive

Scan Parameters

Data Stg Centroid	Threshold
	0

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	15	15
Nebulizer (psi)	20	20
SheathGasHeater	250	250
SheathGasFlow	11	11
Capillary (V)	3000	3000
VCharging	2000	1500

Ion Funnel Parameters

Pos High Pressure RF	200	Neg High Pressure RF	90
Pos Low Pressure RF	110	Neg Low Pressure RF	60

Time Segment 11

Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion MS1 Res	Prod Ion MS2 Res	Dwell	Frag (V)	CE (V)	Cell Acc (V)	Polarity
	Compound 1	No	350 Wide / Unit (6490)	200 Wide / Unit (6490)	200	380	0	5	Positive

Scan Parameters

Data Stg Centroid	Threshold
	0

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	290	290
Gas Flow (l/min)	15	15
Nebulizer (psi)	20	20
SheathGasHeater	250	250
SheathGasFlow	11	11
Capillary (V)	3000	3000
VCharging	2000	1500

Ion Funnel Parameters

Pos High Pressure RF	200	Neg High Pressure RF	90
Pos Low Pressure RF	110	Neg Low Pressure RF	60

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	10	10000000

## Instrument Curves

Actual  
 VCap  
 Capillary Current  
 Chamber Current  
 Gas Flow  
 Gas Temp  
 High Vac  
 MS1 Heater  
 MS2 Heater  
 NebulizerPressure  
 Pump1Current  
 Pump2Current  
 VacuumPressure1  
 Sheath Gas Flow  
 (l/min)  
 Sheath Gas Temp (°  
 C)  
 TurboSpeed1  
 TurboSpeed2

**Name:** HiP Sampler **Model:** G4226A

### Auxiliary

Draw Speed	200.0 µL/min
Eject Speed	200.0 µL/min
Draw Position Offset	0.0 mm
Wait Time After Drawing	0.0 s
Sample Flush Out Factor	5.0
Vial/Well bottom sensing	No

### Injection

Injection Mode	Injection with needle wash
Injection Volume	3.00 µL
<b>Needle Wash</b>	
Needle Wash Location	Flush Port
Wash Time	10.0 s

### High throughput

Automatic Delay Volume Reduction	No
<b>Overlapped Injection</b>	
Enable Overlapped Injection	No

### Valve Switching

Valve Movements	0
<b>Valve Switch Time 1</b>	
Switch Time 1 Enabled	No
<b>Valve Switch Time 2</b>	
Switch Time 2 Enabled	No
<b>Valve Switch Time 3</b>	
Switch Time 3 Enabled	No
<b>Valve Switch Time 4</b>	
Switch Time 4 Enabled	No

### Stop Time

Stoptime Mode	As pump/No limit
---------------	------------------

### Post Time

Posttime Mode	Off
---------------	-----

Name: **Binary Pump** Model: **G4220A**

Flow 0.500 mL/min  
 Use Solvent Types Yes  
 Stroke Mode Synchronized  
 Low Pressure Limit 0.00 bar  
 High Pressure Limit 1000.00 bar  
 Max. Flow Ramp Up 10.000 mL/min<sup>2</sup>  
 Max. Flow Ramp Down 100.000 mL/min<sup>2</sup>  
 Expected Mixer No check

**Stroke A**

Automatic Stroke Calculation A Yes

**Stop Time**

Stoptime Mode Time set  
 Stoptime 22.50 min

**Post Time**

Posttime Mode Time set  
 Posttime 2.00 min

**Solvent Composition**

	Channel	Ch. 1 Solv.	Name 1	Ch2 Solv.	Name 2	Selected	Used	Percent
1	A	5.0 % ACN in Water V.02	Water	100.0 % Water V.03		Ch. 1	Yes	75.00 %
2	B	95.0 % ACN in Water V.02	ACN	100.0 % Acetonitrile V.03		Ch. 1	Yes	25.00 %

**Timetable**

	Time	A	B	Flow	Pressure
1	3.00 min	75.00 %	25.00 %	--- mL/min	--- bar
2	3.10 min	65.00 %	35.00 %	--- mL/min	--- bar
3	9.00 min	62.00 %	38.00 %	--- mL/min	--- bar
4	15.00 min	35.00 %	65.00 %	--- mL/min	--- bar
5	18.00 min	35.00 %	65.00 %	--- mL/min	--- bar
6	20.00 min	0.00 %	100.00 %	--- mL/min	--- bar
7	22.00 min	0.00 %	100.00 %	--- mL/min	--- bar

Name: **Column Comp.** Model: **G1316C**

Valve Position Position 1 (Port 1 -> 2)  
 Ready when front door open Yes

**Left Temperature Control**

Temperature Control Mode Temperature Set  
 Temperature 55.0 °C

**Enable Analysis Left Temperature**

Enable Analysis Left Temperature On Yes  
 Enable Analysis Left Temperature Value 0.8 °C

**Right Temperature Control**

Right temperature Control Mode Combined

**Enable Analysis Right Temperature**

Enable Analysis Right Temperature On Yes  
 Enable Analysis Right Temperature Value 0.8 °C

**Stop Time**

Stoptime Mode As pump/injector

**Post Time**

Posttime Mode Off

**Timetable**

Name: DAD

Model: G4212A

Peakwidth < 0.0016 min (0.016 s response time) (160 Hz)

Slit 4 nm

UV Lamp Required No

**Analog Output**

Analog Zero Offset 5 %

Analog Attenuation 1000 mAU

**Signals**

**Prepare Mode**

Margin for negative Absorbance 100 mAU

**Autobalance**

Autobalance Prerun No

Autobalance Postrun No

**Spectrum**

Spectrum Store None

**Stoptime**

Stoptime Mode As pump/injector

**Posttime**

Posttime Mode Off

**Timetable**

**Signals**

**Signal table**

	Acquire	Signal
1	No	Signal A
2	No	Signal B
3	No	Signal C
4	No	Signal D
5	No	Signal E
6	No	Signal F
7	No	Signal G
8	No	Signal H