

Thermo Scientific SII for Xcalibur Method

---- Overview ----

Name: New Instrument Method

Comment:

Run time: 141.000 [min]

Instrument: NanoLC on b200-pc102

Description:

---- Script ----

```
initial      Instrument Setup
              ColumnOven.TempCtrl: Off
              Sampler.LowDispersionMode: Off
              Sampler.WashSpeed: 4.000 [µl/s]
              Sampler.WashVolume: 100.000 [µl]
              Sampler.PunctureDepth: 8.000 [mm]
              Sampler.SampleHeight: 0.000 [mm]
              Sampler.WasteSpeed: 4.000 [µl/s]
              Sampler.DispenseDelay: 2.000 [s]
              Sampler.DispSpeed: 2.000 [µl/s]
              Sampler.DrawSpeed: 0.200 [µl/s]
              Sampler.DrawDelay: 5.000 [s]
              Sampler.RinseBetweenReinjections: Yes
              Sampler.FlushVolume: 5.000 [µl]
              Sampler.TransVialPunctureDepth: 8.000 [mm]
              Sampler.TransLiquidHeight: 5.000 [mm]
              Sampler.TransportVialCapacity: 99999
              Sampler.LastTransportVial: R1
              Sampler.FirstTransportVial: R1
              Sampler.InjectMode: ulPickUp
              Sampler.LoopWashFactor: 2.000
              Sampler.PumpDevice: "LoadingPump"
              Sampler.TempCtrl: On
              Sampler.Temperature.Nominal: 5.0 [°C]
              Sampler.ReadyTempDelta: 3.0 [°C]
              Sampler.Temperature.LowerLimit: 4.0 [°C]
              Sampler.Temperature.UpperLimit: 45.0 [°C]
              PumpModule.LoadingPump.%A.Equate: "%A"
              PumpModule.LoadingPump.%B.Equate: "%B"
              PumpModule.LoadingPump.%C.Equate: "%C"
              PumpModule.LoadingPump.Pressure.LowerLimit: 0 [bar]
              PumpModule.LoadingPump.Pressure.UpperLimit: 500 [bar]
              PumpModule.LoadingPump.MaximumFlowRampUp: 31 [µl/min²]
              PumpModule.LoadingPump.MaximumFlowRampDown: 31 [µl/min²]
              PumpModule.NC_Pump.%A.Equate: "%A"
              PumpModule.NC_Pump.%B.Equate: "%B"
              PumpModule.NC_Pump.Pressure.LowerLimit: 0 [bar]
              PumpModule.NC_Pump.Pressure.UpperLimit: 800 [bar]
              PumpModule.NC_Pump.MaximumFlowRampUp: 0.300 [µl/min²]
              PumpModule.NC_Pump.MaximumFlowRampDown: 0.300 [µl/min²]
              ColumnOven.ValveLeft: 1_2
```

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0.000 [min] Equilibration
PumpModule.LoadingPump.Flow.Nominal: 30.000 [µl/min]
PumpModule.LoadingPump.%B.Value: 0.0 [%]
PumpModule.LoadingPump.%C.Value: 0.0 [%]
PumpModule.LoadingPump.Curve: 5
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 2.0 [%]
PumpModule.NC_Pump.Curve: 5
0.000 [min] Inject Preparation
Wait Sampler.Ready And PumpModule.LoadingPump.Ready And PumpModule.NC_Pump.Ready And ColumnOven.Ready
0.000 [min] Inject
Sampler.Inject
0.000 [min] Start Run
ColumnOven.ColumnOven_Temp.AcqOn
PumpModule.LoadingPump.LoadingPump_Pressure.AcqOn
PumpModule.NC_Pump.NC_Pump_Flow.AcqOn
PumpModule.NC_Pump.NC_Pump_Flow_LeftBlk.AcqOn
PumpModule.NC_Pump.NC_Pump_Flow_RightBlk.AcqOn
PumpModule.NC_Pump.NC_Pump_Pressure.AcqOn
0.000 [min] Run
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 2.0 [%]
PumpModule.NC_Pump.Curve: 5
3.000 [min]
PumpModule.LoadingPump.Flow.Nominal: 30.000 [µl/min]
PumpModule.LoadingPump.%B.Value: 0.0 [%]
PumpModule.LoadingPump.%C.Value: 0.0 [%]
PumpModule.LoadingPump.Curve: 5
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 2.0 [%]
PumpModule.NC_Pump.Curve: 5
ColumnOven.ValveLeft: 10_1
4.000 [min]
PumpModule.LoadingPump.Flow.Nominal: 5.000 [µl/min]
PumpModule.LoadingPump.%B.Value: 0.0 [%]
PumpModule.LoadingPump.%C.Value: 0.0 [%]
PumpModule.LoadingPump.Curve: 5
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 7.0 [%]
PumpModule.NC_Pump.Curve: 5
124.000 [min]
PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
PumpModule.NC_Pump.%B.Value: 38.0 [%]
PumpModule.NC_Pump.Curve: 5
PumpModule.LoadingPump.Flow.Nominal: 5.000 [µl/min]
PumpModule.LoadingPump.%B.Value: 0.0 [%]
PumpModule.LoadingPump.%C.Value: 0.0 [%]
PumpModule.LoadingPump.Curve: 5

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```
125.000 [min]
    PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
    PumpModule.NC_Pump.%B.Value: 98.0 [%]
    PumpModule.NC_Pump.Curve: 5
    PumpModule.LoadingPump.Flow.Nominal: 30.000 [µl/min]
    PumpModule.LoadingPump.%B.Value: 0.0 [%]
    PumpModule.LoadingPump.%C.Value: 0.0 [%]
    PumpModule.LoadingPump.Curve: 5
    ColumnOven.ValveLeft: 1_2
130.000 [min]
    PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
    PumpModule.NC_Pump.%B.Value: 98.0 [%]
    PumpModule.NC_Pump.Curve: 5
131.000 [min]
    PumpModule.NC_Pump.Flow.Nominal: 0.300 [µl/min]
    PumpModule.NC_Pump.%B.Value: 2.0 [%]
    PumpModule.NC_Pump.Curve: 5
141.000 [min] Stop Run
    ColumnOven.ColumnOven_Temp.AcqOff
    PumpModule.LoadingPump.LoadingPump_Pressure.AcqOff
    PumpModule.NC_Pump.NC_Pump_Flow.AcqOff
    PumpModule.NC_Pump.NC_Pump_Flow_LeftBlk.AcqOff
    PumpModule.NC_Pump.NC_Pump_Flow_RightBlk.AcqOff
    PumpModule.NC_Pump.NC_Pump_Pressure.AcqOff
```

Method Summary

Method Settings

Application Mode: **Peptide**
Method Duration (min): **141**

Global Parameters

Ion Source

Use Ion Source Settings from Tune: **True**
FAIMS Mode: **Not Installed**

MS Global Settings

Infusion Mode: **Liquid Chromatography**
Expected LC Peak Width (s): **30**
Advanced Peak Determination: **True**
Default Charge State: **2**
Internal Mass Calibration: **Off**

Experiment#1 [Orbitrap HCD - High Load (greater than 500 ng)]

Start Time (min): **0**
End Time (min): **141**
Cycle Time (sec): **3**

Master Scan:

MS OT

Detector Type: **Orbitrap**
Orbitrap Resolution: **120000**
Mass Range: **Normal**
Use Quadrupole Isolation: **True**
Scan Range (m/z): **380-1400**
RF Lens (%): **30**
AGC Target: **Standard**
Maximum Injection Time Mode: **Auto**
Microscans: **1**
Data Type: **Profile**

Polarity: **Positive**
Source Fragmentation: **Disabled**
Scan Description:

Filters:

MIPS

Monoisotopic Peak Determination: **Peptide**

Charge State

Include charge state(s): **2-7**
Include undetermined charge states: **False**

Dynamic Exclusion

Exclude after n times: **1**
Exclusion duration (s): **45**
Mass Tolerance: **ppm**
Low: **10**
High: **10**
Exclude Isotopes: **True**
Perform dependent scan on single charge state per precursor only: **True**

Intensity

Filter Type: **Intensity Threshold**
Intensity Threshold: **5.0e4**

Data Dependent

Data Dependent Mode: **Cycle Time**
Time between Master Scans (sec): **3**

Scan Event Type 1:

Sort by Intensity

Precursor Priority: **Most Intense**

Scan:

ddMS² OT HCD

Isolation Mode: **Quadrupole**
Isolation Window (m/z): **1.2**
Isolation Offset: **Off**
Activation Type: **HCD**
Collision Energy Mode: **Fixed**
HCD Collision Energy Type: **Normalized**
HCD Collision Energy (%): **30**
Detector Type: **Orbitrap**
Orbitrap Resolution: **15000**
TurboTMT: **Off**
Mass Range: **Normal**
Scan Range Mode: **Auto**
AGC Target: **Standard**
Maximum Injection Time Mode: **Custom**
Maximum Injection Time (ms): **22**
Microscans: **1**
Data Type: **Centroid**
Scan Description: