Supplementary file S-1

Standardization of PGC-LC-MS-based glycomics for sample specific glycotyping

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Supplementary Abstract: This document contains all supplementary figures and tables and LC-MS parameters used for acquired data unless specified otherwise in the methods section of the manuscript.

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Trend type	Equation	R^2 value
Linear	$y = 2.5582x + 9.5879$	0.9649
Logarithmic	$y = 15.337\ln(x) - 1.4122$	0.9985
Polynomial (order 2)	$y = -0.2317x^2 + 5.5704x + 1.0147$	0.9966
Polynomial (order 3)	$y = 0.032x^3 - 0.8564x^2 + 9.3349x - 5.8574$	0.9987
Power	$y = 7.97x^{0.6445}$	0.9804

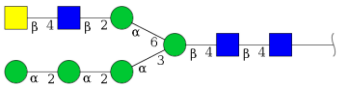
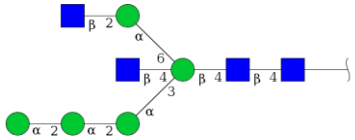
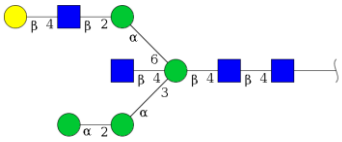
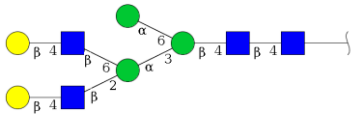
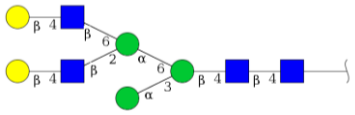
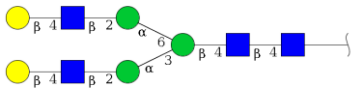
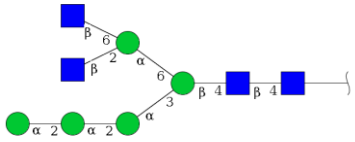
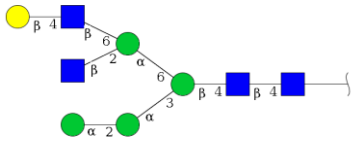
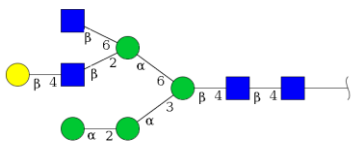
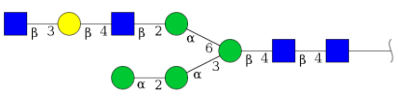
Table S-1 Comparison of several equations for describing dextran ladder elution profile for normalisation of retention time

Standard Glycoprotein	Number of glycan structures	Main type of glycan
Bovine fetuin (*N*-glycans)	17	Complex Sialylated[1]
Bovine lactoferrin (*N*-glycans)	72	Complex Fucosylated[2]
Human neutrophil elastase (*N*-glycans)	16	Paucimannose[3]
Human IgG (*N*-glycans)	6	Complex Non-sialylated[4]
Human IgA (*N*-glycans)	59	Complex Sialylated, Hybrid and Bisected[5]
Human lactoferrin (*N*-glycans)	17	Complex Fucosylated[2]
Fungal cellobiohydrolase I (*N*-glycans)	2	High mannose[6]
Total *N*-glycan unique mammalian structures	189	All types
Porcine gastric mucin (*O*-glycans)	92	*O*-GalNAc, multiple core types[7]
Fungal Cellobiohydrolase I (*O*-glycans)	8	*O*-mannosylation[8]

Table S-2 Purified glycoproteins used for GU library construction of released glycans

Glycan type	Glycan structure/composition	GU value
***N*-glycan**	M3B	3.94
	A4G(4)4F1S4	13.07
	$(Hex)_1$ $(HexNAc)_2$ isomer A	2.17
	$Hex_{(3)}$	2.55
	(Hex)1 (HexNAc)2 isomer B	2.79
	$(Hex)_1$ $(HexNAc)_2$ $(NeuAc)_1$	2.79
	$(Hex)_1$ $(HexNAc)_2$ $(NeuAc)_2$	2.79
***O*-glycan**	(Hex)4 (HexNAc)4 (Deoxyhexose)1 isomer A	13.32
	$(Hex)_4$ $(HexNAc)_4$ $(Deoxyhexose)_3$	13.88
	$(Hex)_4$ $(HexNAc)_4$ $(Deoxyhexose)_1$ isomer B	14.03
	$(Hex)_4$ $(HexNAc)_4$ $(Deoxyhexose)_1$ isomer C	14.62

Table S-3 Glycan compositions and associated glucose unit values with extreme glucose unit values

Theoretical structure accession number	Structure	GlyTouCan Accession	Observed in Glyconnect?
UCORN193		G13151XE	Yes
UCORN194		G20966TZ	Yes
UCORN219		G42175IR	Yes
UCORN511		G43664YB	Yes
UCORN579		G03216SJ	Yes
UCORN380		G36191CD	Yes
UCORN195		G30588SI	No
UCORN222		Not found	No
UCORN223		Not found	No
UCORN209		Not found	No

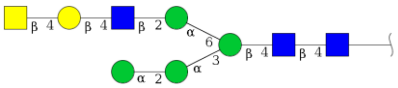
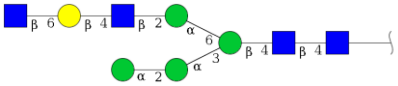
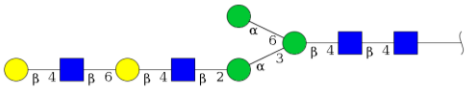
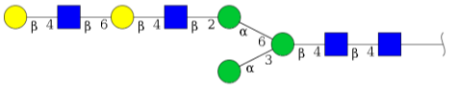
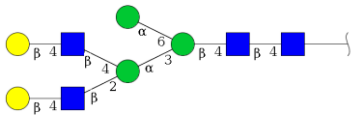
Theoretical structure accession number	Structure	GlyTouCan Accession	Observed in Glyconnect?
UCORN210		Not found	No
UCORN213		Not found	No
UCORN375		Not found	No
UCORN550		Not found	No
UCORN464		G63170HT	No

Table S-4 Example of the gaps in experimental detection of glycan structures predicted bioinformatically for a single glycan composition ($(Hex)_5$ $(HexNAc)_4$) with human biosynthetic constraints (no β1,3 galactose or alpha-gal). Based on database published by Akune et al[9].

Sample Name from Figure 6	Species/ Sex	Location/ Cell-type
HEK293 cell lysate/secreted proteins	Human Female	Kidney Immortalized adrenal cell
BV2 cell lysate/secreted proteins	Mouse Female	Brain Immortalized microglia
PC12 cell lysate	Rat Male	Adrenal gland Cancerous, differentiated into neurites
RVM/PAG	Mouse Male	Brain Mixture of neurons, glia, astrocytes, oligodendrocytes
L5/L4/L3	Mouse Male	Lumbar spinal cord Mixture of neurons, glia, astrocytes, oligodendrocytes

Table S-5 Cell types and tissue samples used in figure 6 with accompanying biological details

Sample	Raw File	Skyline Assay
Bovine Fetuin	CA_fetLAD_NG_270917	Fet NG with ladder_GUnames.sky
Bovine Lactoferrin	CA_BLFLAD_NG_270917	BLF with ladder_GUnames.sky
Human Neutrophil Elastase	CA_HNELAD_NG_270917	HNE with ladder_GUnames.sky
Human IgA	CA_igaLAD_NG_270917	IgA with ladder_GUnames.sky
Human IgG	CA_IggLAD_NG_270916	IgG with ladder_GUnames.sky
Human Lactoferrin	CA_hlfLAD_NG_270917	HNE with ladder_GUnames.sky
Porcine Gastric Mucin	CA_PGMLAD_OG_051017	PGM OG with ladder_GUnames.sky
Cellobiohydrolase I	CA_CBHILAD_NG_270917 CA_CBHILAD_OG_051017	CBHI NG with ladder_GUnames.sky CBHI OG with ladder_GUnames.sky
U87MG cell lysate	CA_U87MG_CL_LAD_NG_090117	U87MG with ladder_GUnames.sky
U87MG secreted proteins	CA_U87MG_sec_LAD_NG_090117	Fig6.sky
BV2 cell lysate	CA_BV2_CL_LAD_NG_090117	Fig6.sky
BV2 secreted proteins	CA_BV2_sec_LAD_NG_090117	Fig6.sky
PC12 cell lysate	CA_PC12_NCF_CL_LAD_NG_090117	Fig6.sky
HEK293 cell lysate	CA_HEK293_CL_LAD_NG_090117	Fig6.sky
HEK293 secreted proteins	CA_HEK293_sec_LAD_NG_090117	Fig6.sky
RVM	CA_RVM_NT_LAD_NG_090117	Fig6.sky
PAG	CA_PAG_NT_LAD_NG_090117	Fig6.sky
L5	CA_L5_NT_LAD_NG_090117	Fig6.sky
L4	CA_L4_NT_LAD_NG_090117	Fig6.sky
L3	CA_L3_NT_LAD_NG_090117	Fig6.sky

Table S-6 Data file links. All *N*-glycan files are annotated in Annotated_NG_structures.gwp and Annotated_U87MG_structures.gwp. All *O*-glycan files are annotated in Annotated_OG_structures.gwp.

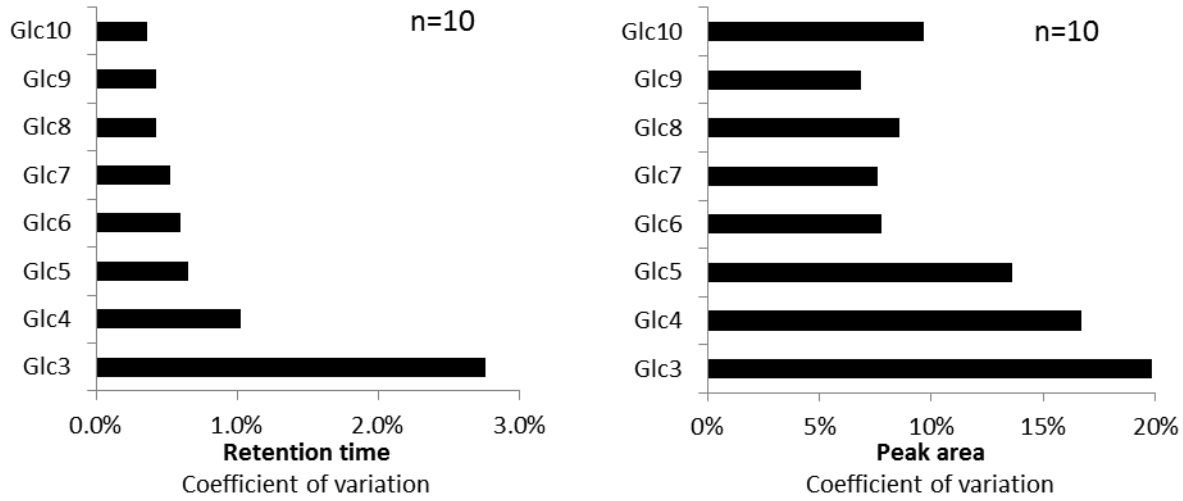


Figure S-1 System-specific variation in retention time and precursor ion peak area between technical replicates

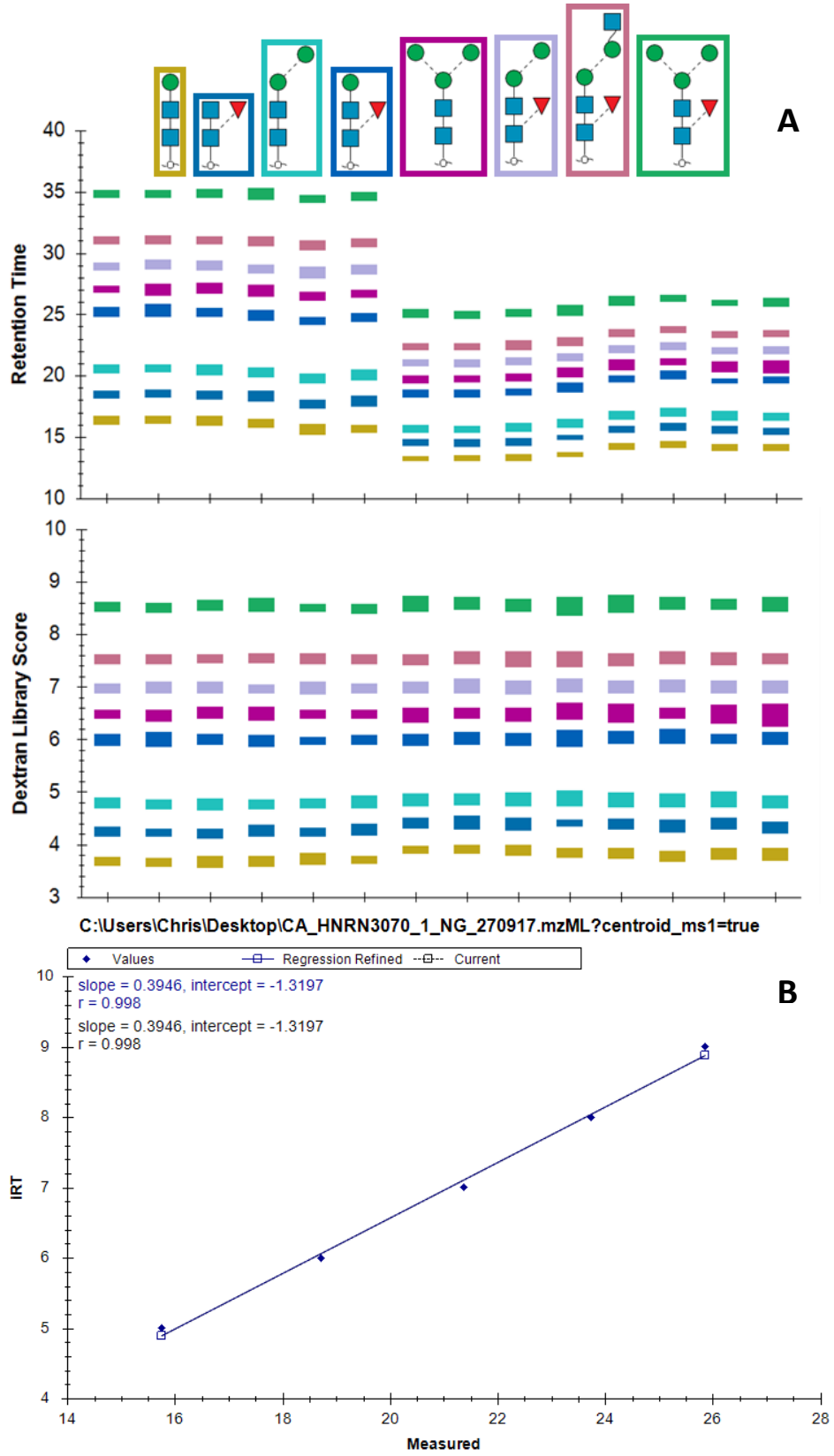


Figure S-2 Application of linear normalisation equation for measured retention time correction to glucose unit values (iRT) using Skyline. **(A)** Retention time shifts normalised using linear retention time normalisation. **(B)** Example of linear retention time normalisation in Skyline

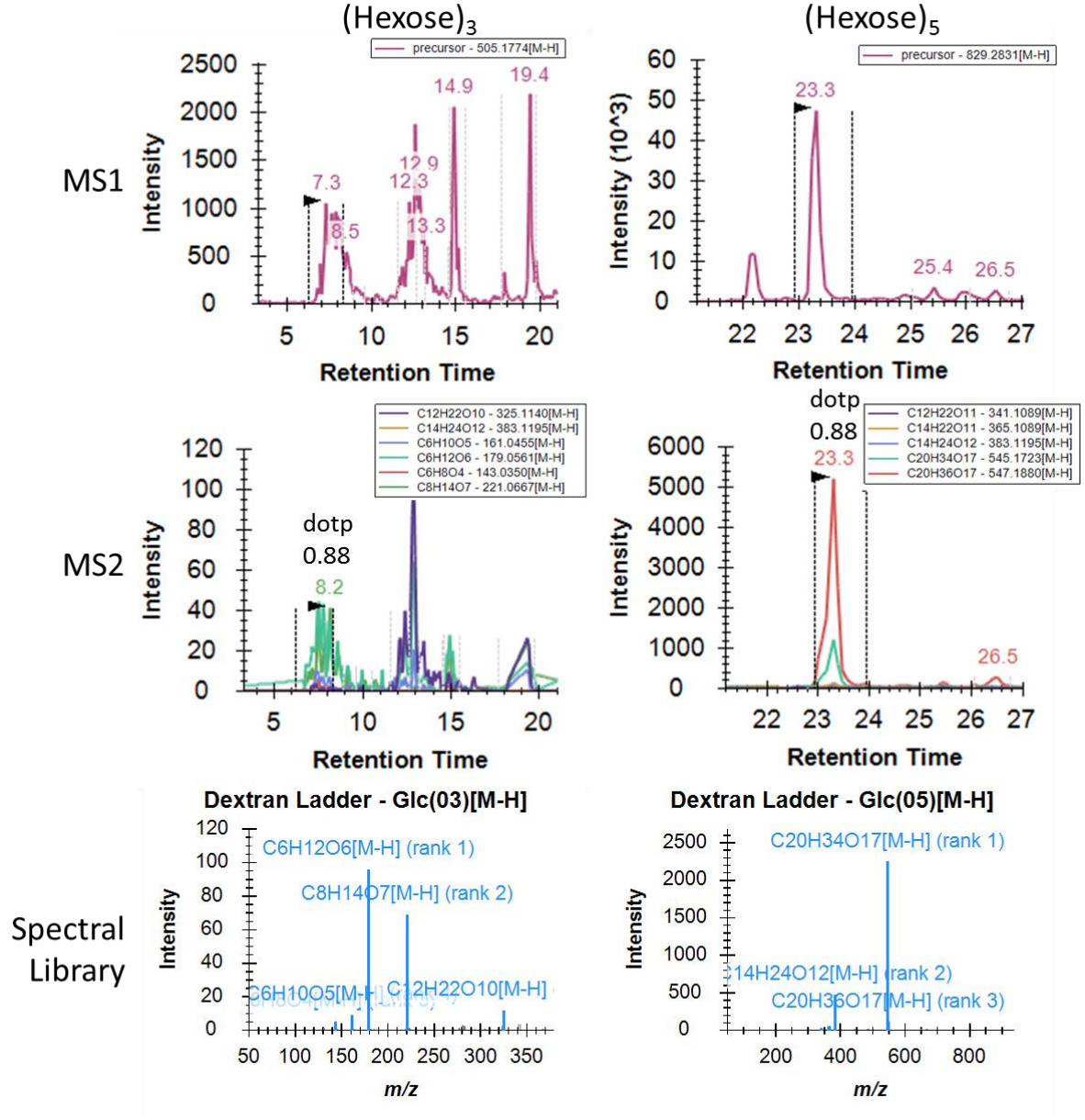


Figure S-3 Correct peak assignment of dextran ladder in isomeric mixtures using diagnostic fragments in Skyline

LC-MS Method Details

Program for Dionex Chromatography MS Link (LC)

ColumnOven.TempCtrl = On
ColumnOven.Temperature.Nominal = 60.0 [°C]
ColumnOven.Temperature.LowerLimit = 55.0 [°C]
ColumnOven.Temperature.UpperLimit = 65.0 [°C]
EquilibrationTime = 0.0 [min]
ColumnOven.ReadyTempDelta = 5.0 [°C]
Sampler.TempCtrl = On
Sampler.Temperature.Nominal = 5.0 [°C]
Sampler.Temperature.LowerLimit = 4.0 [°C]
Sampler.Temperature.UpperLimit = 45.0 [°C]
Sampler.ReadyTempDelta = None
LoadingPump.Pressure.LowerLimit = 0 [bar]
LoadingPump.Pressure.UpperLimit = 500 [bar]
LoadingPump.MaximumFlowRampDown = 998 [µl/min²]
LoadingPump.MaximumFlowRampUp = 998 [µl/min²]
LoadingPump.%A.Equate = "%A"
LoadingPump.%B.Equate = "%B"
%C.Equate = "%C"
NC_Pump.Pressure.LowerLimit = 0 [bar]
NC_Pump.Pressure.UpperLimit = 626 [bar]
NC_Pump.MaximumFlowRampDown = 2.000 [µl/min²]
NC_Pump.MaximumFlowRampUp = 2.000 [µl/min²]
NC_Pump.%A.Equate = "10mM AMBIC"
NC_Pump.%B.Equate = "70% ACNR 10mM AMBIC"
DrawSpeed = 200 [nl/s]
DrawDelay = 5000 [ms]
DispSpeed = 2000 [nl/s]
DispenseDelay = 2000 [ms]
WasteSpeed = 4000 [nl/s]
WashSpeed = 4000 [nl/s]
LoopWashFactor = 2.000
SampleHeight = 2.000 [mm]
PunctureDepth = 7.000 [mm]
WashVolume = 50.000 [µl]
InjectMode = ulPickUp
NC_Pump_Pressure.Step = Auto
NC_Pump_Pressure.Average = Off
RinseBetweenReinjections = Yes
FirstTransportVial = G1
LastTransportVial = G1
TransportVialCapacity = 9999
TransLiquidHeight = 4.000 [mm]

```
    TransVialPunctureDepth =      7.000 [mm]
    FlushVolume =               2.400 [µl]
    LoadingPump.Flow =          0.000 [µl/min]
    LoadingPump.%B =           20.0 [%]
    %C =                    0.0 [%]
    ValveRight =              1_2
    ColumnOven_Temp.Step =       Auto
    ColumnOven_Temp.Average =     On
    NC_Pump_Press_RightBlk.Step = Auto
    NC_Pump_Press_RightBlk.Average =  Off
    NC_Pump_Press_LeftBlk.Step =  Auto
    NC_Pump_Press_LeftBlk.Average =  Off
    Relay_4.State Off

 0.000 Wait                 LoadingPump.Ready and NC_Pump.Ready and ColumnOven.Ready and
Sampler.Ready and PumpModule.Ready
    ;Chromeleon sets this property to signal to Xcalibur that it is ready to start a run.
    ReadyToRun =              1
    ;Xcalibur sets this property to start the run or injection.
    Wait                   StartRun
    NC_Pump.Flow =            4.000 [µl/min]
    NC_Pump.%B =              1.0 [%]
    Wait                   LoadingPump.Ready and NC_Pump.Ready and ColumnOven.Ready and
Sampler.Ready and PumpModule.Ready
    Inject
    NC_Pump_Pressure.AcqOn
    ColumnOven_Temp.AcqOn
    NC_Pump_Press_RightBlk.AcqOn
    NC_Pump_Press_LeftBlk.AcqOn
    ;Chromeleon sets this property to signal the injection to Xcalibur.
    InjectResponse =          1
    ;Depending on your system configuration it might be necessary to manually insert
    ;a "Relay" command below in order to send the start signal to the MS.
    ;Typical syntaxes:
    ;Pump_Relay_1.Closed  Duration = 2.00
    ;UM3PUMP_Relay1.On    Duration = 2.00
    ;Pump_Relay_1.Closed Duration =2.00
    ;UM3PUMP_Relay1.On Duration = 2.00
    NC_Pump.Flow =            4.000 [µl/min]
    NC_Pump.%B =              1.0 [%]

 4.900 NC_Pump.Flow =            4.000 [µl/min]
    NC_Pump.%B =              1.0 [%]

 5.000 NC_Pump.Flow =            4.000 [µl/min]
    NC_Pump.%B =              7.8 [%]
```

73.000 NC_Pump.Flow = 4.000 [µl/min]
NC_Pump.%B = 64.3 [%]

73.100 NC_Pump.Flow = 4.000 [µl/min]
NC_Pump.%B = 99.0 [%]

78.000 NC_Pump.Flow = 4.000 [µl/min]
NC_Pump.%B = 99.0 [%]

78.100 NC_Pump.Flow = 4.000 [µl/min]
NC_Pump.%B = 1.0 [%]

83.000 NC_Pump.Flow = 4.000 [µl/min]
ColumnOven_Temp.AcqOff
NC_Pump.%B = 1.0 [%]
NC_Pump_Pressure.AcqOff
NC_Pump_Press_RightBlk.AcqOff
NC_Pump_Press_LeftBlk.AcqOff
InjectResponse = 0
End

MS Method

Instructment Method
W:\1_RAW_Data\3_Method_Development\Ashwood_Chris\Velos Pro data from previous lab\CA_hlfLAD_NG_270917.raw
Creator: Velos Pro
Last modified: 28/09/2017 by Velos Pro

MS Run Time (min): 90.00
Sequence override of method parameters not enabled.
Divert Valve: not used during run
Contact Closure: not used during run
Syringe Pump: not used during run

MS Detector Settings:
Real-time modifications to method disabled
Stepped collision energy not enabled

Additional Microscans:

MS2	0	0
MS3	0	0
MS4	0	0
MS5	0	0
MS6	0	0
MS7	0	0
MS8	0	0
MS9	0	0
MS10	0	0

Segment 1 Information
Duration (min): 90.00
Number of Scan Events: 6
Tune Method: CA_2017_quant_tunefile_zoom2700V
Scan Event Details:
1: ITMS - p norm !corona !pi oZ(570.0-2000.0)
CV = 0.0V
2: ITMS - p norm !corona !pi Dep Enhanced Wideband MS/MS Most intense ion from (1)
Activation Type: CID
Min. Signal Required: 1.0
Isolation Width: 2.00
Normalized Coll. Energy: 33.0
Default Charge State: 2
Activation Q: 0.250
Activation Time: 10.000
CV = 0.0V
3: ITMS - p norm !corona !pi Dep Enhanced Wideband MS/MS 2nd most intense ion from (1)
Activation Type: CID
Min. Signal Required: 20.0
Isolation Width: 2.00
Normalized Coll. Energy: 33.0

Default Charge State: 2
Activation Q: 0.250
Activation Time: 10.000
CV = 0.0V

4: ITMS - p norm !corona !pi Dep Enhanced Wideband MS/MS 3rd most intense ion from (1)
Activation Type: CID
Min. Signal Required: 20.0
Isolation Width: 2.00
Normalized Coll. Energy: 33.0
Default Charge State: 2
Activation Q: 0.250
Activation Time: 10.000
CV = 0.0V

5: ITMS - p norm !corona !pi Dep Enhanced Wideband MS/MS 4th most intense ion from (1)
Activation Type: CID
Min. Signal Required: 20.0
Isolation Width: 2.00
Normalized Coll. Energy: 33.0
Default Charge State: 2
Activation Q: 0.250
Activation Time: 10.000
CV = 0.0V

6: ITMS - p norm !corona !pi Dep Enhanced Wideband MS/MS 5th most intense ion from (1)
Activation Type: CID
Min. Signal Required: 20.0
Isolation Width: 2.00
Normalized Coll. Energy: 33.0
Default Charge State: 2
Activation Q: 0.250
Activation Time: 10.000
CV = 0.0V

Data Dependent Settings:
Use separate polarity settings disabled
Neutral Loss Mass List: (none)
Product Mass List: (none)
Neutral loss in top: 3
Product in top: 3
Most intense if no parent masses found not enabled
Add/subtract mass not enabled
Charge state screening not enabled
Charge state dependent ETD time not enabled
Charge state rejection not enabled

Global Data Dependent Settings:
Predict ion injection time enabled
Use global parent and reject mass lists enabled
Exclude parent mass from data dependent selection not enabled
Exclusion mass width by mass
Exclusion mass width low: 1.75

Exclusion mass width high: 2.00
Parent mass width by mass
Parent mass width low: 1.50
Parent mass width high: 2.00
Reject mass width by mass
Reject mass width low: 0.50
Reject mass width high: 0.50
Zoom/UltraZoom scan mass width by mass
Zoom/UltraZoom scan mass width low: 1.00
Zoom/UltraZoom scan mass width high: 2.50
Neutral Loss candidates processed by decreasing intensity
Neutral Loss mass width by mass
Neutral Loss mass width low: 0.50
Neutral Loss mass width high: 0.50
Product candidates processed by decreasing intensity
Product mass width by mass
Product mass width low: 0.50
Product mass width high: 0.50
MS mass range: 550.00-1000000.00
MSn mass range by mass
MSn mass range: 0.00-1000000.00
Analog UV data dep. not enabled
Dynamic exclusion enabled
Repeat Count: 100
Repeat Duration: 0.00
Exclusion List Size: 25
Exclusion Duration: 0.00
Exclusion mass width by mass
Exclusion mass width low: 1.75
Exclusion mass width high: 2.00
Expiration: disabled
Isotopic data dependence not enabled
Mass Tags data dependence not enabled

Global Parent Masses:
(none)

Global Reject Masses:

MS Mass	Start (min)	End (min)	Name
588.94	0.000	90.000	Nitrogen source contam 2
641.12	0.000	90.000	Nitrogen source contam.

Custom Data Dependent Settings:
Not enabled